WROCŁAW UNIVERSITY
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M.Sc. Eng. Karolina Sobieraj

Biological production of the carbon monoxide from biowaste

Biologiczna produkcja tlenku węgla z bioodpadów

Doctoral dissertation conducted at
Department of Applied Bioeconomy
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under the supervision of
Prof. Andrzej Białowiec, Eng., PhD
Prof. Jacek Koziel, Eng., PhD

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“

Whether you voice
a well-turned phrase
or write a wise book,
your mind will still be
empty and silent

Czy zdanie okrągłe wypowiesz,
czy księgę mądrą napiszesz,
będziesz zawsze mieć w głowie
tę samą pustkę i ciszę

”

Grzegorz Turnau
"Naprawdę nie dzieje się nic"/
"Nothing Really Happens" (1991)

Abstract

The study aimed to gain new fundamental knowledge in the field of biological production of carbon monoxide (CO) during composting of biowaste, in particular in the field of: (i) determination of optimal aeration level and thermal conditions for CO production during biowaste composting; (ii) isolation and identification of microorganisms responsible for CO production while biowaste composting; (iii) determination of CO emission factors from compost piles on a technical scale. Due to the toxic nature of the CO additional research aim was to assess the potential occupational exposure of composting plant workers to CO during biowaste composting. The state-of-the-art in the field of biological CO production was reviewed, as well as research was designed and performed, including mathematical modeling, laboratory analyses, and experiments on a technical scale, enabling the preparation of the concept of the biowaste composting process focused on CO generation along with recommendations taking into account ensuring the safety of such a process in the context of employees' exposure to this gas.

Research on the CO production potential during biowaste composting under controlled laboratory conditions depending on different aeration rates (2.7, 3.4, 4.8, and 7.8 $L \cdot h^{-1}$) and temperatures (T=35, 45, 55, and 65°C) proved that thermal conditions and aeration level affect CO concentrations but only at the low temperatures and aeration rates (<45°C and <3.4 $L \cdot h^{-1}$).

Analyses of the isolation and identification of microorganisms potentially responsible for CO production from compost samples taken from the pile on a technical scale indicated that all isolates were thermophilic and anaerobic bacteria. Their incubation in laboratory-scale bioreactors allowed the identification of bacterial strains producing CO at concentrations exceeding 1,000 ppm (*Bacillus paralicheniformis*), >800 ppm (*Bacillus licheniformis*), and close to 600 ppm (*Geobacillus thermodenitrificans*).

During research conducted on a technical scale on CO emission from compost piles located in a hermetised composting hall and in an open yard, before and after material turning, using the flux chamber method, it was proved that CO emission is 14x to 39x higher in the closed hall. In addition, net CO emissions to the atmosphere are between 1.7x and 13.7x higher after material turning. The modeling of the CO concentration in the composting hall showed that after 1 h the level of this gas can reach ~50 mg $CO \cdot m^{-3}$ before material turning and >115 mg $CO \cdot m^{-3}$ after, exceeding the World Health Organization (WHO) thresholds for 1-hour and 15-minutes exposure to CO, respectively.

In turn, modeling of the accumulation of CO concentration in a static bioreactor during 14 days of biowaste composting showed that in each of the analyzed variants of the ratio of headspace-to-waste volumes (H:W) (4:1, 3:1, 2:1, 1:1, 1:2, 1:3, 1:4), the CO level exceeded the value of 100 ppmv safe for 15 minutes of work in the composting plant. The CO concentration reached the maximum of 36.1% for the variant without bioreactor ventilation and 3.2% with the daily release of accumulated gas (bioreactor ventilation). Modeling has shown that the airflow required to keep the CO concentration below 100 ppmv should be at least 7.15 $m^3 \cdot (h \cdot Mg \text{ of wet mass of waste})^{-1}$, and that the process with H:W ratio >4:1 and compost pile height <1 m is less susceptible to CO accumulation.

Streszczenie

Celem badawczym rozprawy doktorskiej było zdobycie nowej, podstawowej wiedzy z zakresu biologicznej produkcji tlenku węgla (CO) podczas kompostowania bioodpadów w zakresie: (i) określenia optymalnego poziomu napowietrzenia i warunków termicznych dla produkcji CO podczas kompostowania bioodpadów; (ii) izolacji i identyfikacji mikroorganizmów odpowiedzialnych za produkcję CO podczas kompostowania bioodpadów; (iii) określenia wskaźników emisji CO z pryzm kompostowych w skali technicznej. Ze względu na toksyczny charakter CO dodatkowym celem badań była ocena potencjalnego narażenia zawodowego pracowników kompostowni w związku z ekspozycją na podwyższone stężenie CO podczas kompostowania bioodpadów. W pracy dokonano przeglądu aktualnego stanu wiedzy w zakresie biologicznej produkcji CO, a także zaprojektowano i wykonano badania w skali laboratoryjnej i technicznej oraz przeprowadzono modelowanie matematyczne, umożliwiające przygotowanie koncepcji teoretycznej dotyczącej ukierunkowania procesu kompostowania bioodpadów na generację CO wraz z zaleceniami uwzględniającymi zapewnienie bezpieczeństwa takiego procesu w kontekście narażenia pracowników na ten gaz.

Badania potencjału produkcji CO podczas kompostowania bioodpadów w kontrolowanych warunkach laboratoryjnych w zależności od różnych poziomów napowietrzania (2,7, 3,4, 4,8 i 7,8 $L \cdot h^{-1}$) oraz warunków termicznych (T=35, 45, 55 i 65°C) wskazały, że temperatura i poziom napowietrzenia w procesie wpływają na stężenie CO, ale tylko przy niskich zakresach wartości (<45°C i <3,4 $L \cdot h^{-1}$).

Analizy izolacji i identyfikacji mikroorganizmów potencjalnie odpowiedzialnych za produkcję CO z próbek kompostu pobranych z pryzmy w skali technicznej wskazały, że wszystkie izolaty były termofilnymi beztlenowymi bakteriami. Ich inkubacja w bioreaktorach w skali laboratoryjnej pozwoliła na zidentyfikowanie szczepów bakteryjnych wytwarzających CO w stężeniach przekraczających 1,000 ppm (*Bacillus paralicheniformis*), >800 ppm (*Bacillus licheniformis*) i zbliżonych do 600 ppm (*Geobacillus thermodenitrificans*).

Podczas badań prowadzonych w skali technicznej nad emisją CO z pryzm kompostowych zlokalizowanych w hermetycznej hali kompostowej oraz na otwartym zewnętrznym placu przed i po przerzuceniu materiału, przy wykorzystaniu metody zamkniętej komory badawczej *flux chamber*, dowiedziono, że emisja CO jest od 14 do 39 razy wyższa w zamkniętej hali. Dodatkowo wskaźniki emisji CO netto do atmosfery są od 1.7 do 13.7 razy wyższe

po przerzuceniu materiału. Przeprowadzone modelowanie stężenia CO w hali kompostowej dowiodło, że po 1 h poziom tego gazu może osiągnąć ~50 mg CO·m^{-3} przed przerzuceniem materiału i >115 mg CO·m^{-3} po, przekraczając dopuszczalne przez Światową Organizację Zdrowia (WHO) progi dla 1-godzinnej oraz 15-minutowej ekspozycji na CO, odpowiednio.

Z kolei modelowanie akumulacji stężenia CO w bioreaktorze statycznym podczas kompostowania bioodpadów dowiodło, że w każdym z analizowanych wariantów stosunku objętości headspace bioreaktora do objętości odpadów (H:W równe 4:1, 3:1, 2:1, 1:1, 1:2, 1:3, 1:4), poziom CO przekraczał bezpieczną dla 15 minut pracy w kompostowni wartość 100 ppmv. Stężenie CO osiągnęło maksymalne wartości równe 36,1% dla wariantu bez wentylacji bioreaktora oraz 3,2% przy codziennym uwalnianiu nagromadzonego gazu (wentylacja bioreaktora). Modelowanie dowiodło, że przepływ powietrza niezbędny do utrzymania stężenia CO poniżej 100 ppmv powinien wynosić co najmniej 7,15 m^{3}·(h·Mg mokrej masy odpadów)$^{-1}$, a prowadzenie procesu przy zachowaniu stosunku H:W >4:1 i wysokości pryzmy kompostowej < 1 m jest mniej podatne na akumulację CO.

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1. Introduction

The pressing global problems in recent years related to the depletion of intensively exploited fossil resources, the release of greenhouse gases (GHGs), or excessive waste production have made it necessary to look for a new context and functionalities of previously known processes. In this way, it is possible not only to reduce dependence on non-renewable raw materials but also to increase the efficiency of the processes of valorization of waste materials [1]. One such process, for which a new view, going beyond the existing one, is justified, is the process of biological aerobic treatment of biowaste by composting. As a relatively simple to operate and cheap method, it has become one of the key options for treating organic waste. In Europe, it is considered the most common type of biowaste processing, dealing with >60% of its produced mass (approx. 42 million tons per year) [2]. In 2022, the number of composting plants across the EU27 countries together with Norway, Switzerland, and the UK reached 3,800 installations [3]. Moreover, as predicted, due to the European Union's target to recycle and reuse 65% of municipal solid waste by 2035, it is projected that additional ~40 million tons of biowaste per year will be directed to biological treatment processes, which will result in significant development of composting infrastructure. To process this amount of biowaste, 7,600 installations will be needed [3].

The universality of composting, as well as many years of experience in its conduct around the world, have translated into the perception of this process as reliable in reducing the mass of generated waste and producing organic fertilizer, but at the same time – as a process with a developed and closed formula, known and commonly used since the 20th century [4]. This process, considered to be well known, has been described in the literature by many researchers, including in the field of optimal process parameters [5,6], its modeling [7], optimization [8], composting emissivity [9] or the process additives [10]. The subject of emissivity of the composting process itself includes many substances and gases produced by the decomposition of organic matter contained in waste, such as CO_2, CH_4, N_2O, Volatile Organic Compounds (VOCs), odors, and bioaerosols [9,11–13]. However, the literature ignores one of the important process gases generated during the composting of organic waste with utility potential, which is carbon monoxide (CO).

2. CO production during composting

So far, the production of CO during composting has been observed both on a laboratory and technical scale for various waste fractions as a result of the biological breakdown of organic matter. Composting of green waste [14,15], green waste with manure [16], organic waste [17], and aerobic biostabilization of municipal waste [18,19] have all been linked to CO production.

However, this topic is not often discussed in the literature; the formation of this gas is rather associated with combustion and anaerobic waste treatment processes. Bibliometric analysis of journal articles included in the Web of Science Core Collection without a specific date range using a combination of keywords ‘composting’ and ‘carbon monoxide’ showed that based on parameters such as title, abstract, author keywords, and Keywords Plus, 37 papers on this subject were found (excluding manuscripts which are part of this doctoral dissertation) [20]. A few studies on the generation of CO during aerobic waste treatment were first published in the 1990s, but it wasn't until 2006 that the number of studies on this topic climbed to three per year (Figure 1). Recently, the topic of CO production during composting has become more popular, but the number of articles in this area has not increased significantly (up to 6 articles per year between 2019 and 2022). Although progress in this area of research has been fairly modest, there are still plenty of opportunities to connect the dots and open up new areas of research into the biobased generation of CO from composting.

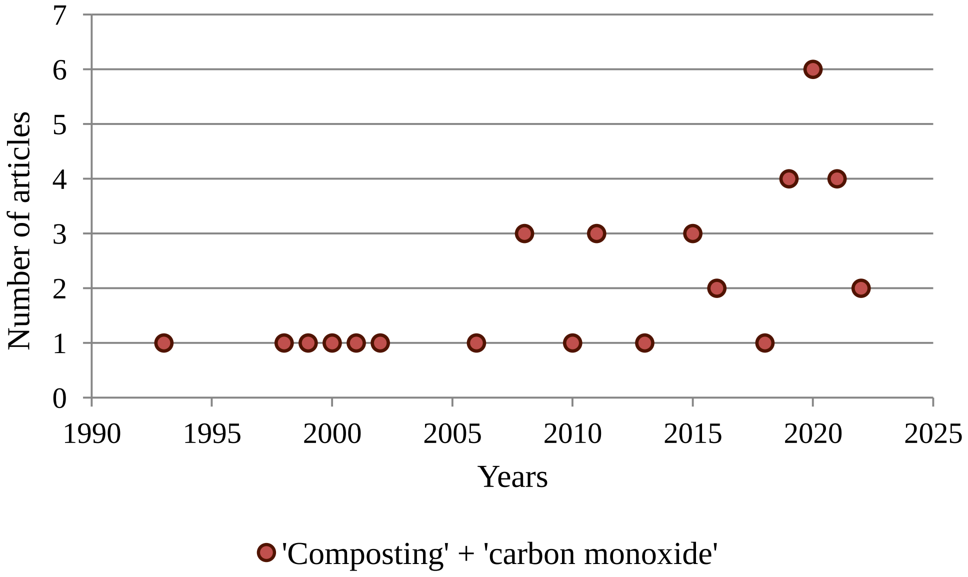

Figure 1. The timeline of published research for 'composting' articles related to 'carbon monoxide' (Web of Science Core Collection) [20]

Two theories on the manner of CO creation can be formed based on the knowledge that has been gathered so far. The abiotic and biotic character of CO formation has been proven through the analysis of samples of sterilized and non-sterile composted material [21]. As a result, it is given that CO production from composting includes a variety of physical and biological processes that are influenced by temperature and O_2 content (abiotic CO production) as well as by the activity of microorganisms (biotic CO formation [22]).

When O_2 is present, CO accumulation in a composted material tends to occur [16,23]. It was also verified experimentally during technical scale aerobic biostabilization of municipal waste; in perforated reactors, where the oxidation of the waste was higher than in tightly sealed material, higher levels of CO were detected [19]. CO production is also influenced by thermal conditions. For instance, the increase in temperature to 80°C caused the second peak of CO generation in the fourth month after starting the process [24]. Because of this, the thermal "chimney effect" in compost piles determines the ideal conditions for CO creation [24]. As a result, thermophilic zones and sections of the composted mass with higher levels of CO overlap [15]. Additionally, CO emission declines at night and below 30°C [25,26]. In compost piles, a link between CO and CO_2 concentrations was also noticed; the lowest CO concentration occurs after the highest CO_2 level. According to Hellebrand [14], this is most likely connected to CO_2 production due to microbial consumption of CO.

Increased production at the beginning of the composting process is characteristic of CO. High CO concentrations were observed both shortly after the formation of the compost pile from green waste [23] and a few hours after starting the experiment using green waste with grass [14,27]. Two peaks of CO production were identified by Hellebrand and Kalk [16] when composting green waste with manure and slurry. The first of them happened between 6 and 12 hours after the start of the process, and the second 5-8 days later. After 1 to 2 weeks in these trials, the CO level started to decline. Additionally, in tests conducted by Stegenta et al. [19], CO emission from the municipal biostabilization process in foil sleeves also started to dramatically decline in the third week of analyses.

Two reasons can be used to explain the reduction in CO concentration that occurs in the subsequent stages of the process. One of these is the aforementioned consumption of CO by bacteria; as a result of their increased activity, this gas may be oxidized to CO_2 [18]. According to the second theory, since the presence of CO depends on the level of O_2 in composted waste, when conditions are aerobic, the amount of CO formed is at its highest level. However, when this component is gradually depleted during the composting process, the concentration of CO declines, and the CO_2 level increases. The O_2 gradient affects also the spatial distribution of CO concentration in the compost pile [15,23,24,26,27]. The reverse tendency in which CO occurs in anaerobic areas has been observed in Haarstad et al. [17]. In this research, the longer period of low O_2 concentration is associated with greater CO concentration. The methanogenic bacteria's ability to produce CO provides an explanation for this [28].

3. Biotic CO production during composting

As mentioned earlier, CO production during composting can be biotic, based on the activity of the microbial community inhabiting the composted material. The biological CO emergence is correlated with the ability of microorganisms to produce the carbon monoxide dehydrogenase (CODH) enzyme [29]. This bidirectional enzyme contributes to both CO generation and oxygenation to CO_2 [30]. Thus, the microorganisms present in the waste composting process, potentially producing CODH, are not only responsible for the net production of this gas but also enable its oxidation to CO_2. The bidirectionality of CODH has fueled the interest in using this enzyme for the biologically mediated water-gas shift (BMWGS) reaction. This process involves converting CO to H_2 and CO_2 according to [31]:

$$CO + H_2O \leftrightarrow H_2 + CO_2$$

The most recent research on CODH has raised the significance of this enzyme and its practical implications [32]. From both an ecological and an economic perspective, the BMWGS reaction may emerge as a preferred technique for the production of biohydrogen [33]. The operations can be carried out locally since they occur at ambient temperatures and air pressure. Transport expenses for the substrate and energy costs will be reduced by producing H_2 from easily accessible biomass and biowaste [34].

In addition to being converted into biohydrogen, the biogenerated CO during composting also has multiple beneficial properties that are used in industrial processes. The created CO can be used as a useful material for additional processes and for the synthesis of fuels and organic compounds. For instance, CO is utilized in metallurgy due to its reducing capabilities; it is a part of wood and synthesis gases, which serve as both fuels and raw materials for technical operations. Moreover, ethanol and methanol can be generated from CO [35,36] at a competitive price [37]. Hydrocarbons and aromatic chemicals are also produced during the conversion of CO [38]. The biological production of CO during composting of biowaste by the activity of microorganisms potentially capable of producing the CODH enzyme is therefore important in implementing the objectives of the circular economy. The composting process, which initially appears to be thoroughly understood and discussed in the literature, will become a lot more attractive as such a novel method for biological CO production.

As reported above, researchers have observed that CO is produced during the composting process, but the often-conflicting results obtained by researchers in the few papers published so far mean that optimal process conditions conducive to CO generation during composting are still not well documented. Although the issue of the biological nature of CO production has been reported, the microbial species responsible for the biological generation of CO have not yet been identified. Therefore, creative strategies for enhancing CO production from composting, and opening new niches of the circular economy, have not yet been established.

Moreover, CO net production from the composting process cannot be avoided since biowaste contains a high amount of organic carbon. The recorded high concentrations of CO in the composted material and the toxic nature of this gas draw attention to the undeniable problem of ensuring the safety of the health and life of composting plant employees. For this reason, the issue of the risk of their excessive exposure to CO indoors also becomes important.

Taking into account the above aspects, as part of this doctoral dissertation, the literature on biological CO production was reviewed, as well as research was designed and performed, including mathematical modeling, laboratory analyses, and experiments on a technical scale, enabling the preparation of the concept of the biowaste composting process focused on CO generation along with recommendations taking into account ensuring the safety of such a process in the context of employees' exposure to this gas.

4. Research problem, the aim of the study, and research hypotheses

4.1.Research problem

Usually CO, as a valuable chemical compound, is produced due to the thermochemical process – gasification of biowaste. However, it requires energy-demanding drying of the feedstock [39]. In contrast, the composting process of biowaste has been reported as a source of CO [15–17]. In addition, CO generation from biowaste is unavoidable, since they contain large amounts of organic carbon.

The knowledge gained so far in the field of CO production from biowaste has proved that this gas can be formed in a biological way, depending on the activity of microorganisms [21].

The first research problem addressed in the doctoral dissertation is acquisition of fundamental knowledge about the optimal parameters and mechanism of biotic CO formation from biowaste. Biowaste composting has been proposed as a new method of biological CO acquisition, being at stage of the fundamental research. However, it requires an increase in CO generation yield. To date, there are no studies focusing on this phenomenon.

The second identified research problem is verification of the safety of the biowaste composting process focused on CO production. CO generation is associated with a threat resulting from the exposure of employees of the composting plant operating the process to high concentrations of this toxic gas.

Therefore, the research carried out as part of the doctoral dissertation raises the research problems of checking the validity of the theoretical concept of using the composting process for biological production of CO along with ensuring process safety.

4.2. Aim of the study

The aim of the study designed and carried out as part of the doctoral dissertation was to gain new knowledge in the field of biological production of CO during composting of biowaste, in particular:

1) Determination of optimal aeration level and thermal conditions for CO production during biowaste composting;
2) Isolation and identification of microorganisms responsible for CO production while biowaste composting;
3) Determination of CO emission factors from compost piles on a technical scale.

Moreover, due to the toxic nature of the CO produced during composting, an additional, fourth research objective was set:

4) Assessment of potential occupational exposure of composting plant workers to CO during biowaste composting.

The research objectives listed above were divided into two groups in accordance with the two research areas addressed in this doctoral dissertation:

I. Characteristics of biological CO production during biowaste composting (research goals: 1-3),

II. Analysis of the exposure of composting plant workers resulting from biological production of CO (research goal 4).

4.3.Research hypotheses

The following research hypotheses have been formulated:

H1: An increase in biowaste composting process temperature above the optimal value of 59°C will increase the intensity of CO production.

H2: An increase in the intensity of biowaste aeration, and hence their oxygenation, will cause a decrease in the intensity of CO production during biowaste composting.

H3: During biowaste composting biotic CO production is caused by activity microorganisms: aerobic bacteria in mesophilic conditions and anaerobic bacteria in thermophilic conditions with a reduced amount of O_2.

H4: Turning the material in a compost pile on a technical scale causes a decrease in CO emission factors due to the increased oxidation of biowaste.

H5: Concentration of CO produced during biowaste composting in the hermetic, closed hall can exceed the safe threshold CO concentration limits for plant workers level of 100 ppm.

5. The structure of the doctoral dissertation

This doctoral dissertation consists of 6 thematically coherent scientific articles published in the years 2021 – 2023 in peer-reviewed journals or being under review, listed chronologically in Table 1. In each of the presented manuscripts, the Ph.D. candidate is the first author. The total IF of the presented publications is 26.229 (published: 18.475), while their total Ministry of Science and Higher Education (MSHE, Poland) score is 720 (published: 480). The topic undertaken in this doctoral dissertation has been discussed in publications on a total of 145 pages using 517 literature sources.

Table 1. Publications included in the doctoral dissertation with bibliometric data

No.	Publication	Pages	Literature sources	IF, points	MSHE, points
M1	**Karolina Sobieraj**, Sylwia Stegenta-Dąbrowska, Jacek A. Koziel, Andrzej Białowiec, 2021. *Modeling of CO Accumulation in the Headspace of the Bioreactor during Organic Waste Composting*, Energies, 14(5), 1-17, doi: 10.3390/en14051367	17	81	3.252	140
M2	**Karolina Sobieraj**, Sylwia Stegenta-Dąbrowska, Gang Luo, Jacek A. Koziel, Andrzej Białowiec, 2022. *Carbon Monoxide Fate in the Environment as an Inspiration For Biorefinery Industry: A Review*, Frontiers in Environmental Science, 10, 1-24, doi: 10.3389/fenvs.2022.822463	24	268	5.411	100
M3	**Karolina Sobieraj**, Sylwia Stegenta-Dąbrowska, Gang Luo, Jacek A. Koziel, Andrzej Białowiec, 2023. *Biological treatment of biowaste as an innovative source of CO — The role of composting process*, Frontiers in Bioengineering and Biotechnology, 11, 1-16, doi: 10.3389/fbioe.2023.1126737	21	36	6.064	100

M4	**Karolina Sobieraj,** Sylwia Stegenta-Dąbrowska, Christian Zafiu, Erwin Binner, Andrzej Białowiec, 2023. *Carbon monoxide production during biowaste composting under different temperature and aeration regimes*, Materials, 16(13), 4551, doi: 10.3390/ma16134551	21	43	3.748	140
M5	**Karolina Sobieraj,** Daria Derkacz, Anna Krasowska, Andrzej Białowiec, 2023. *Isolation and identification of CO-producing microorganisms from compost*, Environmental Microbiology Reports (under review)	37	49	4.006	100
M6	**Karolina Sobieraj,** Karolina Giez, Jacek A. Koziel, Andrzej Białowiec, 2023. *Assessment of emissions and potential occupational exposure to carbon monoxide during biowaste composting*, Materials (under review)	25	40	3.748	140

Due to the two research areas related to the biological production of CO from biowaste included in this doctoral dissertation (listed in Chapter 4), the articles constituting it were divided into two groups and arranged in a logical order. In this way, the data used to implement research area I: *Characteristics of biological CO production during biowaste composting*, contained in the manuscripts numbered M2, M3, M4, and M5, are placed at the beginning of the appendices of the doctoral dissertation. These articles are followed by data presenting research area II: *Analysis of the exposure of composting plant workers resulting from biological production of CO* (manuscripts M1, M6). The structure of the doctoral dissertation is also shown in Figure 2.

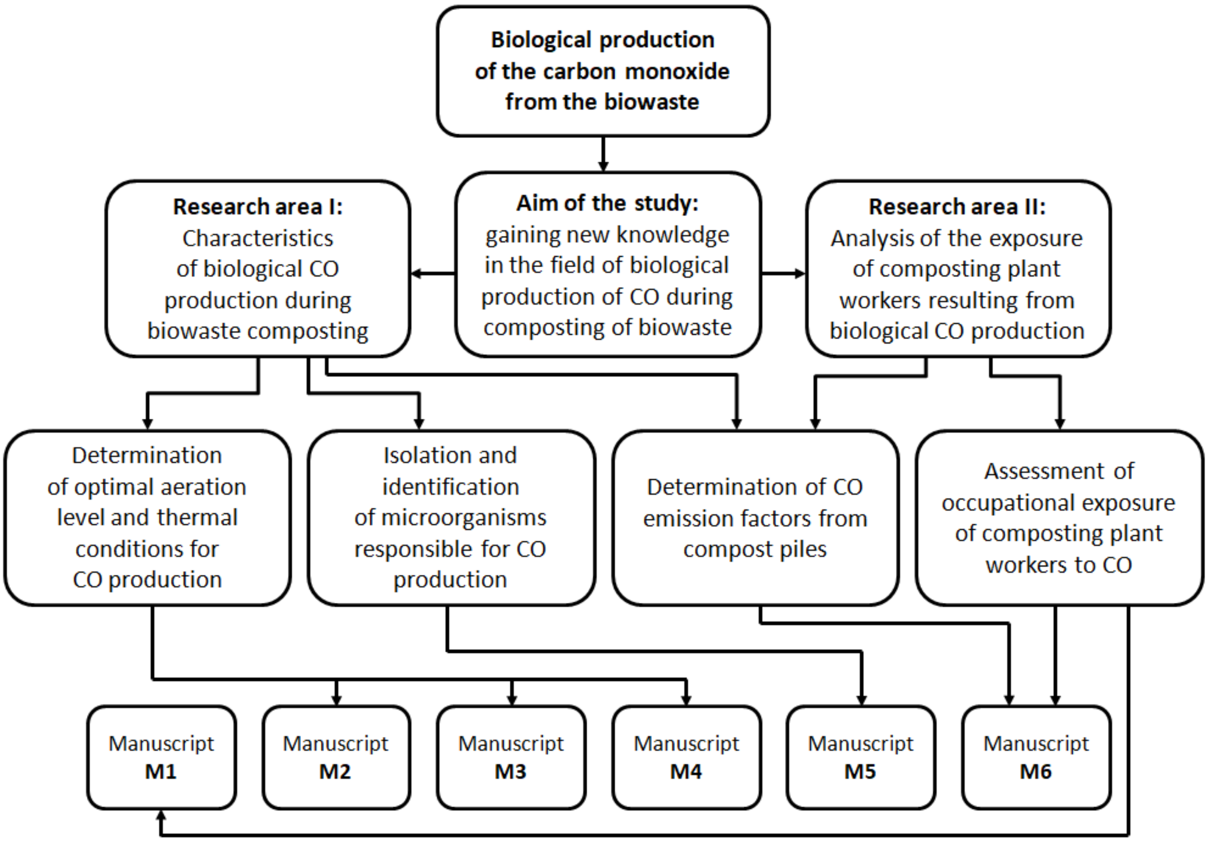

Figure 2. The structure of the doctoral dissertation

The publications that are components of this doctoral dissertation are supplemented by short description. Chapter 6 presents the content of each of the manuscripts, serving to achieve the goals of the doctoral dissertation. The main scientific achievements of each article have been highlighted. The materials and methods are summarized in Chapter 7. The most important effects of the conducted research and their influence on the environmental engineering, mining, and energy discipline are discussed in Chapter 8. Then, in Chapter 9, the conclusions of the doctoral dissertation with the verification of research hypotheses are presented. Based on the summary of conducted study, in Chapter 10 future directions of research in the field of biological production of CO in the composting process are outlined.

6. Content of publications included in the doctoral dissertation

6.1. Research area I: Characteristics of biological CO production during biowaste composting

In order to understand the characteristics of biological CO production, the literature on the subject was reviewed, taking into account various environments, including natural ecosystems and waste treatment processes. The state-of-the-art in this field was collected in manuscripts **M2** and **M3**.

The **manuscript M2** *Carbon Monoxide Fate in the Environment as an Inspiration For Biorefinery Industry: A Review* [40] is a review paper that discusses biological CO transformations in ecosystems and how they can serve as models for the biorefinery sector. The main goal of the study was to provide answers to the scientific questions:

1) Can CO be treated in biorefineries using microorganisms despite its toxicity?
2) What are the potential routes for using CO in biologically mediated processes?
3) What risks are associated with the CO seen as their feedstock?

The analysis of 268 literature sources over a wide time range allowed for the description of CO cycles in the atmosphere, soil, and water, as well as the importance of CO for organisms, including its impact on plants, animals, and humans. Based on data gathered on biological CO formation, consumption in different ecosystems, and its contribution to numerous processes and reactions in the ecosphere chemistry, the next section of this review article discusses the possibility of using CO for industrial purposes in biologically inspired processes in the field of biorefinery. The article highlights the potential of CO use in biologically mediated processes, such as:

1) Water-gas shift bioreaction (BMWGS),
2) Fischer-Tropsch (FT) reaction,
3) gas fermentation,
4) bioelectrochemical syngas conversion (BESs),
5) electro-fermentation (EF)

as a source of added-value biochemicals, fuels, and electricity. In addition, the article addresses current industrial applications of these processes as well as identifies related challenges.

The next review paper is a continuation of the article discussed above. The **manuscript M3** *Biological treatment of biowaste as an innovative source of CO —The role of composting* *process* [20] concentrates on the essential fundamental biochemistry knowledge required for the achievement of the first steps towards the bio-based generation of CO. The manuscript's primary objective was to determine answers to three scientific questions:

1) Is the CO production during aerobic biowaste composting processes dependent on the activity of the same microorganisms and the CODH enzyme they produce in the case of anaerobic processes?
2) Can composting become a leading method for the biobased production of useful CO?
3) Can the knowledge obtained from studying CO generation in anaerobic environments be applied to composting?

With the aid of bibliometric analysis of trends, complex information about CO-metabolizing aerobic and anaerobic microorganisms, pathways, and enzymes of both CO formation and consumption was examined. The manuscript discusses in detail CO generation from biowaste treatment processes, both aerobic and anaerobic, its production during biowaste and biomass storage and transportation, as well as a spatial distribution of CO in composting piles. The potential future paths for CO extraction from biological waste treatment systems, as well as the potential limiting factors, were evaluated.

On the basis of the above-discussed review papers, laboratory scale analyses were proposed. Their results are presented in two articles: **M4** and **M5**.

The study presented in the **manuscript M4** *Carbon monoxide production during biowaste composting under different temperature and aeration regimes* [41] aimed to investigate the CO production potential during biowaste composting under controlled laboratory conditions depending on different aeration rates and temperatures. For this purpose, a series of composting processes were carried out in conditions ranging from ~psychrophilic to thermophilic (T=35, 45, 55, and 65°C), and aeration rates in the range of under optimal, optimal, and exceeding the stoichiometric oxygen demand (2.7, 3.4, 4.8 and 7.8 $L \cdot h^{-1}$). Twelve glass bio-reactors with a volume of about 7 L each were set up for biowaste composting on a laboratory scale. The process lasted for 14 days. Every 24 hours, measurements of CO concentration (ppm) were taken, with the first measurement taking place 24 hours after the bioreactors were placed in the climate chamber and the process began. The gas concentration analyzer was manually inserted into the silicone tubes that were connected to the gas bags collecting the process air from each of the bioreactors separately. Since CO concentration and O_2 levels were observed to be correlated, O_2 concentration (%) was measured concurrently using the same technique and analyzer. An infrared gas analyzer monitored the CO_2 content (%) every 24 hours. The temperature of the compost was also measured during the measurement series. Substrates and compost samples were characterized for water content (WC), pH value, electrical conductivity (EC), loss on ignition (LOI), total organic carbon (TOC), total nitrogen (TN), carbon/nitrogen ratio (C/N), respiration activity AT_4, ammonium nitrogen (NH_4-N) and nitrate nitrogen (NO_3-N). The kinetics of CO concentration decrease during composting (maximum CO concentration C_{COmax} and decrease in CO concentration constant rate k) using linear and nonlinear least-squares regression were determined based on the daily CO concentration values.

The most important scientific achievement of the manuscript M4 was the determination of the optimal aeration level ($<3.4\ L \cdot h^{-1}$) and thermal conditions (<45°C) for CO production during biowaste composting, which contributes to the development of recommendations for a composting process focused on CO production for the first time.

The **manuscript M5** *Isolation and identification of CO-producing microorganisms from compost* [42] is the second paper showing results from laboratory scale analyses of biological formation of CO. Focused on the microbial CO generation during composting, the study aimed to isolate and identify microorganisms potentially responsible for the CO production in compost collected from two areas of the biowaste pile on a technical scale: with low (118 ppm) and high CO concentration (785 ppm). First, the experiment on a composting pile on a technical scale was conducted. To measure and process gas concentrations (CO in ppm, CO_2, and O_2 in %) and the composting pile's temperature (°C), 28 sampling points at four cross sections at 1/5, 2/5, 3/5, and 4/5 of the pile's length and at three heights (0.5, 1.0, and 1.5 m) were tested, along with one deep measurement (pile core, height 1.0 m). The electrochemical analyzer and a thermocouple were attached to a silicone tube that was inserted inside a long stainless-steel probe with holes at the end. The locations with the lowest reported CO concentration (118 ppm) and the composting pile's maximum CO concentration (785 ppm) were chosen for compost sampling. The samples of compost were then subjected to the microorganism's isolation procedure.

After being saturated with physiological fluid, the compost sample material was incubated. Then, each sample was diluted before being placed on YPD plates (fungi) or LB agar plates (bacteria) to check the type of microorganisms inhibiting the compost material. The samples were incubated for 24 hours at different temperatures, including 25°C (YPD plates), 37°C (LB plates), and 62°C and 50°C (temperatures from pile sampling locations; primary culture). Plates, where no growth was noted, were discarded. In the case of plates where the growth of microorganisms was recorded, the mass was seeded on fresh media (YPD or LB, respectively) and incubated under the same conditions (24 h at temperatures 28, 37, 50, or 62°C; reduction plating). Pure cultures were assessed by two aspects:

1) shape and growth resembling the reference strain releasing CO into the environment (*Moorella thermoacetica,* formerly: *Clostridium thermoaceticum*) or other from genus Clostridium,
2) unusual for the environment from outside the compost features: shape, colony colour.

According to these guidelines, 15 strains were selected for DNA isolation for the identification of isolated microorganisms. PCR reactions were performed that multiplied 16S rRNA fragments (bacteria) or ITS (fungi). The results of the PCR were electrophoresized and bands were excised and purified from the agarose gel. PCR products were sequenced.

Isolated and identified bacterial strains were then incubated in sealed bioreactors for 4 days in order to verify their ability to produce CO, first during the initial tests, then after selecting strains characterized by generating increased CO concentration. Bioreactors equipped with metal tight caps with two measuring nozzles were used for this purpose. After 4 days of incubation, the concentration of process gases was measured by connecting the gas concentration analyzer to the silicon tube on one of the nozzles. The measurement of gas samples from the headspace of the bioreactors included concentrations of CO (ppm), $CO_{2,}$ and O_2 (%).

The most important scientific achievement of the manuscript M5 was the identification of bacterial strains colonizing the compost, producing CO in concentrations exceeding 1,000 ppm (*Bacillus paralicheniformis*), >800 ppm (*Bacillus licheniformis*), and close to 600 ppm (*Geobacillus thermodenitrificans*) for the first time in the literature.

6.2.Research area II: Analysis of the exposure of composting plant workers resulting from the biological production of CO

The **manuscript M1** *Modeling of CO Accumulation in the Headspace of the Bioreactor during Organic Waste Composting* [43] presents mathematical modeling describing CO accumulation in the headspace of the bioreactor during organic waste composting modeling depending on the kinetics of CO production inside the pile, constituting the occupational risk to workers that can be exposed to elevated CO concentrations at the early stage of the process. In the article, the effect of headspace ventilation on enclosed compost was evaluated by modeling CO buildup in two different scenarios: one in which there is no ventilation of the bioreactor headspace throughout the process, and the other in which ventilation is defined as a brief daily opening of the bioreactor to release collected CO. The article describes the modeling process, including the analytical procedures and specific inputs used for model calculations (such as waste properties, composting method, CO threshold value, and bioreactor dimensions). The article illustrates CO accumulation levels in the bioreactor during 14 days of composting for seven different headspace-to-waste volume (H:W) ratios, showing that the defined limit value of CO concentration (100 ppm) was exceeded in each of the tested variants. An Excel spreadsheet with the developed mathematical model and guidelines for use is included as Supplementary Material. It enables modeling of all potential CO concentration scenarios and can be modified to meet established standards for the user's maximum CO concentration threshold.

The most important scientific achievements of the manuscript M1 were the development of recommendations regarding the minimum level of air exchange required to maintain a CO concentration below the threshold value ($\geq 7.15\ m^3 \cdot h^{-1} \cdot Mg$ wet mass^{-1}) and the height of the waste pile to reduce the risk of CO accumulation (≤ 1 m), as well as the creation a mathematical model available to readers, which can be used to simulate the exposure of workers in composting plants to CO and to select the efficiency of the ventilation system in composting halls to ensure process safety.

The results of research on the release of CO harmful to composting plant workers are also included in the **manuscript M6** *Assessment of emissions and potential occupational exposure to carbon monoxide during biowaste composting* [44]. The aim of the research was to determine CO net emission rates from compost piles on a technical scale (emission to the atmosphere) and to assess workers' exposure to elevated CO concentration in a closed composting hall. Measurement of CO emissions from biowaste composting was carried out in two plants processing different waste fractions: green waste, sewage sludge, or undersize fraction of municipal solid waste. Using the closed flux chamber method (or flux box), the impact of the location of compost piles and material turning on CO emissions was analyzed, i.e. piles located in a closed hall (hermetised) and in an open yard, both before and after material turning, were examined. A plastic box was modified to serve as a flux chamber ($V=0.071\ m^3$). The chamber was equipped with two valves – one for pressure equalization and the other for gas sampling. By using a silicone tube to connect to the gas analyzer, a gas sample valve allowed for the measurement of CO (ppm), CO_2 (%), and O_2 (%)

concentrations. A thermocouple was used to measure the temperature within the chamber. On each pile, the flux chamber was positioned in three places along its length, on both sides and at the top. Each measurement series consisted of pre- and post-pile turning measurements of CO, CO_2, and O_2 concentrations. A self-propelled turner for windrows provided daily turning during the first stage of composting. The results were utilized to simulate workplace exposures during routine compost management tasks. The modeling of CO emissions over a period of one hour in a hermetized composting hall with a headspace of 1,000 m^3 and a total area of piles of about 1200 m^2 was done. Due to the typical worker time required to turn one pile, the 1 h period was used for the modeling. The "worst-case scenario", wherein the composting hall had no ventilation and CO emissions were permitted to build up, was assumed. The obtained CO concentration values in the enclosed hall were compared with the WHO-acceptable threshold values.

The most important scientific achievements of the manuscript M6 were the determination of the overall net CO emission factors for the first time for both hermetised and open composting plants for the before and after material turning scenarios, as well as the development of recommendations for the biowaste composting process in order to maintain a safe level of CO for employees.

7. Materials and methods

Two of the articles included in the doctoral dissertation are review papers (M2 and M3), while the remaining four are research manuscripts presenting the outcomes obtained as a result of mathematical modeling (manuscript M1), laboratory-scale analyzes (manuscript M4), laboratory and technical scales study (manuscript M5), and research on a technical scale in the composting plants (manuscript M6). Table 2 summarizes the materials used in the research and the implemented methodology for each of the manuscripts M1-M6.

The research presented in the doctoral dissertation focused on analyzing biowaste from three composting plants on a technical scale (manuscripts M4-M6), even if the process was then carried out on a laboratory scale (manuscript M4). In addition to green or plant-based waste, the mixtures used in the experiments contained structural material (branches or pieces of wood). Additional materials in the process were sewage sludge (manuscripts M5 and M6) and undersize fraction of municipal solid waste (manuscript M6).

The research methods used in the doctoral dissertation were broad and included both technical analyzes (measurement of process gas concentrations *in* piles and CO emissions *from* the composted material) and microbiological analyzes (isolation and identification of microorganisms from compost samples). The analyzed material was characterized each time in the research manuscripts (samples of biowaste before the process and composts after the process – manuscript M4; samples of substrates – manuscripts M5 and M6).

Presented articles also focused on the kinetics of CO production during biowaste composting. The kinetics of CO concentration during composting (maximum CO concentration C_{COmax} and decrease in CO concentration constant rate k) were calculated using linear and nonlinear least-squares regression with models of the zero-order and first-order reactions (manuscripts M1 and M4).

Table 2. Summary of materials and methods presented in the manuscripts M1-M6

Manuscript	**Type of the research**	**Materials**	**Methods**
M1	Analytical research	The kinetics data for CO production rates during composting of organic waste (a 1:1:1 mix by mass of dairy cattle manure, grass clippings, and pine sawdust) at temperatures 10, 25, 30, 37, 40, 50, 60, and 70°C reported in previous own study [45]	➢ Modeling performed using the Statistica software 13.3 (TIBCO Software Inc., Palo Alto, CA, USA) and Microsoft Excel spreadsheet. ➢ Mathematical modeling based on the kinetic parameters of CO production during composting determined by using nonlinear least-squares regression and the first-order reaction model. ➢ Assessment of the influence of the composting temperature on CO production constant rate *k*. ➢ The CO potential production as a function of temperature described by Gompertz's model. ➢ The prediction of CO concentration in a headspace calculated according to first-order kinetic with CO mass normalized and converted to ppm. ➢ Calculation of the rate of air exchange to maintain the CO concentration threshold value in the headspace (<100 ppmv).
M2	Review	Analysis of 268 literature sources	➢ Google Scholar database used to identify research papers related to CO fate in the atmosphere, soils, water, CO role for organisms (plants, animals and humans) and biorefinery application of CO.
M3	Review	Analysis of 36 literature sources	➢ Web of Science Core Collection bibliometric analysis for keyword combinations: i) "Carbon monoxide" + "anaerobic", (ii) "Carbon monoxide" + "pathway", (iii) "Carbon monoxide" + "CODH", (iv) "Carbon monoxide" + "microorganisms", (v) "Composting" + "carbon monoxide", (vi) "Composting" + "CODH".
M4	Laboratory scale analyzes	Biowaste from the composting plant of Lobau, Vienna (Austria) (plant-based waste, i.e., vegetables, windfall fruit, leaves, tree and shrub cuttings, lawn clippings, and wilted flowers) with chopped	➢ Biowaste composting carried out for 14 days in 12 adapted glass bioreactors with a volume of ~7 L each with manual turning of the material after 7 days. ➢ Process temperature in the climatic chamber: 35, 45, 55, and 65°C. ➢ Air supply adjusted individually to each bioreactor (2.7, 3.4, 4.8, and 7.8 $L \cdot h^{-1}$). ➢ Measurements of process gas concentrations: CO (ppm), CO_2 (%) and O_2 (%) every 24 hours. ➢ Characterization of substrates and compost samples: water content (WC), pH-value,

		branches; mix shredded, sifted, and screened in the mechanical treatment unit of the Lobau facility	electrical conductivity (EC), loss on ignition (LOI), total organic carbon (TOC), total nitrogen (TN), carbon/nitrogen ratio (C/N), respiration activity AT_4, ammonium (NH_4-N) and nitrate nitrogen (NO_3-N) content. ➢ Determination of the process efficiency by comparing the initial properties of the biowaste and the final compost samples. ➢ Analysis of the kinetics of CO concentration during composting (maximum CO concentration C_{COmax} and decrease in CO concentration constant rate k) using linear and nonlinear least-squares regression with models of the zero-order and first-order reactions.
M5	Technical and laboratory scale analyzes	Biowaste (grass, leaves, and branches) combined in a 4:1 ratio (v/v) with sewage sludge composted in a pile on a technical scale for 7 days; samples from areas with the lowest and highest recorded CO concentration in the composting pile taken to analyzes	➢ Technical scale: measurement of the concentration of process gases (CO in ppm, CO_2 and O_2 in %) and temperature (°C) within the composting pile from 28 points at four cross sections at 1/5, 2/5, 3/5, and 4/5 of the length of the pile and at three heights – 0.5, 1.0 and 1.5 m with one deep measurement (pile core) at height 1.0 m using a long stainless steel probe with holes at the end and a silicone tube inside, connected to the electrochemical analyzer. ➢ Compost sampling from areas with the lowest recorded CO concentration (left side of the pile, 2/5 of the length, height 0.5 m, 118 ppm of CO, T=61.6°C), and the highest CO concentration (right side of the pile, 4/5 of the length, height 0.5 m, 785 ppm of CO, T=50.1°C). ➢ Characterization of the compost samples: water content (WC), pH-value, electrical conductivity (EC), loss on ignition (LOI), total organic carbon (TOC), total nitrogen (TN), carbon/nitrogen ratio (C/N), respiration activity AT_4, ammonium (NH_4-N) and nitrate nitrogen (NO_3-N) content. ➢ Microorganisms isolation from compost samples procedure: material taken into sterile phalcon, flooded with physiological fluid and incubated for 30 min at room temperature while shaking (450 rpm). Preparation of a series of dilutions (10^{-2}, 10^{-3}, 10^{-4}, 10^{-5} and 10^{-6}) plated in YPD and LB agar plates for 24 h at different temperatures, including 25°C (YPD plates), 37°C (LB plates), and 62°C and 50°C (temperatures from pile sampling locations; primary culture). ➢ Selection of pure cultures based on: i) shape and growth resembling the reference strain releasing CO into the environment (*Moorella thermoacetica*, formerly: *Clostridium thermoaceticum*) or other from genus Clostridium; ii) unusual for the

			environment from outside the compost features: shape, colony colour. Selection of 15 strains for DNA isolation. ➢ Identification of microorganisms from compost samples procedure: DNA isolated from grown pre-cultures using a commercial kit for DNA isolation, following the manufacturer's protocols. PCR reactions that multiplied 16S rRNA fragments (bacteria) or ITS (fungi) performed. The results of the PCR electrophoresized using 1% (w/v) agarose gel and visualized. PCR products sequenced using the Sanger method. ➢ Measurements of CO production of isolated strains procedure: incubation carried out in bioreactors equipped with metal tight caps with two measuring nozzles for 4 days. Measurement of process gas concentrations: CO (ppm), CO_2 (%) and O_2 (%) after 4 days by connecting the gas concentration analyzer to the silicon tube on one of the nozzles.
M6	Technical scale analyzes	Composting Plant A, hermitised: green waste (grass, leaves, branches) and sewage sludge from the "Boguszowice" wastewater treatment plant (85 and 15% by fresh mass, respectively); Composting Plant B, open yard: green waste from backyards and parks (5 piles) and undersize fraction of municipal solid waste (1 pile)	➢ The measurement of CO emissions from compost piles using the flux chamber method. A plastic box with a volume of 0.071 m^3 adapted as a flux chamber with two valves installed onto the chamber: for gas sampling with gas analyser and pressure equalization. Flux chamber placed on each pile in three locations along its length, on both sides and in its top (total of $n = 9$). ➢ Measurements of temperature (°C), CO (ppm), O_2 and CO_2 (%) concentrations before and after material turning. ➢ The UK Environmental Agency's methodology (LFTGN07 Guidance on monitoring landfill gas surface emissions) [46] adopted for estimating CO emissions. ➢ Characterization of the compost samples: water content (WC), loss on ignition (LOI), respiration activity AT_4. ➢ Modeling of CO concentration in the hermetised hall with a 1,000 m^3 of headspace and total area of piles of ~1200 m^2 (during 1 h of operation without ventilation) using the Statistica software 13.3 (TIBCO Software Inc., Palo Alto, CA, USA) and Microsoft Excel spreadsheet.

8. Research effects

8.1. Research area I: Characteristics of biological CO production during biowaste composting

8.1.1. Determination of optimal aeration level and thermal conditions for CO production during biowaste composting

According to the literature, composting of biodegradable waste has been reported to produce net amounts of CO. However, despite the advancement of biorefinery processes, the idea of combining the "conventional" composting process with the synthesis of biochemicals has not yet been considered. To change this, it became necessary to determine the optimal conditions of the composting process for CO generation. The key factors influencing CO production so far have been determined to be temperature and O_2 concentration [21], which turned into the starting point for the research described in this doctoral dissertation.

The research carried out on a laboratory scale as part of the doctoral dissertation contributed to the formulation of recommendations for the biowaste composting process focused on CO production for the first time. According to the obtained results, the release of CO is significantly affected by low aeration ($<3.4\ L\cdot h^{-1}$) and low temperature (<45°C) [41]. From a practical point of view, the composting process aimed at obtaining the highest concentration of CO can take place under economically effective conditions, based on low-efficiency aeration systems. At the same time, the highest CO concentrations during biowaste composting under controlled laboratory conditions were obtained at 65°C. The high level of CO production stimulated by oxygen deficits and two thermal ranges, mesophilic and thermophilic conditions, highlights the biological production of CO by microorganisms capable of producing CODH. As indicated in the manuscript M4 [41], this can be proved by three facts: the optimum temperatures for the growth and development of the CODH-producing and CO-converting bacteria identified so far are the thermal ranges of 35 and 65°C [47]. In addition, the efficiency of CODH and conversion of CO_2 to CO increases under thermophilic conditions, and the enzyme itself is tolerant of oxygen [48,49]. This means that after a period of higher oxidation of the material, e.g. after turning the material in a pile, it can be reactivated and take part in the production of CO again. Thus, despite the aerobic nature of composting, the participation of CODH-producing anaerobic bacteria in the process is not excluded.

As indicated in paper M4 [41], composting aimed at CO generation must be characterized by a slightly different system of operating the process, associated with the activity of various CO-producing bacterial strains at different stages of the process. It would be beneficial to extend the mesophilic phase with temperatures close to 35°C (<45°C) when managing the thermal conditions in the pile or bioreactor. The material would next be exposed to a high temperature (thermophilic conditions, 65°C) in order to sterilize the product while simultaneously generating CO.

8.1.2. Isolation and identification of microorganisms responsible for CO production while biowaste composting

The issue of biological CO production during biowaste composting by the activity of microorganisms raised in manuscript M5 [42] also contributed to the acquisition of new knowledge in the field of isolating and identifying species responsible for the release of CO in compost. The research proved that all the isolates from the pile compost samples were bacterial strains with a predominance of Gram-positive bacteria. It was demonstrated that the most common bacteria were representatives of the genus Bacillus (*B. licheniformis, B. haynesii, B. paralicheniformis* and *B. thermolactis*). Based on the research conducted as part of the doctoral dissertation, strains capable of producing CO during incubation in bioreactors were identified for the first time in the literature. CO concentrations produced by isolated strains reached high values, exceeding 1,000 ppm (*B. paralicheniformis*), >800 ppm (*B. licheniformis*), and close to 600 ppm (*Geobacillus thermodenitrificans*). High CO concentrations during incubation were connected with low O_2 (<6%) and high CO_2 levels (>8%).

The results obtained in the paper M5 [42] contributed not only to the first identification of the bacteria responsible for CO production, inhabiting the compost pile. The data acquired also opened new research niches, focusing on the exact understanding of the mechanisms controlling the CO-producing microbial community, including species cooperation and variability during the process. As discussed in the review paper M3 [20], the bacterial strains identified during the research can then be analyzed for the production of specialized biopreparations used at a specific stage of the composting process to intensify the biological production of CO from biowaste. Due to the toxicity of CO, it is also becoming more important to design and modify bacterial strains inhibiting compost so that they can function with a gaseous substrate in a difficult environment, despite problems with gas-to-liquid mass transfer and high affinity of CO to metalloenzymes.

8.1.3. Determination of CO emission factors from compost piles on a technical scale

The research carried out on a technical scale contributed to the acquisition of new, unpublished so far knowledge on CO emission indicators from compost piles. Until now, analyses of this gas formation in large facilities have focused on the concentration of CO within the composted material; however, there were no literature sources discussing the release of this gas into the atmosphere. The study carried out as part of the doctoral dissertation also provided the characterization of CO emissions in terms of their dependence on material turning and pile location, as well as of the spatial distribution of CO from the compost pile. The overall net CO emission factors for both hermetised and open composting plants were developed for the before and after turning scenarios with the range of before/after turning ratios.

Analyzes of the release of CO from full-size compost piles have shown that during the standard process handling, the emission changes and is higher after the material is turned [44]. After this treatment, the maximum average CO flux reaches values from 1.7 to 13.7x higher compared to the indicators before the turning. In addition, studies have shown for the first time that CO is released in a greater amount in an airtight, closed hall compared to emission factors for material composted outside (average CO flux increased approx. 39x before turning the material and 14x after, respectively). Importantly, in the context of the characterization of the biological CO production during biowaste composting, it was reported that the CO emission from the top part of the pile is higher than from its sides. As indicated in the article M6 [44], this proves the existence of the 'chimney effect' in the pile, not only thermal but also related to increased CO release. This confirms the previously mentioned in manuscript M4 role of thermophilic microorganisms in the CO generation [41].

Relevantly, studies have also proven that places that were previously CO emission points can be switched to 'CO sinks'. This tendency has not been reported in the literature so far. From all of the measurement spots on the pile in the hermetized plant, 5% demonstrated a negative CO flux, typically after the material was turned. The 'CO sinks' were not evenly dispersed; the majority of them were found near the sides of the piles (>50% of all locations). The conversion of emission points into CO absorption points is potentially related to the presence of CODH-producing microorganisms in these areas of the pile. The bidirectionality of the enzyme can affect the conversion of CO_2 to CO and *vice versa*, depending on the environmental conditions favorable for both of these reactions.

8.2.Research area II: Analysis of the exposure of composting plant workers resulting from the biological production of CO

The results presented as part of the doctoral dissertation also had a social aspect related to the determination of harmful factors in the work environment in a full-scale composting plant. The research carried out pointed out for the first time that composting of biowaste may pose a threat to the health and life of employees operating the process carried out in the composting hall, both in closed reactors and in the piles.

Composting process hermetization is recommended by the EU's BAT Reference Document for Waste Treatment [50]. Even though this directive can lessen its influence on the ambient environment, enclosed composting has the potential to have unintended consequences, such as the buildup of harmful pollutants. Since CO is heavier than air, it can quickly build up even in closed spaces that are adequately ventilated. Modeling carried out as part of manuscript M1 [43] proved that the composting process carried out in closed bioreactors during the first 14 days leads to the accumulation of CO above the level of 100 ppm (chosen as a safe for 15 minutes of operation), both when the bioreactor is not ventilated and also in the process with ventilation defined as a short, daily opening of the bioreactor. This situation was noted despite considering various ratios of headspace-to-waste volume (H:W) in the reactor (4:1, 3:1, 2:1, 1:1, 1:2, 1:3, 1:4). In each of the analyzed cases of H:W ratios, the concentration of CO in the bioreactor headspace without ventilation quickly significantly exceeded the accepted threshold value of 100 ppm. Research has therefore pointed out that in the event of a failure of the headspace ventilation system, the CO concentration reaches dangerous values for employees within the first 24 h. At the end of composting (14th day), the CO concentration can reach 2.25% for the lowest H:W (4:1) to 36.1% for the highest H:W (1:4), creating lethal conditions for humans. Additionally, modeling showed that even the daily release of gas accumulated in the bioreactor headspace is not sufficient to lower the CO concentration to a safe level. It can reach 3.2%, still being life-threatening for plant workers.

In turn, research on CO emission factors carried out on a technical scale in full-size composting plants proved that the 1-h concentration in a hermetised composting hall can reach max. 59 ppm before turning, and >135 ppm after, raising for the first time concerns about the potential occupational risk during a typical 1 h-long pile turning [44]. This is particularly important due to the WHO threshold values for CO concentration [51]. According to the results obtained, the level of this gas in the closed composting hall exceeds a 1-h and 15-min exposures (25 and 90 ppm, respectively). As indicated in the review manuscript M2, which is part of the doctoral dissertation [40], people working in such an environment may experience various types of symptoms, such as headache, ear ringing, fatigue, weakness, nausea, and vomiting.

Due to the discoveries made in the work, recommendations for the composting process were also developed in order to ensure the safety of employees handling it. As indicated in the manuscript M1 [43], it has been calculated that when the composting process is carried out in a closed reactor, the airflow necessary to keep CO below 100 ppm in the headspace should not be lower than $\geq 7.15\ m^3 \cdot h^{-1} \cdot Mg$ wet $mass^{-1}$. In addition, the optimal H:W volume ratio was indicated, which should be higher than 4:1. If the process is carried out in compost piles, their height should not exceed 1 m to mitigate the risk of excessive accumulation of CO in the composting hall [43]. Protocols for handling biowaste composting in a hermetic hall have also been proposed in the manuscript M6 [44]. It was pointed out that the current system of work, based on 8-hour shifts, during which the same type of activities is performed (including material turning in operating machines with cabins high above the ground or occasional manual labor with high physical effort), is not beneficial in aspect of maintaining safe working conditions. Shortening the amount of time spent in the enclosed composting hall and limiting activities to those requiring only modest physical effort are advised. Due to the higher CO emission from the top part of the piles, proved in the work M6 [44] and the tendency of CO to accumulate in the higher parts of closed halls, it is also recommended that employees responsible for leveling and turning the piles should be equipped with personal protective instruments, such as personal CO detectors and appropriate breathing masks with filters. Composting practices could be improved by the implementation of automating turning and thus reducing worker exposure. Alarms should be installed in the composting hall as well, especially over waste material. Particular attention should be paid to the implementation of failure-free ventilation systems in closed composting plants ensuring the removal of pollutants while ensuring the influx of fresh air. It is advised to regularly monitor CO emissions throughout the composting process, not just in the beginning stages, since they are variable and can increase with temperature, reaching multiple peaks throughout the process.

9. Conclusions and hypotheses verification

The research problem of the doctoral dissertation concerned the knowledge of the optimal parameters and mechanism of biotic CO formation from biowaste in order to check the validity of the theoretical concept of using the composting process for the biological production of CO while ensuring process safety. For this purpose, studies were designed and conducted to enable the determination of optimal aeration level and thermal conditions for CO production during biowaste composting and CO emission factors from compost piles on a technical scale, isolation and identification of microorganisms responsible for CO production while biowaste composting and assessment of potential occupational exposure of composting plant workers to CO during biowaste composting.

According to the research results indicated in chapter 8.1.1 and manuscript M4, the average CO concentration during biowaste composting on the laboratory scale reached the lowest values at 35°C and as the process temperature increased, the CO concentration gradually rose, becoming less stable (the variations between daily measurements for repetitions were higher). The highest values of CO concentration were achieved for the process carried out at 65°C, where the average level of CO release at the beginning of the process was ~300 ppm for the lowest level of aeration. In addition, for each thermal variant, CO production was the highest at aeration below the optimal value, ensuring oxygen deficit (2.7 $L\cdot h^{-1}$). However, as statistical analysis showed, temperature and aeration level affect CO concentrations but only at low temperatures and aeration rates (35°C and 2.7 $L\cdot h^{-1}$). Higher temperatures (>35°C) and aeration rates (>3.4 $L\cdot h^{-1}$) did not influence the CO production during the composting process (there were no statistical differences). Therefore, hypodissertation H1: *An increase in biowaste composting process temperature above the optimal value of 59°C will increase the intensity of CO production* turned out to be false. In turn, the hypodissertation H2: *An increase in the intensity of biowaste aeration, and hence their oxygenation, will cause a decrease in the intensity of CO production during biowaste composting* should be considered correct, since only a low level of aeration, regardless of the thermal conditions of the process, stimulated CO generation.

According to the results presented in chapter 8.1.2 and manuscript M5, production of CO in the compost pile on a technical scale was observed in thermophilic areas (62 and 50°C for low and high CO sites, respectively). It was proved that the microorganisms isolated from the compost samples were bacterial strains. Among the isolates incubated in the bioreactors, *Bacillus paralicheniformis* (producing CO at concentration >1,000 ppm), *Bacillus licheniformis* (>800 ppm), *Geobacillus thermodenitrificans* (~600 ppm), and *Bacillus thermolactis* (>300 ppm) were identified. Since all of the listed species are thermophilic and anaerobic bacteria, hypodissertation H3: *During biowaste composting biotic CO production is caused by activity microorganisms: aerobic bacteria in mesophilic conditions and anaerobic bacteria in thermophilic conditions with a reduced amount of* O_2 should be considered true only in the aspect of the biological CO production by bacteria functioning at high temperatures and the low content of O_2, rejecting the involvement of mesophilic bacteria.

Chapter 8.1.3 and the M6 manuscript present the results of research on the biological CO production from biowaste carried out on a technical scale, obtained to verify hypodissertation H4: *Turning the material in a compost pile on a technical scale causes a decrease in CO emission factors due to the increased oxidation of biowaste*. Based on the data, this hypodissertation was rejected. The average CO flux was higher after the material was turned in both analyzed plants, conducting the composting process in a closed hall and in the open yard. The increase of CO flux after the turning in these plants varied from 1.7x to 7.4x and from 3.5x to 13.7x, respectively, and the developed before/after turning ratio of average CO flux was 0.34 and 0.12.

The social dimension of the research conducted as part of the doctoral dissertation concerned the determination of the level of CO accumulation in a closed composting hall. The hypodissertation H5: *Concentration of CO produced during biowaste composting in the hermetic, closed hall can exceed the safe threshold CO concentration limits for plant workers level of 100 ppm* turned out to be true only for the conditions occurring after material turning. Modeling indicated that after 1 h the maximum CO concentration in the hall headspace before turning didn't exceed 60 ppm (with an average value of 33 ppm). However, this situation changed when the material was turned. During 1 h the concentration of accumulated CO was higher than 100 ppm in 4 cases, with a maximum of 135 ppm.

10.Future research directions

The directions of future research on the biological production of CO from biowaste are related to the analysis of bacterial strains responsible for the release of this gas. As mentioned in the paper [41], due to the special role of two thermal ranges during composting of biowaste on a laboratory scale – mesophilic conditions with a temperature of <45°C and thermophilic conditions at 65°C – further analyzes the efficiency of obtaining CO in these thermal ranges are necessary with the simultaneous characterization of bacterial strains appearing then in the composted mass of the waste. In addition to determining the impact of aeration rate and temperature on CO production, it is necessary to analyze the influence of other key variables in the process, such as the organic matter content in biowaste and their moisture. It is also important due to the variability and seasonality of biowaste directed to the process. In order to increase the amount of CO produced by this process, a mathematical model should be developed taking into account the effects of each individual variable.

Importantly, in the aspect of strains of the genus Bacillus already identified in compost samples and producing CO, extended tests should be carried out, enabling daily measurements of process gas concentrations. The current methodology allowed only to measure of the CO concentration in the headspace of the bioreactor after 4 days of incubation; however, due to the potential activity of the CODH enzyme, there is a premise that the incubated bacterial strains may have produced more CO at the beginning of their growth, and then, after reaching the optimum, began to convert it to CO_2 [42]. It would also be beneficial to analyze the variability of bacterial strains throughout the composting process. This is important due to the tendency for higher CO production in the initial stage of the process, observed in the work [41], which then significantly decreases, as well as the peaks of CO release reported in the literature with increasing temperature. This information should also be supplemented with data resulting from research on the cooperation of microorganisms colonizing the compost. Although the CO concentrations obtained in the headspace of bioreactors during the incubation of the isolated bacterial strains exceeded 1,000 ppm, it is possible that their activity could be reduced due to the lack of interspecies synergy, which was indicated in the paper [42] on the example of the *B. licheniformis* strain.

The results presented in the doctoral dissertation also indicated an important direction for future research on the biological production of CO, which is the analysis of the expression of the gene responsible for encoding the CODH enzyme in CO-producing bacteria. Detailed microbiological and molecular biology-based studies in various conditions of the composting process will allow to determine not only the ability of specific strains to produce this enzyme but also the optimal conditions for its production and the direction of CO and CO_2 conversion [20,41,42].

The issue of the biological production of CO by bacteria should also be supported in the near future by bioinformatics research and omics data, allowing for the characterization of variables affecting the metabolic processes of CO production. This is related to the toxicity of this gas, the increased concentration of which in the liquid phase can inhibit both the growth of CO-producing microorganisms and the activity of bacteria already present in the compost. As indicated in the review paper [40], knowledge in this area can be extended with the help of predictive models that facilitate the control and optimization of strain metabolism. In addition, future studies on strain engineering on the organism model are necessary to obtain bacteria capable of working in the harsh environment of the toxic CO.

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12.List of Annexes

[1]. Karolina Sobieraj, Sylwia Stegenta-Dąbrowska, Gang Luo, Jacek A. Koziel, Andrzej Białowiec, 2022. *Carbon Monoxide Fate in the Environment as an Inspiration For Biorefinery Industry: A Review*, Frontiers in Environmental Science, 10, 1-24, doi: 10.3389/fenvs.2022.822463,

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REVIEW
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Carbon Monoxide Fate in the Environment as an Inspiration For Biorefinery Industry: A Review

Karolina Sobieraj[1], Sylwia Stegenta-Dąbrowska[1], Gang Luo[2,3,4], Jacek A. Koziel[5] and Andrzej Białowiec[1,5]*

[1]Laboratory of Waste and Biomass, Department of Applied Bioeconomy, Wrocław University of Environmental and Life Sciences, Wrocław, Poland, [2]Shanghai Key Laboratory of Atmospheric Particle Pollution and Prevention (LAP3), Department of Environmental Science and Engineering, Fudan University, Shanghai, China, [3]Shanghai Technical Service Platform for Pollution Control and Resource Utilization of Organic Wastes, Shanghai, China, [4]Shanghai Institute of Pollution Control and Ecological Security, Shanghai, China, [5]Department of Agricultural and Biosystems Engineering, Iowa State University, Ames, IA, United States

Carbon monoxide (CO) is ubiquitous in the environment. In this literature review, the biological CO transformations in ecosystems were summarized as an inspiration for the biorefinery industry. Specifically, for the first time, information about CO fate in soil, water, and the atmosphere was collected, and CO impact on plants, animals, and humans was discussed. The review also addresses the need to develop new solutions to implementing circular bioeconomy and highlights the potential of CO use in biologically mediated processes as an untapped valuable resource. Specific key areas of research were identified as 1) development of water-gas shift (WGS) bioreaction, 2) electricity production during bioelectrochemical syngas conversion (BESs), and 3) electro-fermentation (EF) as a source of added-value biochemicals and fuels.

Keywords: carbon monoxide, biorefinery, water-gas shift reaction, CO cycle, CO consumption, CO production

Edited by:
Nsikak U. Benson,
Covenant University, Nigeria

Reviewed by:
Tian Li,
Nankai University, China
Michael Köpke,
LanzaTech, United States

***Correspondence:**
Andrzej Białowiec
andrzej.bialowiec@upwr.edu.pl

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INTRODUCTION

Human's pursuit of knowing and understanding the mechanisms and interdependencies in the environment certainly applies to carbon monoxide (CO). CO is a colorless, odorless, and highly flammable gas. It is considered toxic and threatens air quality and human health (Mahajan and Jagtap, 2020; Ramezani et al., 2020). The harmful effects of CO on higher living organisms are well-documented based primarily on the formation of carboxyhemoglobin (Ruth-Sahd et al., 2011). CO can also be considered an indirect greenhouse gas (GHG). CO reacts with OH radicals in the atmosphere, depleting OH reservoirs, which can effectively control the primary of GHGs such as methane. Atmospheric CO balance can also contribute to the formation of tropospheric ozone (Rozante et al., 2017). On the other hand, the lack of CO classification as a greenhouse gas has led to its omission in many environmental studies, leaving a considerable gap in knowledge about its formation's exact conditions and pathways.

However, CO links and participates in many processes and reactions in the ecosphere, being a part of water, soil, and atmospheric chemistry. The topic of CO formation and consumption in various ecosystems seemed to be well-known, and most of the literature on the subject was published in the 20th century. However, the knowledge about CO gained earlier has the potential to be used in innovative and future-oriented concepts related to gas processing. The natural presence of CO in all elements of the environment has provoked people to ask questions about the possibility of using CO for industrial purposes in biological-inspired processes–in the area of biorefinery.

The concept of biorefinery attracts the widespread interest of researchers, industry, and policy makers, being a part of circular economy, which is currently one of the most significant environmental undertakings and challenges of the 21st century. However, the transition from a linear to a circular model requires a high level of awareness, well-established, science-based, and transdisciplinary approaches that acknowledge multiple ramifications that can be linked into a closed loop. Solutions for moving to green and sustainable production and energy systems from conventional fossil sources are needed. The biorefinery concept is well-positioned to address the increasing demand for energy and materials, natural resource depletion, and societal ecological awareness, with CO as one of the promising chemicals for these purposes. If subjected to biological processing, CO can become a source of energy, fuels such as H_2, and biochemicals (Santoro et al., 2017).

In this literature review, the biological CO transformations in ecosystems were summarized as an inspiration for the biorefinery industry. Specifically, information about CO fate in soil, water, and the atmosphere was collected, and CO impact on plants, animals, and humans was discussed. The review also addresses the need to develop new solutions to implementing circular bioeconomy and highlights the potential of CO use in biologically mediated processes as a valuable resource. Here, for the first time, we aimed to address the questions:

1) Is it possible to treat CO in biologically-mediated biorefinery processes using microorganisms despite its toxic nature?
2) What are the possible pathways of using CO in biologically mediated processes?
3) What are the risks associated with the CO considered for a biorefinery feedstock?

THE FATE OF CO IN THE ENVIRONMENT

CO Fate in the Atmosphere

CO is one of the most common air pollutants, and its concentration in contaminated continental air masses reaches up to several ppmv (Badr and Probert, 1994). CO is emitted to the atmosphere through anthropogenic processes, such as biomass combustion, fossil fuels, waste incineration, industrial processes, and transport. Additional contributors are natural sources (oceans, soils, plants, forest fires), atmospheric oxidation of CH_4, and other non-CH_4 hydrocarbons (NMHC) (Badr and Probert, 1995; Tarr et al., 1995; Schade and Crutzen, 1999; Bruhn et al., 2013). CH_4 oxidation has the largest share of these sources, producing approximately 700 Tg-CO·yr^{-1} (Bergamaschi et al., 2000; Monson and Holland, 2001); fossil fuel combustion together with biofuel use and other industrial emissions are responsible for 500–900 Tg-CO·yr^{-1}, while biomass burning–for 400–800 Tg (Duncan et al., 2007). Photochemical CO production due to the oxidation of naturally emitted and anthropogenic NMHC equals 450 and 110 Tg-CO·yr^{-1}, respectively, (Rozante et al., 2017). In general, global CO levels rose from the Industrial Revolution until 1980; then a gradual decrease in its concentration, especially in the Northern Hemisphere, was observed in measurements from the global surface network from the National Oceanic and Atmospheric Administration (NOAA), caused by both the use of catalytic converters in cars and technological advances in combustion since 2000 (Bakwin et al., 1994; Voiland, 2015; Gaubert et al., 2017). More recently, the downward trends in CO observed for both the Northern and Southern Hemispheres have shown good consistency with long-term trends in bottom-up emissions in Europe, the United States, and China; where an improvement in combustion efficiency and a reduction in emissions from anthropogenic sources was observed (Gaubert et al., 2017). It is worth noting, however, that despite its main global sources, atmospheric CO levels show spatial as well as seasonal variability. While the oxidation of CH_4, a gas that is evenly distributed around the world, provides a similarly constant CO background of around 25 ppb, the remaining emission groups depend on space-time aspects. Thus, inter alia, CO from fossil fuels shows higher levels in the northern mid-latitudes, mainly in winter, while biomass combustion in tropical continents contributes to higher CO concentrations in the summer, during the dry season, along with the rainforests (Andreae et al., 2012). Additionally, the spatial variation in CO concentration is also characteristic on a smaller scale, e.g., in urban areas, where it depends not only on meteorological conditions or thermal inversion but also directly on atmospheric turbulence and traffic intensity (Oliveira et al., 2003).

Nevertheless, the determination of the atmospheric CO concentration is still a challenge; despite the variety of analytical techniques available, there is still remaining uncertainty. Therefore, in addition to the most commonly used methods, such as gas chromatographic combined with a mercuric oxide (HgO) reduction detection or a flame ionization detector (FID), non-dispersive infrared absorption (NDIR), vacuum ultra-violet resonance fluorescence (VURF), and tuneable diode lasers spectroscopy (TDLS), comparative studies were also performed (Ou-Yang et al., 2009; Zellweger et al., 2012). In addition to the analytical methods of CO measurement, further possibilities were gradually added, such as the use of closed path Fourier Transform Infrared (FTIR) absorption, cavity-enhanced off-axis integrated cavity output spectroscopy (ICOS), or multi-path quantum cascade laser (QCL) absorption in the mid-infrared range, and cavity ring-down spectroscopy (CRDS) in the near-infrared range (Zellweger et al., 2012).

CO is mainly utilized by the tropospheric reaction with the OH hydroxyl radical (Logan et al., 1981; Khalil and Rasmussen, 1984; Badr and Probert, 1995). CO is oxidized to CO_2 in the stratosphere, where it migrates *via* convection, turbulence, and mixing (Seiler and Warneck, 1972; Seiler, 1974). The reaction is fast and independent of temperature; thanks to it, the residence time of CO in the atmosphere is relatively short, from 2 weeks to 3 months (Rozante et al., 2017; Rakitin et al., 2021). Soils and oceans are also involved in CO capture (Ingersoll et al., 1974; Conrad et al., 1982) and higher plants and algae (Krall and Tolbert, 1957; Chappelle, 1962).

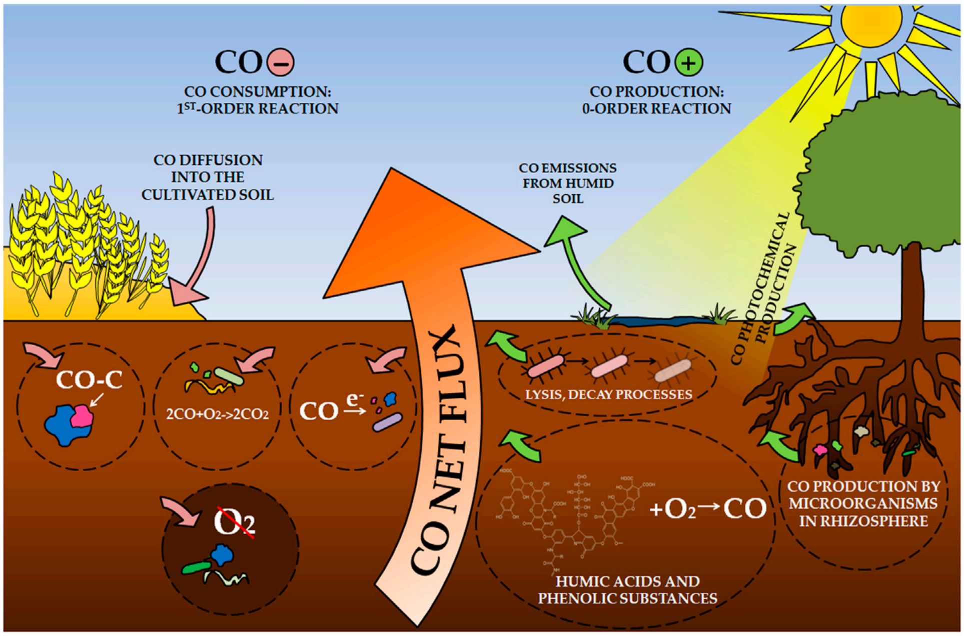

FIGURE 1 | Carbon monoxide cycle in soil.

Taking into account the characteristics of greenhouse gases, CO is not considered one of them as it is not capable of absorbing infrared radiation (Rozante et al., 2017). However, because of the primary mechanism for removing atmospheric CO by reaction with the OH radical, CO is recognized as an essential trace gas that controls the oxidative ability of the atmosphere (Bruhn et al., 2013). An increase in CO concentration in the troposphere causes changes in the distribution and amount of OH; reactions of the radical with CO and CH_4 constitute about 97% of its destruction (Logan et al., 1981; Levine et al., 1985; Badr and Probert, 1995). The change in the atmospheric OH affects the concentration of other gases, including CH_4 and O_3 (Hameed et al., 1980). Thus, CO indirectly affects the energy budget of the atmosphere (Evans and Puckrin, 1995), increasing the concentration of GHGs and the time of their utilization in the troposphere, as well as controlling the transfer to the stratosphere, which in turn has an impact on stratospheric O_3 (Ramanathan et al., 1985; Thompson, 1992; Bruhn et al., 2013). The radiative forcing from CO is estimated more than from N_2O and halogenated hydrocarbons (Rakitin et al., 2021).

CO Fate in Soil

CO is constantly supplied to the atmosphere, which results from both natural and anthropogenic sources. Despite the significant amount of gas emitted, its concentration in the atmosphere does not seem to increase (Bartholomew and Alexander, 1979). It's because natural processes are responsible for utilizing CO shortly after its release (Inman et al., 1971).

Soil is considered one of the main sinks of atmospheric CO, responsible for 40% of total consumption (Seiler, 1978). However, soils can also be a CO source in the global CO cycle, as noted, especially in the savannas and deserts (Conrad and Seiler, 1985a; Kuhlbusch et al., 1998). For this reason, CO uptake by soils is a net flux consisting simultaneously of consumption and production (**Figure 1**) (Seiler, 1978; King and Crosby, 2002; Bruhn et al., 2013; van Asperen et al., 2015; Pihlatie et al., 2016). Simultaneous chemical, physical and microbiological processes (Kuhlbusch et al., 1998) depend on many climatic, biological, and physical soil factors, making the equilibrium CO vary between a few ppbv up to hundreds of ppbv (parts per billion by volume) (Conrad and Seiler, 1979; Conrad and Seiler, 1980b; Conrad and Seiler, 1982a). The most important soil parameters include water content, temperature, organic matter content, pH, soil type, the depth of CO consumption horizon, and CO concentration in the gas phase (Potter et al., 1996). Even small changes in this balancing between CO production and soil uptake can severely impact tropospheric chemistry (Moxley and Smith, 1998b).

Soils have been analyzed for the production and consumption of CO for over 40 years. The soil processes were considered for most CO budget analyses in the atmosphere (Crutzen and Gidel, 1983; Conrad, 1996). Soil CO absorption studies were conducted in static and dynamic chamber systems to investigate gross and net exchanges or both (Ingersoll et al., 1974; Conrad and Seiler, 1985b; Sanhueza et al., 1994a). CO consumption was defined as a first-order process, while CO production is a zero-order process for CO (Conrad and Seiler, 1982a; Duggin and Cataldo, 1985; King, 1999b). Gross CO exchanges were determined mainly based on ^{14}CO oxidation, while both gross production and consumption were estimated using models and empirical estimates of net uptake rate constants and steady-state headspace CO concentrations (Bartholomew and Alexander, 1981; King, 1999b).

CO uptake by soils is mainly due to the activity of microorganisms (Inman et al., 1971; Conrad and Seiler, 1980b; Sanhueza et al., 1998; Whalen and Reeburgh, 2001). The biological nature of the CO consumption has demonstrated, among others, the fact that CO use activity in autoclaved soils disappears and returns when they are inoculated again with fresh and non-sterile soil (Inman et al., 1971; Ingersoll et al., 1974; Liebl and Seiler, 1976; Seiler, 1978). However, CO uptake may be limited at elevated CO levels due to microbial metabolic limitations [above 1,000 ppbv, (Bartholomew and Alexander, 1979)]. When CO levels reach lower values that are closer to atmospheric levels of this gas (100–200 ppbv), indicators of CO consumption by soils are higher than its production into the atmosphere (Potter et al., 1996).

The process of CO consumption by microorganisms is considered a result of several mechanisms. CO can be metabolized and incorporated into cellular material, catabolicly oxidized to CO_2 by autotrophs as a source of energy or carbon, and used as an additional electron donor by heterotrophic microorganisms; the first of these processes appearing to be dominant (Bartholomew and Alexander, 1979). The assumption that CO uptake by soils is based on the metabolism of the microorganisms is confirmed by research using antibiotics that caused immediate inhibition of this process. For this reason, it is assumed that the CO consumption is controlled by the anabolic activity of microorganisms that consume it during growth and not in the case of protein biosynthesis (Conrad and Seiler, 1980b). In this way, microorganisms are responsible for removing CO from 10% to as much as 50% of its global emissions, depending on sources (Heichel, 1973; Liebl and Seiler, 1976; King, 1999a).

CO consumption is correlated with the total organic carbon present in soil (Inman et al., 1971; Ingersoll et al., 1974). It is associated with organic matter as a source of energy and carbon by CO oxidizing microorganisms (Heichel, 1973; Kiessling and Meyer, 1982; Meyer and Schlegel, 1983; Moxley and Smith, 1998a). The opposite tendency was observed by (Conrad and Seiler, 1985b), proving that the rate of CO uptake depends only on soil enzymes and microorganisms that are not limited by the available organic matter. It is also worth emphasizing that the CO exchange between soil and atmosphere is mainly determined by the top 5 cm of soil (King, 1999a); concentration profiles of this gas do not show a significant amount at greater depth, and below 10 cm they are close to zero (Sanhueza et al., 1998). CO consumption and production processes in these few upper centimeters of soil are also most vulnerable to changes in temperature and humidity and other factors such as fires (Kuhlbusch et al., 1998).

Soil CO consumption based on the activity of microorganisms can occur both under aerobic and anaerobic conditions; in the latter case, it occurs at a lower rate (Conrad and Seiler, 1980b). It is assumed that there are anaerobic sites in the soil in which certain groups of microorganisms break down CO without the presence of O_2 (Zavarzin and Nozhevnikova, 1977). This is confirmed by observations of CO consumption in anaerobic conditions in fresh soil, which was just directed to incubation in an anaerobic atmosphere. Anaerobic CO consumption then increased by 15%, while aerobic CO consumption decreased by 40% (Conrad and Seiler, 1980b). The conversion of ^{14}CO to CO_2 in anaerobic conditions has also been noted (Bartholomew and Alexander, 1979).

CO absorption rates are controlled by environmental factors such as humidity and temperature (Conrad and Seiler, 1985b; Moxley and Smith, 1998b; King, 1999a). Soil absorption of this gas reaches its maximum value under optimal humidity and temperature conditions. However, these processes have not been extensively studied for various soil types (Moxley and Smith, 1998b; Sun et al., 2018). CO consumption was reported under temperature conditions in the range of 20–30°C with an optimum uptake at 30°C (Inman et al., 1971; Heichel, 1973; Ingersoll et al., 1974; Liebl and Seiler, 1976; King, 1999a). The temperature reactions of the CO flux between soil and atmosphere are complex and include the consumption of this gas and its production by an abiotic path (Conrad and Seiler, 1985b). According to (King, 1999a), CO formation processes become more and more important at temperatures above 30°C and become dominant when the temperature exceeds 35°C. Data reported by (Scharffe et al., 1990) agree with this observation, according to which 30°C is the temperature limit at which the ability to consume CO is exceeded. In addition (Inman et al., 1971) found that at 40°C, CO was about 30% higher in the presence of light than in the dark. Soil heating above 40°C resulted in CO production (Inman et al., 1971; Ingersoll et al., 1974).

Due to the possibility of lower than optimal humidity of the annual soil moisture range in natural conditions, field observations of the impact of this parameter on CO flux are challenging (Sun et al., 2018). Nevertheless, it has been investigated that wetland soils have low CO uptake (Moxley and Smith, 1998b). This tendency may be caused by limiting O_2 diffusion by filling the soil pores with water and hindering gas transport. Reduced water content causes an increase in CO consumption up to an optimum value at soil moisture in the range of 30–60% (King, 1999a). In addition, CO absorption ceases when the soil water freezes or the soil is desiccated (Potter et al., 1996; Moxley and Smith, 1998b). A renewed increase in humidity in dry soils leads to the restoration of CO uptake. However, the hysteresis becomes visible. Water stress contributes to this, resulting from increased water content to optimal values immediately after its low level (King, 1999a).

The combination of the above factors contributes to the seasonality of CO consumption in soils. CO intake shows daily variability as a function of CO concentration changes in the atmosphere and temperature (King, 1999b). For this reason, soil can be seen as a source of CO during the day, while it absorbs it at night (Conrad and Seiler, 1982a). In addition, changing weather conditions also affect soil moisture, which translates into the activity of microorganisms. For this reason, lower CO uptake rates in winter were observed when the microbial activity is relatively small (Conrad and Seiler, 1980b).

CO production in soils is mainly abiotic, although there are also reports of the production of this gas in laboratory conditions by root microorganisms (Conrad and Seiler, 1985b; Zepp et al., 1997; King and Crosby, 2002). The non-biological nature of CO formation is confirmed by the tendency to increase it after autoclaving or other sterilization processes of soil samples, such as the use of heat, ultraviolet light, or fumigation (Smith et al., 1973; Conrad and Seiler, 1980b; Conrad and Seiler, 1982a). CO emission is based on chemical and biochemical reactions leading to loss of organic matter by rotting, lysis of microorganisms, and oxidation of dead microbiological material (Conrad and Seiler, 1980b). Heat treatment of dry soil stimulates the efficiency of CO production, which is caused by the breakdown of complex organic substances into simpler particles–including plant residues (Conrad and Seiler, 1982a).

Photochemical production has an important share, as evidenced by the linear relationship of CO emissions from the intensity of solar radiation and the lack of this gas production in the dark (Seiler et al., 1978; Conrad and Seiler, 1980b; Yonemura et al., 2000; van Asperen et al., 2015). Illumination of mineral soils, litter, and plant tissue with light at < 400 nm wavelength stimulates CO production (Valentine and Zepp, 1993). There are also conflicting reports regarding this parameter; (Conrad and Seiler, 1985c) observed CO production both in light and in the dark, which they explained by moisture rather than the photochemical nature of CO forming processes. The stimulating effect on CO production was, among others, an increase in soil moisture–those saturated with water emitted CO into the atmosphere (Conrad et al., 1988). Higher moisture may involve the activation of soil enzymes and dormant microorganisms, which may multiply and reach other habitats in the soil (Conrad and Seiler, 1985b). In addition, moist organic matter is better for microorganisms compared to a dry substrate for CO production.

Data on the impact of soil pH on the amount of CO stream between soil and atmosphere can be found in the literature. An increase in soil pH causes an increase in this gas stream by stimulating abiological CO production (Conrad and Seiler, 1985a). According to the authors, this abiological CO production in soil follows the Arrhenius equation, and increasing pH causes a decrease in energy and entropy of activation. Studies by (Inman et al., 1971) proved that acidic soils participate in more active CO utilization.

CO formation in soils is also based on the chemical oxidation of humic acids and other phenolic substances in an O_2 atmosphere (Miyahara and Takahashi, 1971). One of the most important factors affecting the activation energy of CO production is the physicochemical state of humic acid. Its decrease was noted simultaneously with the increase of dissolved humic acid polymers in soil (Conrad and Seiler, 1985a). The CO production from humic acids is a heat-stimulated reaction (Conrad and Seiler, 1985a). In addition, this reaction was stimulated by the presence of O_2, but the addition of chemicals quenching singlet oxygen, superoxide, or hydroxyl radicals did not inhibit it (Conrad and Seiler, 1985a).

The CO flux between soil and atmosphere is also affected by how the soil is cultivated. However, these trends are not clear; some research shows an increase in CO use by arable land compared to non-cultivated soils (Scharffe et al., 1990; Sanhueza et al., 1994b; Sanhueza et al., 1994a). It is explained by the loss of organic matter in the soil and changes in pH and water conditions in cultivated soils (King, 2000). In addition, plowing buries the surface layer of soil and mulch, which reduces CO production in the surface layer and leads to diffusion of CO deep into the soil, where it is consumed by microorganisms (Sanhueza et al., 1994b).

For this reason, forests and native grasslands are seen as areas with lower CO consumption (King, 2000). However, the data obtained in studies of temperate soils underline the minimal impact of agriculture on CO flux. Evidence grows that soil cultivation reduces CO consumption (Moxley and Smith, 1998a; Moxley and Smith, 1998b).

CO Fate in Water

The ocean has been recognized as a source of CO released into the atmosphere since the early 1970s (Swinnerton et al., 1970; Lamontagne et al., 1971). Despite the low ocean share (0.4–9%) among all sources of CO in the atmosphere (Bates et al., 1995), it can constitute up to 50% of the load in the marine boundary layer (Erickson and Taylor, 1992; Stubbins et al., 2006a). The southern hemisphere is particularly important here, in which CO production accounts for almost 60% of the total CO flux from the ocean surface (Erickson, 1989). Surface ocean waters are saturated with CO compared to atmospheric equilibrium, which causes a net flux of this gas at the ocean-atmosphere interface (Linnenbom et al., 1973; Logan et al., 1981; Zuo et al., 1998). The CO emissions to air are controlled mainly by the concentration of this gas in water (Bates et al., 1995). It depends on several factors such as photochemical production, consumption by microorganisms, exchange between air and water, and physical mixing (**Figure 2**) (Wilson et al., 1970; Conrad et al., 1982; Butler et al., 1987; Jones, 1991). Due to the impact of these factors, CO concentration in waters shows diurnal, seasonal, and regional diversification (Bates et al., 1995).

CO was identified as the second most important product of dissolved organic matter (DOM) photolysis in water bodies (Mopper and Kieber, 2000; Stubbins et al., 2006b). The rate of CO formation is one order of magnitude higher compared to other low molecular weight carbon photoproducts produced under aqueous conditions (Mopper et al., 1991; Zuo and Jones, 1995). It is the photodegradation of the DOM by part of the UV solar radiation that is indicated as the main source of CO from both ocean and sea waters, as well as from the surface of lakes, rivers, wetlands, and coastal waters (Zuo and Jones, 1997; Pos et al., 1998; Zuo et al., 1998; Stubbins et al., 2006a; Blomquist et al., 2012).

The rate of CO production is linearly dependent on the concentration of DOM, mainly derived from the degradation of dead biomass, fragmented organic matter particles, and droppings of living organisms, as well as light absorption and water fluorescence (Zuo and Jones, 1997). Carbonyl compounds and phenols are an important part of the soluble organic matter in waters; they build aqueous fulvic acids, then oxidize to quinones. It is phenols and carbonyls found in the surface layer of water that

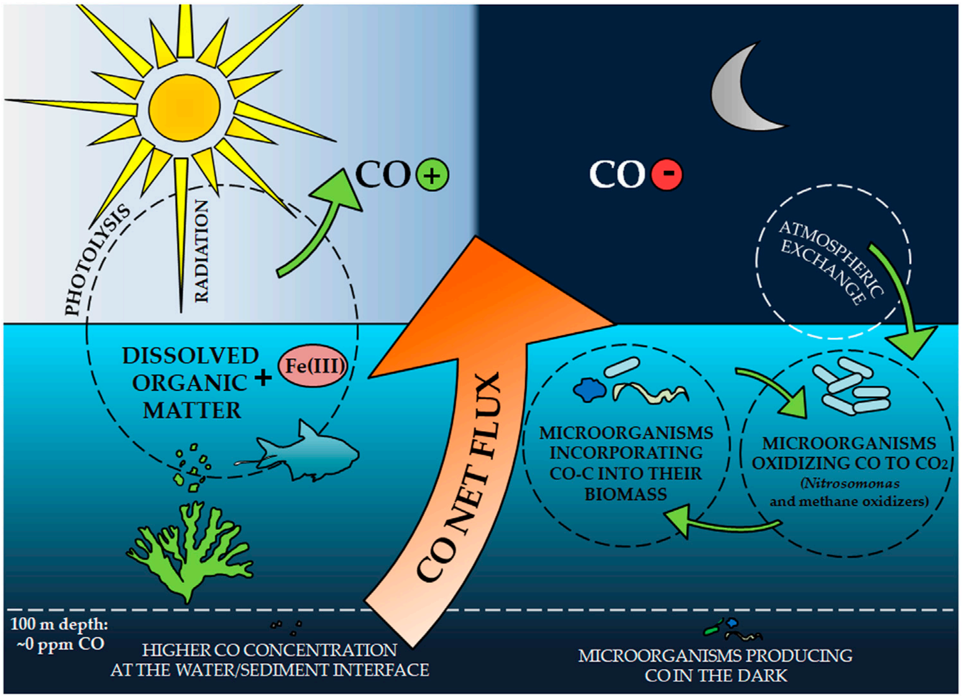

FIGURE 2 | Carbon monoxide cycle in water.

absorb solar rays, producing CO. Their mechanism as photosensitizers is also a possible pathway, thanks to which solar energy is transferred to other carbonyl compounds (Zuo and Jones, 1997).

Ozone depletion and the resulting increase in UV-B radiation that reaches the earth's surface have been noted as a factor increasing CO photoproduction in ocean waters (Erickson, 1989; Pos et al., 1998). Additionally, the photodegradation of DOM and thus the associated CO production in waters are affected by dissolved iron. It forms complexes with organic matter (including carbonyls and carboxylates) that are highly photoreactive (Zuo and Hoigne, 1992), which speeds up CO formation. This is confirmed by studies of (Zuo and Jones, 1997), who showed that CO photoproduction increases with increasing Fe (III) concentration.

Due to the dependence of the CO flux on the photolysis processes of soluble organic materials and thus on the light intensity, CO photoproduction in the water surface proceeds according to the daily cycle. Field studies showed maximum CO production in the early afternoon, fall in the evening, and minimum around dawn (Swinnerton et al., 1970; Conrad et al., 1982; Jones, 1991; Bates et al., 1995; Zuo and Jones, 1995; Pos et al., 1998; Xie et al., 2002; Zafiriou et al., 2003; Stubbins et al., 2006a). CO production is also characterized by a time delay relative to light intensity, which draws attention to its mechanism–gas production is initiated by light, and then CO is released within a few hours (Conrad and Seiler, 1980a; Conrad et al., 1982). In addition, the highest daily CO concentration amplitudes were recorded during sunny days, while on rainy days, when the light intensity was lower, the dissolved CO content in water showed lower fluctuations or was constant (Conrad et al., 1982). This demonstrates the short-term residence of CO in the water column of about 3–4 h, strongly coupled with the day-night cycle (Zuo et al., 1998; Tolli and Taylor, 2005).

The correlation between the CO production rate and dissolved organic carbon concentration affects the regional flux. Higher rates of CO formation are observed in coastal places rich in organic matter and other chemical and physical compounds, compared to open oceanic spaces (Lamontagne et al., 1971; Linnenbom et al., 1973; Jones and Amador, 1993; Johnson and Bates, 1996; Zuo et al., 1998). Within the latter, higher CO saturation rates were observed in the waters of the North-West region of Africa and in the equatorial area, which is explained by the activity of equatorial currents mixing surface waters with nutrient-rich waters (Voituriez and Herbland, 1979; Katz et al., 1980; Conrad et al., 1982).

The CO concentration in waters varies with depth. Its gradient can be observed in the euphotic zone, with the highest values at or near the water surface, where the light is not suppressed (Tolli and Taylor, 2005). At greater depths, the CO concentrations decrease, reflecting decreasing light intensity (Seiler, 1978). Below the mixed layer (> 100 m depth), CO concentration is low and constant. The exception is higher concentrations occurring at the water/sediment interface (Swinnerton and Lamontagne, 1974; Johnson and Bates, 1996), as well as small maximum CO concentrations, which are explained by the functioning of microorganisms producing CO very slowly in the dark (Conrad et al., 1982). This indicates a very low net CO exchange between these two zones, which does not significantly affect the daily variability in the surface water layer (Conrad et al., 1982; Mopper et al., 1991; Zuo et al., 1998).

The CO flux from the water surface depends on transfer velocity, which is affected by wind speed and water temperature (Erickson, 1989). The first factor affects the intensity of light–when the wind speed drops to about 2 m s^{-1} on calm days, the CO concentration increases; the rough surface of the water causes a significant reduction in the amount of CO (Conrad et al., 1982).

Microbiological consumption influences the cyclic daily variability of CO concentration at the water surface (Conrad et al., 1982; Blomquist et al., 2012) and atmospheric exchange (Zuo and Jones, 1995). CO consumption by microorganisms follows the Michaelis-Menten kinetics; the CO oxidation rate increases linearly with increasing CO concentration and depends on the size and type of microbial population (Conrad and Seiler, 1982b; Tolli and Taylor, 2005). However, their species are not known. Chemolitotrophicamonnium oxidizers (*Nitrosomonas*) and methane oxidizers (methanothrops) have a high affinity for CO in waters, and they are mainly suspected of CO consumption processes (Zuo et al., 1998). The first group includes, for example, *Nitrosococcus oceanus* and *N. europaea*, the activity of which is catalyzed by ammonia monooxygenase; on the other hand, in the methanotrophs group, CO oxidation was confirmed in the case of, among others, *Methylomonas methanica*, *Methylococcus capsulatus* and *Methylomonas albus*, and the responsible enzyme was not precisely indicated (Ferenci, 1974; Hubley et al., 1974; Bédard and Knowles, 1989; Jones and Morita, 2011). These microorganisms are capable of oxidizing CO at concentrations <100 nM (Jones et al., 1984).

During the day, sunlight has been shown to significantly inhibit the activity of microorganisms responsible for CO oxidation in the surface water layer (Zuo et al., 1998; Tolli and Taylor, 2005). The opposite situation can be observed at night. The sharp decrease in CO concentration is caused by the lack of CO photoproduction and the resumption of the first-order reaction of gas oxidation by microorganisms. Their activity is no longer inhibited, and the decreasing level of dissolved CO does not cause the supersaturation of enzymes responsible for its consumption (Tolli and Taylor, 2005). The microbiological consumption of CO *in situ* by converting it to CO_2 is the dominant mechanism for the utilization of this gas; it is generally not included in microbial biomass, although there are observations that some autotrophic bacteria may build in CO-C after it has been oxidized. However, they are not necessarily those strains that participated in this process (Tolli and Taylor, 2005).

CO ROLE FOR ORGANISMS

CO Impact on Plants

Biosynthesis and photoproduction of CO in plants were observed in the second half of the 20th century (Wilks, 1959; Schade et al., 1999). This compound is formed during oxidative heme catabolism due to the activity of the enzyme heme oxygenase (HO). The result is three products: CO, biliverdin, and free iron Fe^{2+} (**Figure 3**). The second one is immediately transformed into bilirubin, while the iron is involved in ferritin induction (Bilban et al., 2008). Among the three isoforms of HO discovered so far, HO-1, HO-2 and HO-3 have been distinguished, the last two of which are characterized by low activity (Maines, 1997).

Additionally, (Muramoto et al., 2002) found AtHO1, plastid heme oxygenase, hemoprotein forming a complex with heme in 1:1 ratio. Their research led to the production of CO by catalyzing the heme to biliverdin IX conversion reaction, which was catalyzed by the AtHO1 mentioned above. In addition to producing CO in plants using HO enzyme-catalyzed reactions, which are the predominant method of producing this compound, researchers also found other ways to form it. (Zilli et al., 2014) observed an increase in CO levels in soybean during the first week after planting, but this effect was not correlated with the increase in HO activity. The authors indicated lipid peroxidation and ureide metabolism as an alternative source of CO in this plant.

Research on the effects of CO on plants covers many aspects of their development, including seed germination. While investigating the influence of gases such as O_2, N_2, and CO on *Setaria faberii*, it was observed that the latter might have a twofold influence (Dekker and Hargrove, 2002). Depending on the CO concentration, it stimulated seed germination (it increased from 37 to 56% at 1% CO) or inhibited it (75% CO added resulted in a decrease in germination from 37 to 14%). The authors explain the negative CO effect on seed development by inhibiting the plant's mitochondrial respiration; in terms of promoting germination, the researchers rejected the idea that this gas acted on a respiratory apparatus, leaning more towards CO interacting with CO, an undefined physiological factor in the seed.

CO has also been found to influence the response of plants to abiotic stress caused by environmental salinity, drought, ultraviolet radiation, and heavy metal pollution (Wang and Liao, 2016). These factors, influencing plant growth, resistance, and yield, can significantly limit their development. One of the most frequently analyzed elements from the above mentioned is salt stress. The researchers conducted experiments on plants such as *Triticum aestivum, Oryza sativa,* and *Cassia obtusifolia*, analyzing both their seeds, leaves, and roots (Xu S. et al., 2006; Liu et al., 2007; Xie et al., 2008; Ling et al., 2009; Zhang et al., 2012). Research on the influence of CO on sprouting was conducted by (Liu et al., 2007) by analyzing the reaction of *Oryza sativa* seeds to the addition of this compound's donors (hematin and its aqueous solution) under salt stress. CO suppressed the adverse effects of NaCl salinity, mitigating the inhibition of sprouting.

Additionally, CO limited oxidative damage by activating antioxidant enzymes. A similar situation was observed in *Cassia obtusifolia* seeds and seedlings, where CO ensured cytoprotection, activated anti-oxidant enzymes, and increased osmotic substances (Zhang et al., 2012). In an analysis of the effect of a salted environment on seeds of wheat by (Xu S. et al., 2006), low CO concentrations counteracted lipid peroxidation in germinating seeds by enhancing catalase (CAT), ascorbate peroxidase (APX), superoxide dismutase (SOD), and guaiacol peroxidase (GPOX) activities. Similar to the mitigation of

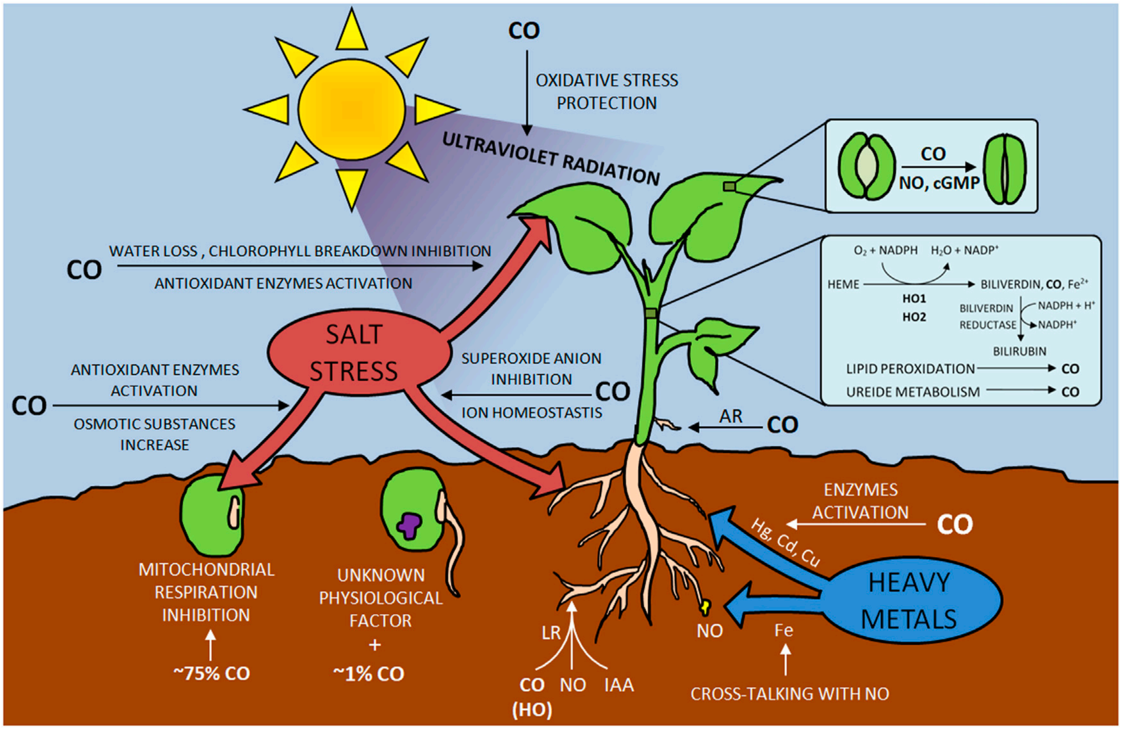

FIGURE 3 | Impact of CO on plants development.

oxidative damage in the seeds of *Oryza sativa*, CO caused the same effect in the leaves of *Triticum aestivum* (Huang et al., 2006). When treated with a hematin CO donor, they showed a reversal of chlorophyll breakdown and water loss previously experienced due to the treatment of wheat tissues with NaCl solution. Moreover, the authors noted an increase in enzymes, as reported by (Xu S. et al., 2006).

Additionally, the mechanism of mitigating the inhibition of germination of its seeds under simulated osmotic stress conditions was analyzed by (Liu et al., 2010). The beneficial CO effect based on the endogenous HO/CO signal, possibly integrated with NO, was proven. *Triticum aestivum* was also analyzed for the effects of salt stress on the roots. (Xie et al., 2008) and (Ling et al., 2009) found that CO was involved in maintaining ion homeostasis and reduced superoxide anion production.

The ability to mitigate its response to environmental pollution with heavy metals such as Hg, Cd, Cu, or Fe is an important aspect of CO activity in the context of increasing plant resistance to stress conditions. These metals, which pose a threat to plants and the organisms consuming them, could be mitigated thanks to both NO and CO (Wang and Liao, 2016). The experiments conducted by (Han et al., 2007) involving *Medicago sativa* L. plant exposed to mercury (in the form of $HgCl_2$) showed that the increase of lipid peroxidation and limitation of root growth could be inhibited by water solution of CO or hematin. Researchers noted the HO-1 transcript induction cells in *Alfalfa* after 12–24 h of increased activity of the enzymes glutathione reductase (GR), monodehydroascorbate reductase (MDHAR), and SOD. Plant exposure to Hg in *Brassica juncea* was also carried out by (Meng et al., 2011), who reported the activation of CAT and APX in addition to the SOD. Additionally, it was proven that reducing the toxic effect of Hg due to the CO activity is also based on proline and reduced non-protein thiols.

The addition of exogenous CO improves the accumulation of chlorophyll, contributing to the prevention of chlorosis as reported by (Kong et al., 2010), investigating the regulation of iron homeostasis by CO in *Arabidopsis*. Moreover, the CO effect on the plant contributed to the accumulation of NO in the root tips. These results prove not only the beneficial effect of CO on plant adaptation in a Fe-deficient environment but also cross-communication with NO. The role of CO in signaling oxidative damage was also confirmed in the environment with an increased concentration of Cd in *Medicago sativa* roots (Han et al., 2008). Among the stressors for plant function, researchers also analyzed UV-B radiation, which leads to the formation of reactive oxygen species (ROS). An increase in HO-1 protein expression was noted in the Glycine max L. leaves subjected to irradiation (Yannarelli et al., 2006), demonstrating the mechanism of plant protection tissues against oxidative damage.

The interaction between CO and other signaling molecules, such as NO, phytohormones, or H_2S, aroused the broad interest of researchers (Wang and Liao, 2016). It was found that phytohormones can induce various distinct developmental CO-dependent responses in plants. Research by (Guo et al., 2008) on lateral tomato roots (LR) formation showed that CO is partly involved in this process. The researchers, analyzing haem oxygenase-1 (LeHO-1), the source of intracellular CO in tomatoes, found that an increase in proteins and transcripts of

LeHO-1 caused a simultaneous increase in LR. This effect was shown for tomato mutants with loss of LeHO-1 function, during which the development of impaired LR was observed. Treatment with CO helped restore LR's normal development. Further analyses of the CO role in LR development were based on auxin (indole-3-acetic acid, IAA) and the NO mediator (Correa et al., 2004). The tests showed that the influence on the tissues of tomatoes with CO increased the level of IAA in them; however, intracellular NO generation induced by CO was observed in the roots of this plant. Moreover, the activity of CO was inhibited by N-1-naphthylphthalamic acid and carboxy PTIO [cPTIO, [[2-(4-carboxylatophenyl)-4,4,5,5-tetramethyl-2-imidazoline 3-oxide]-1-yloxy] radical], which are known inhibitors of auxin and NO (Fricker, 1999). These observations were also supported by (Cao Z.-Y. et al., 2007; Xuan et al., 2008). The use of naphthylphthalamic acid by the former reduced the HO activity, the CO content was lower, and auxin-mediated induction of cucumber rooting (*Cucumis sativus*) was inhibited. This effect was mitigated by adding IAA, CO water solution, and hematin (HO-1 activator and CO donor), leading to a reduction in root growth inhibition. Greater CO levels were observed due to the use of IAA and/or hematin and an increase in HO activity (or HO-1 expression). The effect of Zn protoporphyrin IX (ZnPPIX), an inhibitor of HO-1, was also tested (Liu et al., 2007) to determine its inhibitory effect on IAA and hematin. The use of CO aqueous solution did not significantly inhibit accidental rooting of the cucumber. Moreover, the aqueous CO solution increased the endogenous CO content. A similar effect was achieved by (Cao Z. et al., 2007). During their experiments, exogenous CO, dependent on NO, increased the number and length of six lateral roots of *Brassica napus* L. Yangyou. Analyzes of the influence of CO on plant root growth also concerned the development of root hair and root tip segments [*Solanum lycopersicum* and *Triticum aestivum*, respectively, (Xuan et al., 2007; Guo et al., 2009)], as well as adventitious root development [*Cucumis sativus* and *Phaseolus radiates*, (Xu J. et al., 2006; Xuan et al., 2008; Xuan et al., 2012; Lin et al., 2014; Cui et al., 2015)]. It has been proven that CO can regulate the expression of target genes CSDNAJ-1 and CSCDPK1/5 (Xuan et al., 2008), restore the ability to develop them after the use of inhibitors during treatment in CH_4-rich water (Cui et al., 2015), and take part in with the growth of cucumber adventitious roots in hydrogen-rich water (Lin et al., 2014).

The relationship between the activity of CO, NO, HO, cPTIO, and ZnPP were also investigated by (Cao Z. et al., 2007). Their research tested the HO response to abscisic acid (ABA) in the context of stomatal closure in *Vicia faba* leaves. Their research proved that the addition of ZnPP or Hb (CO/NO scavenger) blocked this ABA-induced process, while, as in the case of previous authors, the application of hematin or CO aqueous solution increased the CO-generated and stimulated stomatal closure. The authors also declared that the CO generated by HO activity is involved in the stomata closure process and that NO and cyclic guanosine monophosphate (cGMP) act as downstream intermediates. In addition, HO-1/CO was analyzed in the context of programmed cell death (Xie et al., 2014). Observations of ZnPP interactions were performed to test their role during (H_2S)-induced cytoprotection. The authors observed that the addition of CO aqueous solution or bilirubin mitigated the negative effect of this HO-1 inhibitor on NaHS responses.

CO Impact on Animals

CO is considered an important endogenous signaling gas with similar properties to NO, produced in their organisms through heme degradation (Dulak and Józkowicz, 2003; Verma et al., 1993). Some of the CO can be generated *in vivo* by non-enzymatic haem metabolism by hydrogen peroxide or ascorbic acid. CO is then produced by breaking methylene bridges (Dulak and Józkowicz, 2003). The similarity to NO is due, *inter alia*, to the ability of both of these compounds to bind the iron atom derived from the heme moiety, which is linked to the soluble guanylate cyclase. This binding activates this enzyme and thus stimulates the production of intracellular cGMP (Cao Z. et al., 2007). The relationship between CO and NO in animal organisms has been confirmed in the studies of bovine pulmonary artery endothelial cells, which proved that 11–110 nmol/L of CO in them increases the concentration of released NO (Thom et al., 1997).

Research on the influence of CO on animals was indirectly initiated at the beginning of the 20th century by using them as detectors of vitiated air in mines (Burrell and Seibert, 1914). The analyses were conducted on commonly available animals, such as chickens, dogs, mice, pigeons, rabbits, canaries, and guinea pigs. One of the first reports on the experiments taking place in 1914 in the United Kingdom proved that the most sensitive to the effects of CO were mice and canaries, which meant that they were repeatedly used in rescue operations in mines as organisms experiencing stress caused by CO faster than humans (Burrell and Seibert, 1914).

Most of the studies concerned the effect of high concentrations of CO on animals. Only a few sources reported the effects of prolonged or repeated exposure to CO (Jones et al., 1971), and the results were often contradictory (Preziosi et al., 1970). Analyzes conducted in the mid-20th century showed that 11-weeks exposure of dogs for 6 days a week for 5.5 h to 100 ppm CO caused brain and heart muscle damage (Lewey and Drabkin, 1944). In the latter case, CO poisoning in these animals led to many pathological changes, such as muscle fibers degenerative changes, necrosis, and hemorrhage in the ventricle, associated with a COHb level of 75% (Ehrich et al., 1944). Myocardial changes have also been reported in other animals, such as rabbits, that showed necrosis after 30–60 min exposure to 3,000 ppm CO (Takahashi, 1961) or, *inter alia*, myofibrillar disintegration after exposure to 100 ppm CO for 4 h (Hugod, 1981). (Preziosi et al., 1970) reported the mortality of the studied dogs of 31%; the remaining surviving animals showed extensive central nervous system and myocardial fiber degeneration pathologies, similar to hypoxia-induced lesions. The authors also noted that the most severe changes occurred in animals exposed to CO for 60 min or more but were less severe than those exposed to short-term exposure at high gas concentrations. In addition, CO can reduce vascular resistance in the coronary vessels of animals, leading to too high blood flow, which has been studied in dogs

(Einzig et al., 1980; Kleinert et al., 1980), and it can also reduce myocardial contractility [seen in both dogs and goats, (Erickson and Buckhold, 1972; James et al., 1979)]. Experiments on the exposure of monkeys and dogs to CO also proved that this gas reduces the required threshold leading to ventricular fibrillation and arrhythmia (DeBias et al., 1976; Vanoli et al., 1989). On the other hand, it was later proven that the produced CO dilates blood vessels and prevents platelet aggregation, which maintains tissue microcirculation at an appropriate level (Katori et al., 2002).

CO has also been identified as affecting the respiratory system of animals. Researchers found many changes, including capillary and alveolar epithelial endothelial cell edema in the lungs of rats exposed to 0.5–1% CO (Niden and Schulz, 1965). A higher concentration of 95% led to a 56% decrease in ATP (Bassett and Fisher, 1976). In rabbits, CO exposure resulted in endothelial and epithelial swelling, and additionally, the endothelium was detached from the basement membrane (Fein et al., 1980). A similar effect was also observed in rats (Smialek et al., 1973). Changes caused by CO led to a decrease in dynamic lung compliance and increased resistance in the respiratory tract of animals (Fein et al., 1980). Results opposite to those presented above were obtained by (Musselman et al., 1960). 3-months long exposure of rats, rabbits, and dogs to 50 ppm CO did not cause any observed side effects in these animals. The absence of toxic effects of long-term exposure of rats, dogs, monkeys, and guinea pigs to three different concentrations of CO (51–200 ppm) was also observed by (Jones et al., 1971). No changes in lung tissues were observed in the analyzed dogs, despite being exposed to a 20–30 times higher CO dose than the lethal dose in humans (8–14%) (Fisher et al., 1969). A similar lack of toxic effects in the same animals was observed by (Halebian et al., 1984).

CO also has metabolic effects on animal organisms. It was reported that it changes blood glucose levels in rats (Smith and Penrod, 1940), rabbits (Gothert et al., 1972), and dogs (Schrenk et al., 1932). The hyperglycemia due to CO poisoning was explained by the animals' livers' decreased ability to produce and store glycogen. It is also influenced by an increased adrenaline level in the central nervous system and not, as initially thought, disruption of insulin production (von Oettingen, 1944). Additionally, after injection of CO under the skin of rats, catecholamine excretion in the urine significantly increased (400–600%) a few hours after the procedure (Pankow and Ponsold, 1978).

CO can dissolve in tissue fluids, which has been observed in rabbits, guinea pigs (Göthert et al., 1970), rats (Savolainen et al., 1980), and dogs (Coburn et al., 1971) exposed to CO followed by measuring carboxymyoglobin (COMb) levels (Sokal et al., 1984). The COMb concentration after CO exposure is lower than the COHb level in the blood and skeletal muscles. In addition, long-term exposure does not cause CO accumulation in the latter and the extravascular heart compartment (Sokal et al., 1984). Importantly, however, exposure of animal organisms to CO in combination with their workload increases the risk of tissue hypoxia due to the increased tissue level of CO, as shown in studies on rats (Sokal et al., 1986). Additionally, exposure to CO causes changes in specific enzymes in animal organisms, such as plasma leucine aminopeptidase (Katsumata et al., 1980), lactate dehydrogenase, creatine phosphokinase (Penney and Maziarka, 1976), cardiac cytochrome oxidase (Fukui et al., 1987) and cerebral cytochrome oxidase (Savolainen et al., 1980). In the first three enzyme cases, CO increased their activity, and the plasma leucine aminopeptidase level tested in rats exposed to CO depended on the duration of absorption (Katsumata et al., 1980). Enzymes susceptible to CO activity reached a high level of activity, reaching 117 and 132% above controls (lactate dehydrogenase and creatine phosphokinase, respectively, (Penney and Maziarka, 1976).

Behavioral studies of the effects of CO on animals were carried out as early as the second half of the 20th century. These analyses covered a broad spectrum of behaviors but were mainly based on rats, mice, monkeys, and pigeons (Laties and Merigan, 1979). For the first two groups, it was reported that CO interfered with several activities such as food and water intake and activities such as running, swimming, or digging. The eating disorder was associated with exposure of rodents to a CO concentration from 50 ppm for 120 h per week (Stupfel and Bouley, 1970), through 250 ppm per day (Koob et al., 1974; Annau, 1975) to 400–500 ppm (Theodore et al., 1971) and in each case, it was correlated with a reduction in weight gain. The running performance of rats showed a decrease in both long-term exposure of rodents to CO (200 ppm per day) and acute but high gas concentration (700 ppm for 30 min), while in the second case, it was lower (Plevova and Frantik, 1974). CO also limited the mice's ability to move; in the final stage of the 17-h treatment with CO, the distance covered was shortened from 33% (55 ppm), 50% (84 ppm) to 75% (160 ppm) (Malorny, 1972). A similar inhibitory effect on the activity of animals was observed during rats swimming (over 50% decrease in swimming ability after 1-h and 7-h exposure to CO concentrations of 300 and 100 ppm, respectively), and hamster's digging [decrease by 75% after exposure to 50 ppm CO, (Malorny, 1972)]. The behavior of the animals' conditioned reflexes was also analyzed. After the rats were exposed to CO, researchers observed a significant decrease in their responses, including pressing on a lever to obtain food [exposure to 500 ppm CO (Teichner, 1967)]. The animals were also negatively affected with a gas concentration of 250, 500, and 10,000 ppm (Goldberg and Chappell, 1967; Annau, 1975). Analyzes of this type were also carried out on monkeys, which showed reduced responsiveness after being exposed to 383 ppm CO for 1 week (Back and Dominguez, 1968; Theodore et al., 1971).

Accidental animal poisoning with CO is rarely described in the literature. However, due to their cohabitation with people, pets such as cats and dogs are also exposed to elevated CO concentrations due to poor ventilation, faulty heating installations, or fires (Sobhakumari et al., 2018). Few reports are addressing the toxicity threshold of CO for pets, and no evidence of pathology and histological changes induced by CO have been reported (Berent et al., 2005). The analysis showed that after 6–8 h of exposure of cats and dogs to exhaust gases containing CO, the animals were confused, inclined to a lying position, showed stiffness, and suffered from dyspnea (Berent et al., 2005). On the other hand, cats found dead after a fire in

TABLE 1 | Levels of carboxyhemoglobin (COHb) saturation (%) and symptoms (Kinoshita et al., 2020).

COHb (%)	Clinical symptom
<1	normal range (due to endogenous production)
<10	smoker's blood (no symptom)
10–20	headache, fatigue, ear ringing
20–30	headache, weakness, nausea, vomiting
30–40	severe headache, dizziness, nausea, vomiting
40–50	syncope, confusion, increased respiration, and heart rate
50–60	coma, convulsions, depressed respiration
60–70	coma, convulsions, cardiopulmonary depression, often fatal
70<	respiratory failure, death

their habitats were characterized by visible signs of poisoning, such as irregular red skin spots on the abdominal surface and the auricle, as well as a bright color of blood and muscles (Sobhakumari et al., 2018). Smoke inhalation also caused epileptic seizures in dogs and, as a consequence, their death (Kent et al., 2010).

Additionally, the literature reported human-analogous delayed neurological symptoms in dogs after exposure to smoke (2–6 days after the animals initially improved). Of the dogs tested, 60% either died or were euthanized because of their injuries (Jackson and Drobatz, 2002). Slightly different results were obtained by (Mariani, 2003). The initial acute condition of the Australian shepherd found during the fire showed that dementia and pneumonia were alleviated during 7 days of treatment.

In addition to inducing toxic effects in animal organisms, CO has functional properties. It has been indicated as a potent inhibitor of cell apoptosis (Katori et al., 2002; Liu et al., 2003), which, by activating the cGMP signaling pathway, alleviates vascular disorders, including lesions caused by arterial damage (Liu et al., 2003). In addition to preventing programmed cell death, CO can regulate inflammation (Otterbein, 2002). In mice, this immunoregulatory role of CO was confirmed during the analysis of sensitized to ovalbumin (Chapman et al., 2001), while in miniature swine in ischemia-reperfusion injury lungs (Sahara et al., 2010). By modulating cytokine production and cell proliferation, CO creates protection against stressors and increases organ transplants' effectiveness in rodents (Nakao et al., 2006). Additionally, it was found that endogenous CO influences contractile responses by regulating vascular tone (Wang et al., 1997). However, the above effects induced by CO require an appropriate and significantly reduced concentration of this compound.

CO Impact on Humans

CO is known to be toxic because of its ability to interfere with oxygen delivery at high concentrations. CO is inhaled from the lungs into the bloodstream. Since the affinity of CO for hemoglobin (Hb) is 230- to 270-times greater than that of oxygen, COHb is formed in erythrocytes (**Eq. 1**):

$$\mathrm{CO} + \mathrm{Hb} \rightarrow \mathrm{COHb} \qquad (1)$$

The formation of COHb in the blood depends on various factors, including the concentration of inspired CO, duration of exposure, pulmonary ventilation, exercise, and health status (Kinoshita et al., 2020). Tissue hypoxia is the main toxic effect of acute CO poisoning due to the formation of COHb. It causes decreases the oxygen transport capacity, resulting in insufficient oxygenation at the tissue level. Typical symptoms of CO poisoning are headache, dizziness, weakness, vomiting, chest pain, and confusion was often mistaken for flu. Prolonged exposure causes brain damage and death (Adach et al., 2020) (**Table 1**).

In urban areas, high concentrations of inhaled CO can have a negative impact on human health. CO is often formed due to the incomplete combustion of carbon-containing compounds, primarily in internal combustion engines. Studies by Mandal et al., 2011 show that between 6 and 20% of homes in London have CO levels above World Health Organization (WHO) upper limits (**Table 2**) (Mandal et al., 2011). The worldwide incidence of CO poisoning has remained stable during the last 25 years (Kinoshita et al., 2020). Moreover, results from China show that a 1-mg m^{-3} increase of CO concentrations was associated with ~2–3% increments in daily years of life lost (YLL) from non-accidental causes, cardiovascular diseases, respiratory diseases, coronary heart disease, stroke, and chronic obstructive pulmonary disease, respectively, (Wang et al., 2021). Associations were more robust in the elderly (≥65 years), females, populations with low education attainment, and those living in southern regions. The role of expired-air CO as an independent marker of 16-y all-cause, cardiovascular, and cancer mortality in a substantial sample of apparently healthy subjects after adjustment for smoking was also evaluated (Bérard et al., 2015). Results provided a better understanding of the potential CO role on mortality and developing new prognostic and therapeutic tools for prevention.

Additionally, the newest studies show a strong correlation between the concentration of air pollutants such as CO and the number of SARS-CoV-2 cases. A report by (Meo et al., 2021) shows that the PM2.5 concentration increased by 221%, O_3 by 20%, and CO concentration increased by 151% after the California wildfire. This was compared with the number of cases, and deaths due to COVID-19 increased by 56.9 and 148.2%. An increase in ambient concentrations of toxic pollutants, which were temporally associated with an increase in the incidence and mortality of COVID-19, could also depend on other reasons, including temperature, humidity, changes in societal patterns of social distancing, and mass gatherings or adherence to wearing masks. Nevertheless, researchers obtain similar results from London (Ayoub Meo et al., 2021). A one AQI unit in the increase in CO level significantly increased the number of cases and deaths by 21.3 and 21.8%, respectively. Air pollutants, such as PM2.5, CO, and O_3, are positively associated with increased SARS-CoV-2 cases and daily deaths in London, United Kingdom, but the CO influences the most on new cases.

Despite the toxic effects on the nervous system and the cardiovascular system, CO also plays an essential role in the proper functioning of the human body, where it is also produced

TABLE 2 | Indoor carbon monoxide guidelines World Health Organization (WHO) (World Health Organization, 2010).

Averaging time	Concentration (mg·m^{-3})	Comments
15 min	100	Excursions to this level should not occur more than once per day Light exercise
1 h	35	Excursions to this level should not occur more than once per day Light exercise
8 h	10	Arithmetic mean concentration Light to moderate exercise
24 h	7	Arithmetic mean concentration Awake and alert but not exercising

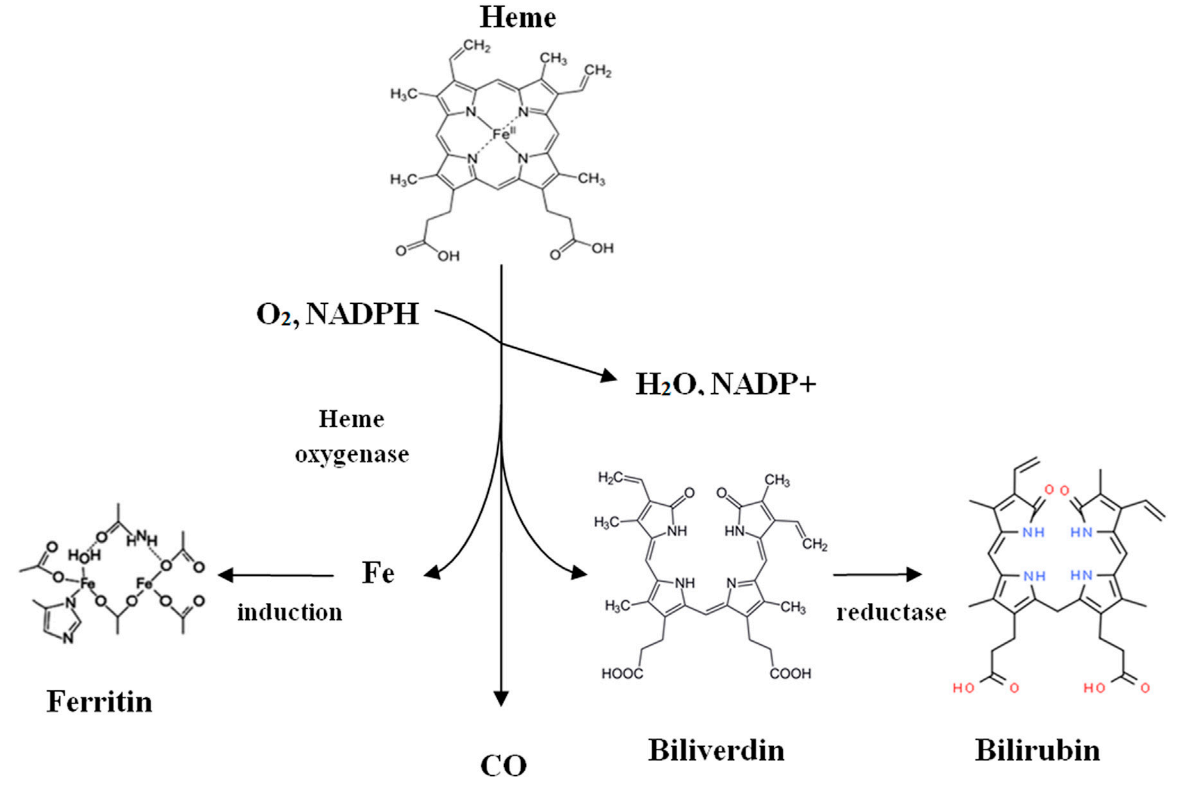

FIGURE 4 | Heme catabolism (Adach et al., 2020).

endogenously. Like plants and animals, the primary source of endogenous CO in the human body is heme degradation, catalyzed by HO (Olas, 2014). This enzyme breaks up porphyrin in the presence of NADP and molecular oxygen, resulting in the formation of various so-called primary degradation products (HDP): CO, ferrous cations (Fe^{2+}), and biliverdin (**Figure 4**). The next stage in decay is catalyzed by biliverdin reductase, which begins the reaction of biliverdin to the 2nd-degree metabolite bilirubin in the presence of NADPH and H+. This bilirubin is then secreted into the bile and excreted in the urine (Adach et al., 2020).

Recent findings have indicated that heme oxygenases and generation of CO serve as a critical mechanism to maintain the integrity of the physiological function of organs and supported the development of a new paradigm that CO, at low concentrations, functions as a signaling molecule in the body and exerts significant cytoprotection (Adach et al., 2020). Gaseous modulators, such as CO, NO, and H_2S, are important physiological mediators in the body. CO has antiapoptotic, signaling, and anti-inflammatory effects; hence, pharmacological agents that can imitate its action may yield therapeutic benefits. Pharmacists and biochemists have extensively studied such practical applications for many years (Kramkowski et al., 2012). Significant amounts of preclinical data indicate that exogenously provided CO can ameliorate I/R injury associated with organ transplantation. A study examining the safety and tolerability of inhaled CO in kidney transplant patients was reported by (Ozaki et al., 2012). However, the basic problem with administering CO is that it exists in gaseous form at room temperature. Although inhalation would commonly result in toxic effects, devices have been invented that strictly control the concentration of inhaled CO and automate the level of its release. The need to avoid the toxic effects of CO administration is not the only complicating aspect of inhalation therapy (Adach et al., 2020).

CO has also held great promise in cancer and other treatments due to its multifaceted regulation of cellular function and the tumor microenvironment. These days, it is growing at a somewhat accelerated pace, including the fast progress in a wide range of intelligent CO donors and CO delivery-related nanoplatforms (Zhou et al., 2020).

BIOREFINERY APPLICATION

The natural processes of CO transformation taking place with the participation of microorganisms in media (e.g., soil or water) inspired researchers to try to restore them under controlled conditions. In this way, biologically-mediated processes compete with inorganic ones. For example, the growing demand for H_2 in the 20th and 21st centuries began to be increasingly satisfied by the water-gas shift reaction (WGS) (Reddy and Smirniotis, 2015). This exothermic reaction, taking place according to **Equation 2**, is carried out at high temperatures and a pressure of 1.0–1.6 MPa (Cui et al., 2019):

$$CO + H_2O \rightarrow H_2 + CO_2 \tag{2}$$

Gradually, however, the possibility and advantages of using anaerobic bacteria enabling CO metabolization began to be noticed, indicating the economic and ecological effectiveness of the biologically-mediated WGS (BMWGS). Studies have shown that CO is oxidized to CO_2 by a broad range of aerobic and anaerobic microorganisms from various physiological and taxonomic groups, treating CO as an energy or carbon source necessary for growth (**Table 3**). The majority of those microorganisms use The Wood-Ljungdahl (acetyl-CoA) pathway both in the metabolism and production of CO. The Wood–Ljungdahl pathway is the central metabolism for acetogenic growth, mainly in different types of fermentation by mesophilic and thermophilic bacteria and thermophilic Archaea (Diender et al., 2015).

Thus, the use of bacteria in the BMWGS reaction fits in with the goals of the circular economy by using biomass and bio-waste as raw materials for the production of fuels and organic compounds (Henstra et al., 2007). These raw materials are also available in developing countries where the bioeconomy can be advantageous. Process conditions such as ambient temperature and pressure also influence the biological competitiveness of a method (Alfano and Cavazza, 2018). The advantage of BMWGS is the natural regeneration of microorganisms, which, compared to inorganic catalysts, can quickly replicate their cells to avoid poisoning (Henstra et al., 2007). Notably, from the point of view of industrial use of WGS, this reaction occurs in the dark so that it can be carried out in closed reactors. Thus, for this reason, process costs are minimized, including the elimination of the need for photo-bioreactors (Amos, 2004).

Another beneficial method of CO use with the participation of microorganisms is the Fischer-Tropsch (FT) reaction, taking place at a temperature of 150–300°C and using syngas as a substrate converted catalytically (Maitlis and Klerk, 2013; Selvatico et al., 2016). The primary purpose of FT is to make liquid hydrocarbons by converting CO and H_2 mixture (Alfano and Cavazza, 2018). In this reaction, both waste and biomass can be carbon sources (Maitlis and Klerk, 2013), and depending on the material used, the required H_2/CO ratio changes (Selvatico et al., 2016). Initially, this reaction, especially in the commercial sector from 1936, used mainly conventional non-renewable substrates such as coal or natural gas to ensure economic viability (Köpke and Simpson, 2020). An example of a mass-scale operation is The Shell Pearl Gas-to-Liquids installation located in Qatar, which since 2012 uses a natural gas well to produce up to 140,000 barrels per day (Shell, 2022). However, due to environmental reasons, based on the concern to reduce carbon emissions, the spectrum of materials used in the FT process has been expanded to various groups of waste, including agricultural, organic industrial, or municipal solid waste; the use of the latter is implemented at the Fulcrum Bioenergy plant, whose production capacity is <1,000 barrels per day (Fackler et al., 2021).

One of the newest technologies aimed at producing sustainable fuels and chemicals using the abundant above-ground carbon feedstocks is gas fermentation. The metabolism of chemolithoautotrophic microorganisms enables the utilization

TABLE 3 | Microorganisms involved in different CO production pathways.

Microorganism	Pathway/cycle	Reference
Clostridium thermoaceticium	The Wood-Ljungdahl (acetyl-CoA)	Schlegel, (2004)
Clostridium formicoaceticum		Drennan et al. (2001)
Rhodospirillum rubrum		Drake and Daniel (2004); Pierce et al. (2008)
Moorella thermoacetica		Wu et al. (2005)
Carboxydothermus hydrogenoformans		
Klebsiela pneumoniae	Conversion of S-methylthioadenosine to methionine	Dai et al. (1999)
Morganella morganii	Aromatic amino acid metabolism by bacteria	Hino and Tauchi, (1987)
Clostridium aceticum	Homoacetate fermentation	Schlegel, (2004)
Clostridium thermoaceticum		
Clostridium formicoaceticum		
Moorella thermoacetica		

of carbon oxides, including CO (Fast and Papoutsakis, 2012; Claassens et al., 2019). Thanks to this, gas fermentation offers a wide range of potential substrates, including 1) industrial waste gas, e.g., from steel and ferroalloy production, refinery processes, 2) syngas formed from organic waste, biomass residues, and municipal solid waste, as well as 3) CO_2 from various processing plants, processes of ethanol production from corn biomass or direct air capture (Köpke and Simpson, 2020). Such a wide variety of input materials, however, makes them compositionally variable, and additionally, the syngas used in the process is characterized by a high level of impurities. They include, among others, heavy metals, nitric oxides, NH_3, aromatic compounds, and sulfur compounds (Infantes et al., 2020). While FT processes encounter technical and hence economic problems resulting from the need to provide a purified feedstock to produce high-purity gases, gas fermentation shows high process tolerance to contaminants (Köpke and Simpson, 2020). As in the case of the BMWGS reaction mentioned above, it results from the ability of the microbial catalyst to self-replicate and the process tolerance is based on the binding of pollutants with microorganisms, which are washed out of the reactor after being killed (Köpke and Simpson, 2020). This is facilitated by the continuous process lasting several weeks or months (Fackler et al., 2021). This situation does not lead to the accumulation of unfavorable substances in the process, unlike in traditional chemical thermocatalytic processes. This has its economic consequences, making the fermentation process possible to reduce capital expenditure by keeping the high selectivity of the products despite the variability of the substrates, e.g., by lowering the required level of raw materials (Clomburg et al., 2017). Moreover, this high selectivity increases the conversion efficiency due to the formation of fewer by-products than is the case with catalyzed chemical processes (Fackler et al., 2021). In addition, the features of the process enable the creation of modern facilities, which, unlike the previously adopted classic industrial model, are able to flexibly operate with various groups of microorganisms using one infrastructure. Thus, depending on the type of microbial strain, the same gas composition can lead to a variety of products (Köpke and Simpson, 2020).

Learning about acetogenesis in 1932, willingness to find the added value of fossil sources, and to ensure energy security, were the driving force behind scientific research on syngas fermentation as early as the 1980s. After groundbreaking events, such as the demonstration of the use of gas fermentation to produce ethanol in 1989 and the isolation of the first acetogens producing various compounds in 1990, gas fermentation was considered ready to be attempted on a larger scale (Köpke and Simpson, 2020; Fackler et al., 2021). The top three organic solvents from syngas identified based on their octane values were methanol, ethanol, and iso-propanol (Fackler et al., 2021). The efforts and knowledge gained from the laboratory studies were then transferred to the Bioengineering Resources, Inc., pilot plant in 2003 (which obtained rights to commercialize the technology in 2008), Coskata company (established in 2006, since 2015 as Synata Bio), and INEOS New Planet BioEnergy commercial venture in 2011 (closed in 2016 due to problems related to with a high content of hydrogen cyanide in syngas). Importantly, in 2005, research on *C. autoethanogenum* at LanzaTech in New Zealand led to the up-scaling of the technology using a 500-L pilot fermenter (Liew et al., 2016) and, consequently, to the creation of a commercial installation in China in 2018, occupying ethanol production with a capacity of 16 million gallons per year, jet fuel and plastic and nylon precursors (Beijing Shougang LanzaTech New Energy Science & Technology Co., Ltd.) (Fackler et al., 2021). The aforementioned growing interest in using municipal solid waste and agricultural waste in the process is also visible. The first of these groups was processed into ethanol by the Japanese company Sekisui Chemical in cooperation with LanzaTech in 2017, which led to the establishment of SEKISUI Bio-Refinery CO., Ltd., a company that verifies the developed technology, 3 years later. One of the above-mentioned shareholders, LanzaTech, also started a joint operation with Indian associates in the same year to implement the process on agricultural substrates (Fackler et al., 2021). Another alternative and innovative course are the *in vitro* utilization of enzymes present in the BMWGS reaction. The research undertaken concerns hydrogenases; due to the difficulties in understanding the biogenesis of CODH, this enzyme is not yet considered ready to be used on an industrial scale (Alfano and Cavazza, 2018).

However, the industrial applications of CO and CO-transforming bacteria are still being explored and described, and so far, several problems related to these processes have been identified.

One of the limitations is the toxicity of CO, which, due to its high affinity to metalloenzymes, may inhibit the growth and catabolic activity of bacteria (Alfano and Cavazza, 2018). The concentration of this gas in the liquid phase greater than 0.15 m mol L^{-1} decreases the reaction rate, which may result in the complete closure of BMWGS (Amos, 2004). Therefore, researchers point to the need for an in-depth understanding of syngas metabolism, which should allow the development of microorganisms resistant to the toxic effects of CO (Ismail et al., 2008). On the other hand, CO availability to the bacteria could be a problem since their activity potential depends on the CO content. It is necessary to optimize the distribution of microorganisms, the CO concentration in the substrate, and the gas-to-liquid mass transfer (Alfano and Cavazza, 2018). The latter is one of the most problematic aspects affecting the operation of the BMWGS on an industrial scale in large reactors. Due to the low solubility of CO, this reaction is characterized by slow, diffusion-limited mass transfer from the bulk gas into the pores of the catalyst; but the rate of mass transfer is much higher than the rate of mass transfer from a gas into a liquid. In effect, for most reactor configurations, microorganisms have to "wait" for a dose of CO in the solution due to the slow mass transfer rate, which significantly reduces the rate of BMWGS (Amos, 2004). Another potential threat is also CO_2, the presence of which at higher process pressure leads to the formation of, among others, carbonic acid. In general, biological WGS is highly active when the pH of the process is in the range of 6.8–8. When it is less than 6.5, the microorganisms are lysed;

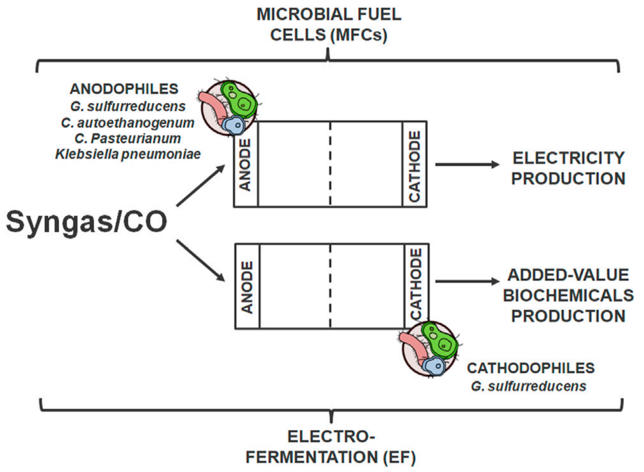

FIGURE 5 | Bioelectrochemical syngas conversion in BESs using electrochemically active bacteria (EAB) applied at the anode and cathode to produce bioenergy and biochemicals, respectively.

similar inhibition occurs when pH > 8 (Amos, 2004). Higher molecular weight contaminants may also cause concern when the BMWGS reaction is used in syngas conditioning, which may negatively affect microbial cells (Amos, 2004). An important aspect indicated by the researchers is that BMWGS kinetics can only be determined experimentally (Amos, 2004).

Too high a concentration of CO in the process is also a problem in the case of syngas fermentation, affecting its efficiency. This is based on the inhibitory effect of this gas on the activity of the key enzyme, hydrogenase, and hence the H_2 utilization rate (Devarapalli and Atiyeh, 2015). Therefore, in industrial applications, it would be necessary to use fast-response sensors, which by measuring CO and H_2 concentrations, would be able to accurately determine them online, enabling high yields of ethanol and stability of the process (Dang et al., 2021). However, currently, dedicated sensors are not available on the market; solutions such as membrane-coated electrochemical sensors in the power industry are not suitable for the CO and H_2 concentration measurements required in the process due to the slow reaction time and labor-intensive operation (Dang et al., 2021). Additionally, the challenge for this type of device is the ability to measure both compounds simultaneously; the cross selectivity of CO and H_2 is currently not developed, which does not allow for the precise differentiation of these gases by one sensor.

As mentioned earlier, it is necessary to consider the appropriate H_2/CO ratio in the FT reaction. The most popular substrate for the production of syngas used is wood and straw, and the syngas produced in this way is characterized by a too low substrates ratio for industrial purposes (Sansaniwal et al., 2017). Therefore, the currently preferred direction is to use the BMWGS reaction and control it to obtain a specific and desired H_2/CO ratio with the help of microorganisms (Bukur et al., 2016).

What was mentioned before, syngas fermentation has an advantage over FT processes due to, i.a. higher biocatalysts specificity (Henstra et al., 2007; Wainaina et al., 2018). However, the traditional approach to fermentation is associated with a few significant problems, such as redox imbalance and limited growth and effectiveness of CO-converting microorganisms due to the limited reducing power of the Wood-Ljungdahl pathway (Barbosa et al., 2021). For this reason, in 2009, research on bioelectrochemical syngas conversion (bioelectrochemical systems, BESs) in dedicated systems began (Kim and Chang, 2009). BESs technology, based on integrated biological conversion and production of electricity, uses the activity of electrochemically active bacteria (EAB) capable of the use of insoluble electron acceptors or donors-anodophiles and cathodophiles-biocatalysts that are applied at the anode and cathode, respectively, (**Figure 5**) (Barbosa et al., 2021). In these processes, the chemical energy stored in compounds is transformed into bioenergy; both for electricity production thanks to oxidation (anode) and for production of added-value biochemicals thanks to reduction reactions [cathode, (Logan et al., 2008; Lovley, 2011; Choi and Sang, 2016)].

The first solution, electricity production from syngas/CO using microbial fuel cells (MFCs), is the most popular and tested BESs method (Mehta et al., 2010; Hussain et al., 2011b; Neburchilov et al., 2011; Hussain et al., 2012; Kumar et al., 2017; Santoro et al., 2017). Researchers have proposed three theories about the mechanism of this process. The first is the direct electricity generation by transferring electrons by Fe (III)-reducing carboxydotrophic bacteria to the anode; this process occurs in the one-stage chamber (Mehta et al., 2010). Another assumes that this process is indirectly based on the change of CO to compounds such as H_2 or acetate (CO fermentation products, resulting from acetogenic carboxydotrophic microorganisms, such as *Alkalibaculum bacchi, C. ljungdahlii, Acetobacterium spp.* or *C. carboxidivorans*). Anodophilic bacteria then convert acetate as a substrate for electricity production in MFCs (e.g., *Geobacter sulfurreducens*) (Kim and Chang, 2009; Hussain et al., 2011a; Hussain et al., 2014). The third theory allows for the possibility of producing energy from the resulting H_2 or converting it and CO_2 into acetate (Mehta et al., 2010). Additionally, the influence of temperature on CO in MFCs conversion was analyzed. Greater process efficiency and higher power density were observed in thermophilic conditions (Hussain et al., 2012).

Researchers are increasingly interested in biochemicals production (e.g., CH_4, acetate, or H_2) in the electrochemically-assisted fermentation process (electro-fermentation, EF) (Rabaey and Rozendal, 2010). This technology is based on the introduction into electric circuits an electrode to provide additional energy and induce the transformation of the substrate into the expected product. Its advantage over conventional fermentation is based on increased production efficiency while reducing costs and increasing product purity (Engel et al., 2019). This is possible thanks to modifying redox balances and fine-tuning metabolic pathways with the need to use special additives (Barbosa et al., 2021).

Analyzes of the use of syngas/CO for the production of biochemicals were initiated in 2010 (Köpke et al., 2010; Nevin et al., 2011), and the process itself is considered future-oriented due to carbon capture and storage in the form of valuable products (Barbosa et al., 2021). Related reactions may become a support for the 4th generation biofuels (Barbosa et al., 2021). Several compounds have been obtained in EF processes using syngas/CO, including, e.g., 1,3-propanediol [*G. sulfurreducens*, (Moscoviz et al., 2018)], 3-hydroxypropionic acid (3-HP) [*Klebsiella pneumoniae*, (Kim et al., 2017)], lactate and 2,3-butanediol [*Clostridium autoethanogenum*, (Kracke et al., 2016)], butanol [*C. pasteurianum*, (Choi et al., 2014)]. Promisingly, the amounts of these compounds exceeded those obtained with conventional fermentation, for example:

- Production of 1,3-propanediol was improved by the yield of 10% [*G. sulfurreducens*, (Moscoviz et al., 2018)],
- 1.7-fold enhancement of 3-hydroxypropionic acid (3-HP) production was observed by (Kim et al., 2017),
- Production of lactate and 2,3-butanediol increased by 35-fold and 3-fold, respectively, in electrically enhanced fermentation of fructose (Kracke et al., 2016),
- Improvement of butanol production from glucose and 1,3-propanediol production from glycerol by 20 and 21%, respectively, was noted by (Choi et al., 2014).

A wide spectrum of waste that can be used in biorefinery processes, including municipal, industrial, agricultural waste, etc., is a valuable alternative to the use of fossil resources for the production of fuels, chemicals, or electricity. Above mentioned results indicate the need to develop described technologies and to scale up for industrial applications in the near future. Introducing them to mainline production can bring benefits on the economic and environmental side, possibly through credit and tax incentives. However, these solutions have limitations that affect the economic aspects of implementing it at a commonly used, commercial level. Streams of this waste are abundant, but their characteristic feature is significant dispersion. In order for them to compete with traditional feedstocks in petrochemical processes, they must be delivered to the plant in an integrated manner in quantities that exceed the current capacity (hundreds or thousands of tons per day compared to hundreds of thousands of tons for fossil raw materials). Increased efficiency and competencies of biorefineries largely depend on the availability of these resources, i.e., on a consistent logistic system for collecting, storing, and transporting waste to processing sites. It is also important for each industrial process to be stable and handle to produce large amounts of a uniform product; process specifications also need to consider the variability in raw material, which is extremely important when the substrate is waste (high possibility of inhomogeneity). What is also important, is that biorefinery technology should provide easy and inexpensive storage and transport of gases like H_2; new technology like proton-exchange-membrane (PEM) is still not cost-competitive to the alternative steam-methane-reformation (SMR) process that uses natural gas (Takors et al., 2018). Additionally, the competitiveness of biorefinery plants could be based on the economic benefits of recovering CO_2 as a by-product of the processes carried out. For this purpose, it is necessary to analyze the possibilities of implementing various methods, depending on the composition of the substrates, e.g., waste or synthesis gas.

An interesting direction is also the integration of aerobic treatment of organic waste with biorefinery processes. The expanding range of possible routes to use biological CO as a valuable resource in reference to recent reports on the production of this gas from the composting process (Stegenta et al., 2019a; Stegenta et al., 2019b) raises a new, important question: is it possible to control and enhance the biological CO production from aerobic processes for the biorefinery industry and/or H2 production? Observations made for green waste (Kurola et al., 2010), their mixture with manure (Hellebrand and Kalk, 2001), organic waste (Haarstad et al., 2006), the municipal waste ((Phillip et al., 2011; Stegenta et al., 2018) and studies on the dual nature of CO production biotic and abiotic, (Stegenta-Dąbrowska et al., 2019) indicate that the composted mass may contain bacteria responsible for metabolizing CO, capable of producing the enzyme carbon monoxide dehydrogenase (CODH). If this hypothesis was confirmed, it would significantly increase the potential sources of CO to be used in the further development of biorefineries. Therefore, laboratory-scale studies are needed to understand in detail the process and biological factors influencing CO production during composting.

Additionally, due to the described earlier problems associated with CO high affinity to metalloenzymes, there is a concentration barrier for this compound in the liquid phase that limits the growth and activity of microorganisms. Therefore, it is essential to develop strain engineering using tools developed on the organism model, which will allow not only to prepare the bacteria to work in the harsh anaerobic environment of the process with gaseous substrates but also to obtain new products from the same substrates. The support may be predictive models based on omics data and the use of bioinformatics tools that enable learning about the rules and variables affecting metabolic processes, as well as their control and optimization. Moreover, one of the biggest problems with the efficiency of biorefinery processes, mass transfer limitations, is being gradually addressed by the latest technological advances such as the use of high mass-transfer bioreactors and anaerobic biofoundries. However, further efforts are needed to develop technologies on a larger scale, which is linked to the need to finance pilot operations.

CONCLUSION

As shown in this review article, such an inconspicuous molecule as CO plays an essential role in each of the elements of the environment, directly and indirectly influencing the processes taking place. Moreover, its absence would disrupt natural changes in nature and affect plants and the organisms' functioning—both animals and humans.

Ignored in many aspects of pro-environmental and economic activities, CO is becoming noticed by an increasing number of researchers, activists, and industry representatives, gaining more and more importance, e.g., in medicine, veterinary medicine, and the chemical industry and energy industries. The initial neglect of CO as a harmful gas over many years of research conducted on plants, animals, and humans has turned into an approach consisting of minimizing the effects of CO and the pathways of its effective use and achievement of intended goals. Similarly, the first observations and research conducted on CO fate in the atmosphere, soils, and water bodies became the basis for the currently extended analyzes of the ways of metabolizing CO in a biological way using microorganisms, which are crucial for the development of the biorefinery industry. The first laboratory analyses, as well as experiments carried out in pilot plants, subsequently led to the transfer of CO as a substrate to commercial plants, which proves that despite its toxic nature, this compound is a valuable material for biological processes.

Because of that, CO application in biorefinery can be a part of the circular economy. However, the technically and economically successful implementation of such processes has been revealed to be very challenging and requires continuous progress. Like every industrial process, using CO in biorefinery should be economically efficient, which is connected with the possibility of stable production of large amounts of a standardized product. Additionally, process specifications need to consider the variability in raw waste material. Therefore, further studies on the processes' kinetics in fully controlled and well-mixed laboratory-scale stirred-tank bioreactors are needed to provide the data allowing for the modeling of the biorefinery concept on an industrial scale.

AUTHOR CONTRIBUTIONS

KS: Conceptualization, Data curation, Funding acquisition, Resources, Writing–original draft. SS-D: Conceptualization, Data curation, Resources, Writing–original draft. GL: Conceptualization, Writing–review and editing. JK: Conceptualization, Supervision, Writing–review and editing. AB: Conceptualization, Data curation, Funding acquisition, Resources, Supervision, Writing–review and editing.

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Appendix No. 3

Karolina Sobieraj Wrocław, 22.06.2023
(Name and surname) (Place and date)

Department of Applied Bioeconomy
Wrocław University of Environmental and Life Sciences
Wrocław, Poland
(Affiliation)

STATEMENT

I hereby declare that in the publication:

Karolina Sobieraj, Sylwia Stegenta-Dąbrowska, Gang Luo, Jacek A. Koziel, Andrzej Białowiec, 2022. *Carbon Monoxide Fate in the Environment as an Inspiration For Biorefinery Industry: A Review*, Frontiers in Environmental Science, 10, 1-24, doi: 10.3389/fenvs.2022.822463

my contribution included:
developing the concept and design of the study. I prepared the entire text of the article as well as Figures 1-3 and 5. During the review process, I made corrections to the text of the manuscript and prepared responses to the reviewers. I obtained funding for the publication of the manuscript.

Signed by /
Podpisano przez:

Karolina Natalia
Sobieraj

Date / Data:
2023-06-22 21:04

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(Date and signature)

Appendix No. 3

Sylwia Stegenta-Dąbrowska
(Name and surname)

Wrocław, 21.06.2023
(Place and date)

Department of Applied Bioeconomy
Wrocław University of Environmental and Life Sciences
Wrocław, Poland
(Affiliation)

STATEMENT

I hereby declare that in the publication:

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my contribution included:
assistance in preparing the text of the manuscript included in the chapter *CO Impact on Humans*, including preparing Figure 4 with Tables 1 and 2. During the review process of the article, I assisted in making corrections to the text of the manuscript and in preparing responses to the reviewers.

21.06.2023, Sylwia Stegenta-Dąbrowska
(Date and signature)

Appendix No. 3

Luo Gang
(Name and surname)

Shanghai, China,...2023/5/31
(Place and date)

Shanghai Key Laboratory of Atmospheric
Particle Pollution and Prevention (LAP3),
Department of Environmental Science and Engineering,
Fudan University, Shanghai, China
(Affiliation)

STATEMENT

I hereby declare that in the publication:

Karolina Sobieraj, Sylwia Stegenta-Dąbrowska, Gang Luo, Jacek A. Koziel, Andrzej Białowiec, 2022. *Carbon Monoxide Fate in the Environment as an Inspiration For Biorefinery Industry: A Review*, Frontiers in Environmental Science, 10, 1-24, doi: 10.3389/fenvs.2022.822463

my contribution included: revision of the final version of the manuscript before submission and participation in the process of reviewing the article and related modifications to the manuscript.

Gang Luo 2023/5/31
(Date and signature)

USDA
United States Department of Agriculture

Research, Education, and Economics
Agricultural Research Service

Appendix No. 3

Jacek A. Koziel
(Name and surname)

Bushland, Texas, June 22, 2023.
(Place and date)

U.S. Department of Agriculture, Agricultural Research Service
Conservation and Production Research Laboratory
Bushland, Texas
(Affiliation)

STATEMENT

I hereby declare that in the publication:

Karolina Sobieraj, Sylwia Stegenta-Dąbrowska, Gang Luo, Jacek A. Koziel, Andrzej Białowiec, 2022. *Carbon Monoxide Fate in the Environment as an Inspiration For Biorefinery Industry: A Review*, Frontiers in Environmental Science, 10, 1-24, doi: 10.3389/fenvs.2022.822463

my contribution included:

revision of the manuscript, in particular preparation of the text of the manuscript included in the introduction part and the abstract. While working on the manuscript, I participated in English language editing. I participated in the review process and the related modifications to the manuscript.

JACEK KOZIEL Digitally signed by JACEK KOZIEL
Date: 2023.06.22 17:47:53 -05'00'

……………………………..
(Date and signature)

Jacek Koziel, Ph.D., Research Leader
Livestock Nutrient Management Research Unit
USDA-ARS Conservation & Production Research Lab
Bushland, TX 79102
Phone: 806-356-5744
Email: jacek.koziel@usda.gov
Google Scholar – ORCID – Web of Science

Appendix No. 3

Andrzej Białowiec Wrocław, 21.06.2023
(Name and surname) (Place and date)

Department of Applied Bioeconomy
Wrocław University of Environmental and Life Sciences
Wrocław, Poland
(Affiliation)

STATEMENT

I hereby declare that in the publication:

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my contribution included:
developing the concept and design of the study. I participated in the revision of the manuscript. I participated in the review process and the related modifications to the manuscript. I was also the corresponding author.

Signed by /
Podpisano przez:

Andrzej Białowiec

Date / Data:
2023-06-21
08:19

……………………………….

(Date and signature)

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EDITED BY
Abdelfatah Abomohra,
University of Hamburg, Germany

REVIEWED BY
Nabin Aryal,
University of South-Eastern Norway (USN), Norway
Liwen Luo,
Hong Kong Baptist University, Hong Kong SAR, China
Ihana Aguiar Severo,
Federal University of Paraná, Brazil

*CORRESPONDENCE
Andrzej Białowiec,
✉ andrzej.bialowiec@upwr.edu.pl

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Biological treatment of biowaste as an innovative source of CO—The role of composting process

Karolina Sobieraj[1], Sylwia Stegenta-Dąbrowska[1], Gang Luo[2,3,4], Jacek A. Koziel[5,6] and Andrzej Białowiec[1,6]*

[1]Department of Applied Bioeconomy, Wrocław University of Environmental and Life Sciences, Wrocław, Poland, [2]Shanghai Key Laboratory of Atmospheric Particle Pollution and Prevention (LAP3), Department of Environmental Science and Engineering, Fudan University, Shanghai, China, [3]Shanghai Technical Service Platform for Pollution Control and Resource Utilization of Organic Wastes, Shanghai, China, [4]Shanghai Institute of Pollution Control and Ecological Security, Shanghai, China, [5]USDA-ARS Conservation and Production Research Laboratory, Bushland, TX, United States, [6]Department of Agricultural and Biosystems Engineering, Iowa State University, Ames, IA, United States

Carbon monoxide (CO) is an essential "building block" for producing everyday chemicals on industrial scale. Carbon monoxide can also be generated though a lesser-known and sometimes forgotten biorenewable pathways that could be explored to advance biobased production from large and more sustainable sources such as bio-waste treatment. Organic matter decomposition can generate carbon monoxide both under aerobic and anaerobic conditions. While anaerobic carbon monoxide generation is relatively well understood, the aerobic is not. Yet many industrial-scale bioprocesses involve both conditions. This review summarizes the necessary basic biochemistry knowledge needed for realization of initial steps towards biobased carbon monoxide production. We analyzed for the first time, the complex information about carbon monoxide production during aerobic, anaerobic bio-waste treatment and storage, carbon monoxide-metabolizing microorganisms, pathways, and enzymes with bibliometric analysis of trends. The future directions recognizing limitations of combined composting and carbon monoxide production have been discussed in greater detail.

KEYWORDS

carbon monoxide, anaerobic, aerobic, CODH, composting, bibliometric analysis

1 Introduction

The global consumption and demand for resource-intensive goods, energy and raw materials continues to grow, largely exceeding current renewable pathways. There is a need to search for innovative and more sustainable ways to address these pressing challenges. The circular economy is now considered not as an option, but a necessity. Sustainable resource management and recovery, including waste and biomass, scaling up biotechnological and microbiological processes to biorefineries, can improve cycling loops in bioeconomy-driven future.

Current advances in circularity and bioeconomy models require continuous ground-truthing and refinement on scales that are relevant to be impactful. The scale of environmental challenges requires well-informed decisions, which must nevertheless be based on basic and applied research. Prioritizing research can enable more sustainable technologies in different sectors of the economy.

A fresh look at commonly known materials, substrates, by-products can provide "waste," "bio-waste," or "pollutant" a new meaning. The substrate that deserves a refreshed focus is carbon monoxide (CO). Recognized as a primary air pollutant, CO is also purposefully generated through thermochemical reactions and appreciated for its numerous industrial

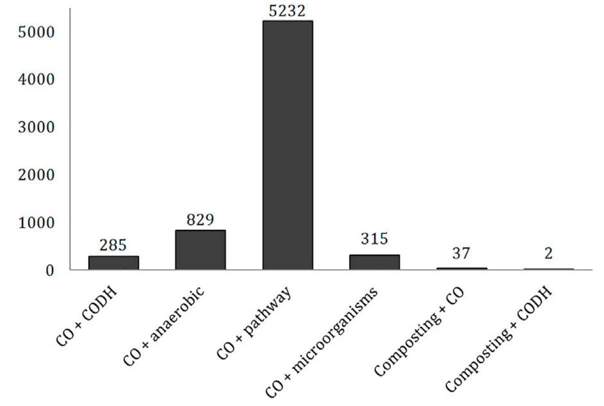

FIGURE 1
Number of publications in the Web of Science Core Collection for chosen combination of keywords.

applications, including in metallurgy, as a component of synthesis gas, and production of common chemicals such as ethanol, methanol, hydrocarbons and aromatic compounds (Perondi et al., 2017; Yang et al., 2017).

However, it is less-known that CO can be produced biologically, as a by-product of biological waste treatment processes (Stegenta et al., 2018; Stegenta et al., 2019a; Stegenta-Dąbrowska et al., 2019). Biological processes of organic matter (OM) decomposition, both under aerobic and anaerobic conditions can generate CO. While anaerobic CO generation is relatively well understood (Oelgeschläger and Rother, 2008; Andreides et al., 2022), the aerobic is not. Yet many industrial-scale bioprocesses involve both conditions and can be difficult to control.

It has been reported that CO is present during biowaste composting in aerobic piles and bioreactors, with concentration exceeding 1,000 ppm (Stegenta et al., 2019a; Stegenta-Dąbrowska et al., 2022). Thus, there appears to be sufficient amount of CO generated in a common bio-waste treatment process that could be further explored as a pathway for industrial scale biorenewable CO production. This is particularly interesting considering that its biological production is mainly associated with the presence of microorganisms that produce the enzyme CO dehydrogenase (CODH), responsible for both the production and metabolism of CO under anaerobic conditions (Abubackar et al., 2011; Jeong et al., 2015).

Considering that in the composted pile there are both aerobic and anaerobic areas, inhabited by microorganisms capable of functioning in both conditions, important research direction-type questions arise:

(i) Is the CO production during the aerobic biowaste composting processes based on the activity of the same microorganisms and the CODH enzyme produced by them as in the case of anaerobic processes?
(ii) Can the existing knowledge gained while analyzing CO generation under anaerobic conditions, be applied for composting that is both aerobic and anaerobic?
(iii) Can a "lowly" composting become a leading process for the biobased production of valuable CO?

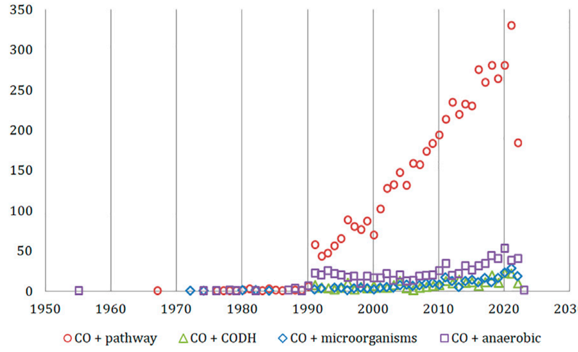

FIGURE 2
The timeline of research areas for "carbon monoxide" articles relevant to the scope of this review (Web of Science Core Collection).

This review summarizes the necessary basic biochemistry knowledge needed for realization of initial steps towards biobased CO production. The limited information about CO sources, mechanisms, microorganisms involved, and optimal conditions for its formation during bio-waste aerobic biostabilization, including composting, is summarized. We analyzed for the first time, the complex information about CO production during aerobic, anaerobic bio-waste treatment and storage, CO-metabolizing microorganisms, pathways, and enzymes with bibliometric analysis of trends. The future directions recognizing limitations of combined composting and CO production have been discussed in greater detail.

2 Methods

The Web of Science Core Collection was searched to find journal articles (without a specific date range). "Topic," which included title, abstract, author keywords, and Keywords Plus has been set as the search parameter. The bibliometric analysis was performed for the combination of keywords:

(i) "Carbon monoxide" + "anaerobic",
(ii) "Carbon monoxide" + "pathway",
(iii) "Carbon monoxide" + "CODH",
(iv) "Carbon monoxide" + "microorganisms",
(v) "Composting" + "carbon monoxide",
(vi) "Composting" + "CODH".

The data were transferred to Microsoft Excel 2007. Google Scholar was used to document the original and foundational research on this topic, which was relatively old and outside the time range of the Web of Science Core Collection (Haddaway et al., 2015). Inclusion on these older references was crucial to connect the key established facts present only in the earlier literature, bridge the gap in knowledge, and attempt to chart the future research direction.

3 Results

3.1 Bibliographic record on carbon monoxide

The highest number of records was found for "carbon monoxide" + "pathway" (5,232), following by "carbon monoxide" + "anaerobic"

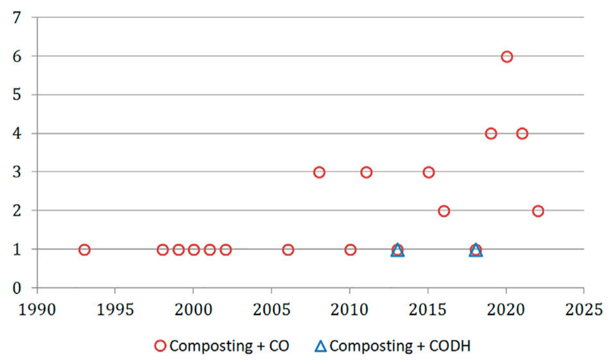

FIGURE 3
The timeline of published research for "composting" articles related to CO and CODH (Web of Science Core Collection).

(829; Figure 1). A much lower and similar number of scientific papers was found for the combination of "carbon monoxide" with "microorganisms" and "CODH" keywords (315 and 285, respectively). The main publishing form was research article (85%, 84%, 77%, 91%, respectively), followed by reviews (12%, 13%, 17%, 6%, respectively).

First reported studies were noted for "carbon monoxide" + "anaerobic" keywords (1955, Figure 2). The most frequently discussed topic was the CO metabolic pathways where the earliest works dated back to the 1960s and 1970s. This was followed by first publications in the field of microorganisms involved in the CO cycle (1972–1974). Two decades later, the role of the CODH enzyme started to emerge in reports from the 1991 initial studies. The key breakthroughs for each of the above-mentioned topics occurred in the 1990s and the 1st decade of the 20th century. This was evidenced by the increase of number of the articles published (by ~870% between 1990 and 1991 for "pathway" articles, 120% in case of "CODH" keywords in 1995–96 and 100% for "microorganisms" between 2010/2011). The highest variations in the published output from year-to-year were observed for the CODH enzyme, likely due to the relatively narrow focus of this field of study.

3.2 Bibliographic record on composting and CO

While a high number of records were found for articles focusing on the CO biochemistry, the subject of composting in combination with CO and the CODH enzyme was much less discussed by researchers (Figure 1). The CO production was described in 37 articles, while the production of CODH by microorganisms in the composted biomass was reported in only two.

The formation of CO during the aerobic waste treatment was first published in the 1990s; only a handful of studies were published until 2006, after which the number of reports on this topic increased to 3 research articles per year (Figure 3). The most popular combination of the words "composting" + "carbon monoxide" was achieved recently (up to 6 articles per year published between 2019–2022). On the other hand, after the publication of two articles on the CODH enzyme in the context of the composting process (dated 2013 and 2018), this topic was not raised again.

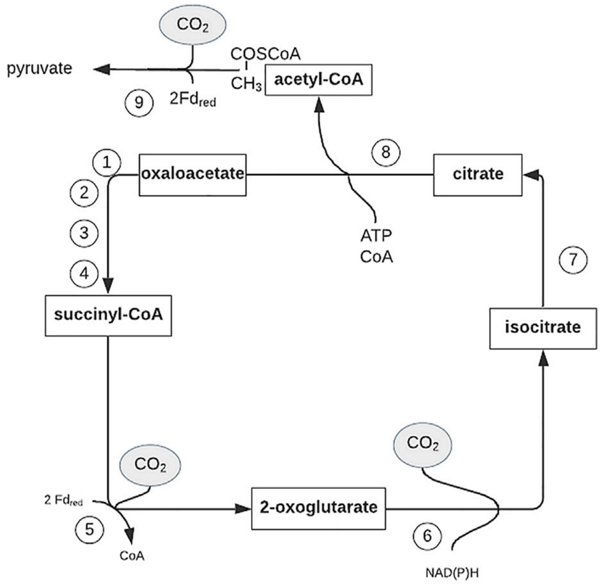

FIGURE 4
Outline of the reductive citric acid cycle for autotrophic CO_2 fixation. Figure redrawn after reference (Hügler et al., 2005). Bold arrows indicate the reactions catalyzed by key enzymes. Enzyme activities: 1, malate dehydrogenase (EC 1.1.1.37); 2, fumarate hydratase (fumarase) (EC 4.2.1.2); 3, fumarate reductase; 4, succinyl-CoA synthetase (EC 6.2.1.5); 5, 2-oxoglutarate:ferredoxinoxidoreductase (EC 1.2.7.3); 6, isocitrate dehydrogenase (EC 1.1.1.42); 7, aconitate hydratase (aconitase) (EC 4.2.1.3); 8, ATP citrate lyase (EC 2.3.3.8); and 9, pyruvate: ferredoxin oxidoreductase (EC 1.2.7.1); Fd red, reduced ferredoxin.

In general, it is important to recognize that this field of study and the scope of this review, have been accelerating rather slowly, and that large opportunities for connecting the facts and charting new research directions aiming at biobased production of CO still exist.

4 Discussion

4.1 Microorganisms involved in the CO metabolism

4.1.1 Aerobic bacteria

Aerobic bacteria utilizing CO for growth were isolated from garden soil and described in (Beijerinck and van Delden, 1903) for the first time in 1903. Then, bacteria which could grow in air enriched with CO (Lantzsch, 1922), and Hydrogenomonas carboxidovorans bacteria, capable of oxidizing CO and H_2 were found in 1953 (Kistner, 1953). CO-oxidizing bacteria were also isolated from urban soils during their enrichment with a mixture of 20% O_2 and 80% CO in 1973 (Nozhevnikova and Zavarzin, 1973). By the end of the 20th century, additional CO oxidants were discovered, called carboxydotroph, included Actinobacteria, Proteobacteria, and Firmicutes (Gadkari et al., 1990). The growth of these bacterias was observed with increased CO concentration (>10%) (Gadkari et al., 1990). Today, carboxydotrophs are viewed as a group of bacteria that use carbon and CO energy as their sole source when present in concentrations >1% (King, 2003). Although many of their groups are not closely related, they share the same metabolic profile, based on

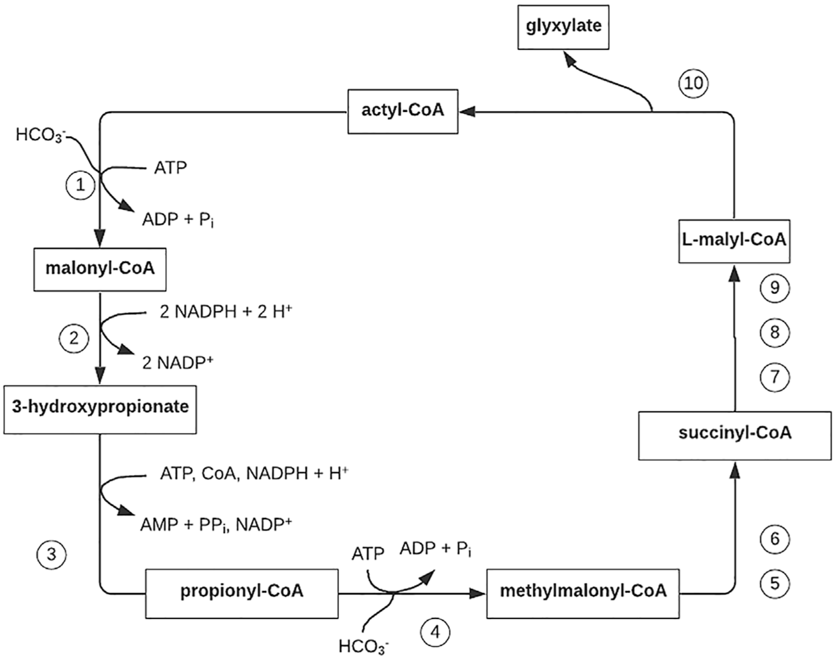

FIGURE 5
3-Hydroxypropionate cycle of autotrophic CO_2 fixation in the phototrophic green non-sulfur eubacterium *Chloroflexus aurantiacus.* Figure redrawn after reference (Alber et al., 2008). Step 1, acetyl-CoA carboxylase; step 2, malonyl-CoA reductase (bifunctional; step 3, propionyl-CoA synthase (trifunctional); step 4, propionyl-CoA carboxylase; step 5,methylmalonyl-CoA epimerase; step 6, methylmalonyl-CoA mutase; step 7, succinyl-CoA–L-malate CoA transferase; step 8, succinate dehydrogenase (electron acceptor unknown); step 9, fumarase; step 10, L-malyl-CoA/β-methylmalyl-CoA lyase (bifunctional).

the possibility of aerobic growth with CO levels as high as 90% (v/v) (Meyer et al., 1986). This process involves directing electrons to a CO-insensitive terminal oxidase with a high affinity for O_2 through a CO-insensitive branch of the respiratory chain (Cypionka and Meyer, 1983).

Subsequent discoveries proved that some of the CO oxidants could not function under such extreme conditions. Due to their growth only at low CO concentration and taking up gas as an additional source of energy for survival, they were referred to as carboxidovores [including *Mesorhizobium plurifarium* and *Bradyrhizobium* spp., CPP from the rhizosphere, *Stappia aggregata* from marine sediment, *Silicibacter pomeroyi* from seawater, *Burkholderia xenovorans* from soil or *Mycobacterium* spp. RIM from volcanic soil (Weber and King, 2007)]. However, they play an important role in the biogeochemistry of CO, representing a group of facultative lithotrophs and taking CO from many natural systems, such as sediments, plant roots, or oxic soils (Moran et al., 2004).

Continued analysis of various environments, such as compost, sewage, sewage sludge, or freshwater sediment, allowed, however, to find also more carboxydotrophs, the diversity of which turned out to be very rich [incl. *Oligotropha carboxidovorans*, *Pseudomonas thermocarboxydovorans*, *Pseudomonas carboxydohydrogena*, *Bacillus schlegelii* (Krüger and Meyer, 1984)]. Most carboxydotrophs are mesophilic, with few exceptions as *P. thermocarboxydovorans* (Lyons et al., 1984), *B. schlegelii* (Krüger and Meyer, 1984), and *Streptomyces thermoautotrophicus* (Gadkari et al., 1990). However, no extreme species such as acidophiles, psychrophiles, hyperthermophiles, and extreme halophiles were reported (King and Weber, 2007). Carboxydotrophs also include plant pathogens and symbiotes (Tiquia-Arashiro, 2014).

4.1.2 Anaerobic bacteria

The anaerobic carboxydotrophic bacteria that metabolize CO as the only carbon source include acetogens, methanogens, sulfate and elemental sulfur reducers, phototrophic bacteria, and hydrogenogens (Techtmann et al., 2009). Like aerobes, they are widespread in natural habitats, but their rich cultures' preferred locations are not fully understood (Nguyen et al., 2013).

Among the groups mentioned above, researchers are most interested in the obligatory anaerobes—acetogens. Their use can lead to obtaining useful substances, such as chemicals and fuels (Henstra et al., 2007). During syngas fermentation, acetogens produce, among others, acetate, butanol, and butyrate (Jeong et al., 2015). On the other hand, ethanol from CO can generate such homoacetogens as *Alkalibaculumbacchi, Butyribacterium methylotrophicum, Clostridium ljungdahlii, Clostridium carboxidivorans* P7^T, *Clostridium ragsdalei, C. autoethanogenum* and *Clostridium drakei* (Liu et al., 2012; Mohammadi et al., 2012). Members of acetogens have been isolated from such media as composts, wastewater, and the rhizosphere, volcanic soil, hydrotherms, water sediments, or coal heaps (King and Weber, 2007). The utilization of CO for acetogens is a way to provide energy, cellular material, but also CO_2 and acetate (Mörsdorf et al., 1992). For example, some acetogens may consume CO as an electron donor and produce H_2 when oxidizing this compound, such as *C. thermoaceticum* (Kerby and Zeikus, 1983). Other acetogens, including *Acetogeniumkivui*, cannot do so and only utilize CO in the material and acetate produced (Daniel et al., 1990). Acetogenic bacteria can grow at a high CO environment. The "record holders" are representatives of *Peptostreptococcus productus* (Ma et al., 1991),

TABLE 1 Carbon monoxide metabolism reactions (Diender et al., 2015).

Conditions	Metabolism	Reaction equation
Anaerobic	Hydrogenogenic	$CO + H_2O \rightarrow CO_2 + H_2$
	Methanogenic	$4CO + 2H_2O \rightarrow CH_4 + 3CO_2$
	Acetogenic	$4CO + 2H_2O \rightarrow CH3COO^- + H^+ + 2CO_2$
	Solventogenic (ethanol)	$6CO + 3H_2O \rightarrow C_2H_5OH + 4CO_2$
Anoxic	Sulfate	$4CO + SO_4^{2-} + H^+ \rightarrow 4CO_2 + HS^-$
Aerobic	Oxygen	$2CO + O_2 \rightarrow 2CO_2$

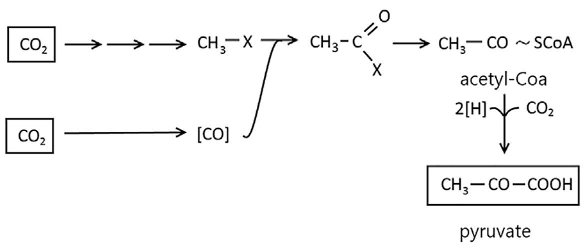

FIGURE 6
Autotrophic CO_2 fixation in reduction acetyl-CoA pathway. Figure redrawn after reference (Schlegel, 2004).

which can thrive at up to 90% CO (v/v), and they also show the fastest growth on CO (Mörsdorf et al., 1992).

Methanogenic bacteria are obligate anaerobes that produce CH_4 from CO_2, other carbon compounds, or acetate (Mörsdorf et al., 1992). This group's first representative to utilize CO for growth was discovered in 1977 (Daniels et al., 1977). Like acetogens, methanogens use CO as a source of energy, cellular material, and CO_2, but their main product is CH_4 instead of acetate. Methanogens are also more sensitive to CO (Mörsdorf et al., 1992). Representatives of this group include: *Methanothermobacter thermautotrophicus, Methanosarcina barkeri*, and *M. acetivorans* (Rother et al., 2004).

The use of CO as an energy source by converting it into CO_2 and H_2 is characteristic of desulfuricans (Sipma et al., 2006). They are obligate anaerobes capable of autotrophic growth (Fauque et al., 1991). Their ability to oxidize CO was observed in the 1950s by Yagi's research team (Yagi, 1959). The generated H_2 is later used to reduce sulfates (Rabus et al., 2013). Some of these bacteria, such as *Desulfotomaculum thermobenzoicum* and *Desulfotomaculum kuznetsovii*, are also produced by acetate (Parshina et al., 2005a). Because sulfate-reducing bacteria can generally only tolerate low CO concentrations [up to a few percent (Jansen et al., 2004)], it is believed that the H_2 production by CO oxidation (biologically-induced water gas-shift—BWGS reaction) is by detoxification (Oelgeschläger and Rother, 2008). Higher CO concentrations inhibit the growth of, among others, *Desulfotomaculum* species or *Desulfovibrio vulgaris* strain Madison (Lupton et al., 1984; Klemps et al., 1985). On the other hand, *Desulfotomaculum carboxydivorans* is capable of growth at 100% CO, which consequently draws attention to use this strain in BWGS reaction. Without sulfate, CO is converted into H_2 and CO_2, while in the presence of sulfate, some of the produced H_2 is used for sulfate reduction (Parshina et al., 2005b). Thermophilic bacteria have been discovered amongst desulfuricants, including *D. thermoacetoxidans* and *T. yellowstonii, D. kuznetsovii,* and *D. thermobenzoicum* subsp. *Thermosyntrophicum* (Parshina et al., 2005a).

CO tolerance by phototrophic bacteria was noted in 1968 (Hirsch, 1968), and less than a decade later, it was discovered that CO could be the only source of carbon and energy under dark conditions for *Rubrivivaxgelatinosus* and *Rhodospirillum rubrum* (Uffen, 1976; Dashekvicz and Uffen, 1979; Uffen, 1981). It has been reported that the former is capable of developing at 100% CO in the gas-phase, and by oxidizing it, it produces CO_2 and H_2 (Techtmann et al., 2009). The microbial capabilities of the BWSG reaction were confirmed by (Younesi et al., 2008). *R. rubrum* showed a higher rate of CO conversion yield compared to other similar microorganisms (Alfano and Cavazza, 2018). For this reason, CO has become the subject of research on the production of biohydrogen from syngas (Kerby et al., 1995), which indicated that it requires an additional carbon source for CO conversion and growth, and it works most efficiently using acetate as a substrate (Najafpour and Younesi, 2007).

Laboratory studies of BWGS reaction conducted with *R. rubrum* were extended to industrial scale; bacteria proved to be suitable for large-scale biohydrogen production in continuous bioreactors, opening the door to the development of H_2 production technology using living microorganisms (Alfano and Cavazza, 2018). *Citrobacter* sp. Y19 obtained three times higher level of produced H_2 compared with *R. Rubrum* (Jung et al., 2002).

The hydrogenogens term originates in the 21st century, refers to anaerobic thermophilic bacteria and archaea that, as they grow, oxidize CO using H_2O as an electron acceptor, producing molecular hydrogen and CO_2 (Oelgeschläger and Rother, 2008). These reactions resemble BWGS (Oelgeschläger and Rother, 2008), and researchers suggest that high temperature facilitates hydrogenogens' CO metabolism due to increased gas diffusion rate (Diender et al., 2015). Among the hydrogenogens, the *Carboxydothermus hydrogenoformans, Thermosinus carboxydivorans* or *Thermococcus* AM4 are well-known (Techtmann et al., 2009). Hydrogenogens can be found in hydrothermal, geothermal, and volcanic environments (Wu et al., 2005). *C. hydrogenoformans* have become of interest as this microorganism is likely to enable the production of biohydrogen from syngas due to the rapid growth and CO as a source of sole carbon and energy catalyzing in the dark BWGS reaction (Wu et al., 2005). Although researchers have not yet documented the ability to convert CO contained in it to H_2, there are sources relating to pure CO use by these bacteria (Tiquia-Arashiro, 2014).

However, what is important when discussing the anaerobic conversion of CO by bacteria is the diversity and variability of microbial communities during this process, explored for syngas

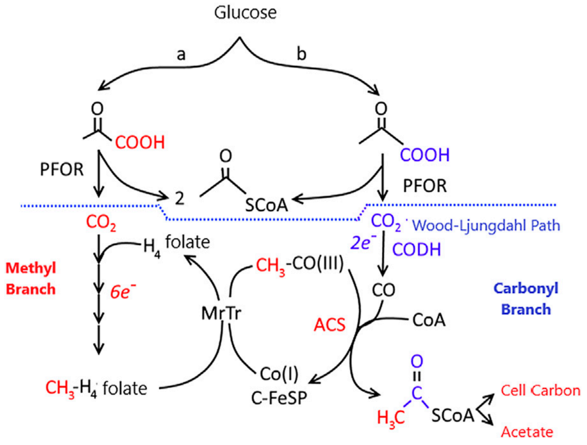

FIGURE 7
The Wood-Ljungdahl pathway of autotrophic CO and CO_2 fixation. CODH, CO dehydrogenase; ACS, acetyl-CoA synthase; MeTr, methyltransferase; CFeSP, Corrinoid iron-sulfur protein; PFOR, pyruvate ferredoxin oxidoreductase. Letters **(A,B)** indicate two branches of the Wood-Ljungdahl pathway. Reactions leading to the formation of the methyl group of acetyl-CoA are colored red, while those leading to the carbonyl group are colored blue. Figure redrawn after reference (Ragsdale, 2004).

biomethanation. CO can be converted directly or indirectly *via* other pathways, also leading to intermediate products such as H_2, CO_2, formate, acetate, butanol, ethanol, propionate or butyrate (Sancho Navarro et al., 2016; Aryal et al., 2021). It is these intermediary metabolites that contribute to the development of a variety of bacterial strains in the bioreactor. However, what needs to be emphasized is that the biological reactions of CO conversion conducted by different microbial groups have different energy balances (Asimakopoulos et al., 2020). The standard change of Gibbs free energy for these biocatalytic reactions indicates that the activity of carboxydotrophic methanogens, converting CO to CO_2 and CH_4 is the most favorable, since the $\Delta G\underline{o}$ reaches a value of -210.9 kJ·mol^{-1} [compared to -165.4 and -135.6 kJ·mol^{-1} for the next two most preferred bacteria, acetogens, and hydrogenotrophic methanogens (Grimalt-Alemany et al., 2018)]. The multiplicity of syntrophically coexisting bacteria can also be explained by the fact that they use CO both as a carbon and energy source (Asimakopoulos et al., 2020). It was also proven that bacterial CO-converting community composition changes depending on the type of substrate used in the process of syngas upgrade to biomethane; these observations were made with manure and sludge-based inoculum (Grimalt-Alemany et al., 2018).

4.2 CO microbiological consumption—Pathways and enzymes

The microbiological CO consumption depends on the O_2 availability and follows the first-order kinetics (Conrad and Seiler, 1980). This was also confirmed by another report (Rich and King, 1999), where the CO consumption in anaerobic conditions was lower than that carried out in aerobic conditions. It has also been found that CO is metabolized under all oxidation-reduction conditions. All these observations are in line with our previous study, where we showed that maximum CO concentration was observed at ~5% O_2 in the composting pile (Stegenta-Dąbrowska et al., 2019).

However, the enzymes used under aerobic and anaerobic conditions differ in terms of chemical structure and presence of Ni-Fe clusters in active centers. CO is metabolized both under aerobic and anaerobic conditions by (Jeong et al., 2015):

(i) CO dehydrogenase (CODH)—acceptor oxidoreductase as the systematic name for the activity that catalyzes CO oxidation to CO_2 or its reverse,
(ii) Acetyl-CoA synthase (ACS)—enzyme that assembles acetyl-CoA from enzyme-bound methyl, CO, and CoA groups,
(iii) Bifunctional (CO dehydrogenase/acetyl-CoA synthase or CODH/ACS)—neither ACS nor CODH alone would suffice because they describe only the partial reactions. CODH/ACS is preferable to ACS/CODH, because CODH precedes ACS in function.

CO metabolism is linked to the global carbon cycle, which involves the oxidation of organic carbon to CO_2 by heterotrophic organisms as an energy source and the replenishment of fixed organic carbon by autotrophic organisms in a reductive process called CO_2 fixation. CO_2 is returned to the carbon cycle by one of the following pathways (Ragsdale, 2004):

(i) The Calvin-Benson-Basham cycle,
(ii) The reductive tricarboxylic acid (TCA) cycle (Figure 4),
(iii) The 3-hydroxypropionate cycle (Figure 5),
(iv) The autotrophic CO_2 fixation in reduction acetyl-CoA pathway or
(v) The Wood-Ljungdahl (acetyl-CoA) pathway.

Three types of CO metabolism are recognized: aerobic, anoxic, and anaerobic (Table 1). The aerobic matabolism involves exogenous electrons, while the anoxic uses the internal generation of intermediates as an electron acceptor (Diender et al., 2015). A relatively well-studied example of respiratory CO metabolism is CO oxidation coupled with oxygen reduction (Meyer and Schlegel, 1983). Oxygen microbes of the genus *Carboxydotrophic* use CO as a source of carbon and energy. They transfer electrons from CODH by catalyzing the oxidation of CO through the respiratory chain, which eventually reduces O_2 according to the equation shown in Table 1. CO_2 is assimilated as a source of cellular carbon *via* the Calvin-Benson Bassham pathway. These bacteria are well adapted to the role of CO detoxification in the environment as they are highly prone to CO uptake (Ragsdale, 2004).

CO can also be converted to CH_4 under anaerobic conditions by a range of microorganisms, including methanogenic archaea, as described in Section 4.1.2. These microorganisms use CODH, an enzyme that allows CO as a carbon source and its oxidation (Navarro et al., 2014). However, the efficiency of methanogenesis with CO as a substrate is not very high, and only three microorganisms have been marked as capable of producing CH_4 from CO: *M. thermoautotrophicus, Methanosarcina acetivorans*, and *Methanosarcinabarkeri*. Most of these organisms use CO for growth and metabolizing in the Wood-Ljungdahl pathway (Ragsdale and Pierce, 2008).

Autotrophic CO_2 fixation by methanogenic microorganisms, sulfate-reducing and acetogenic bacteria occurs without

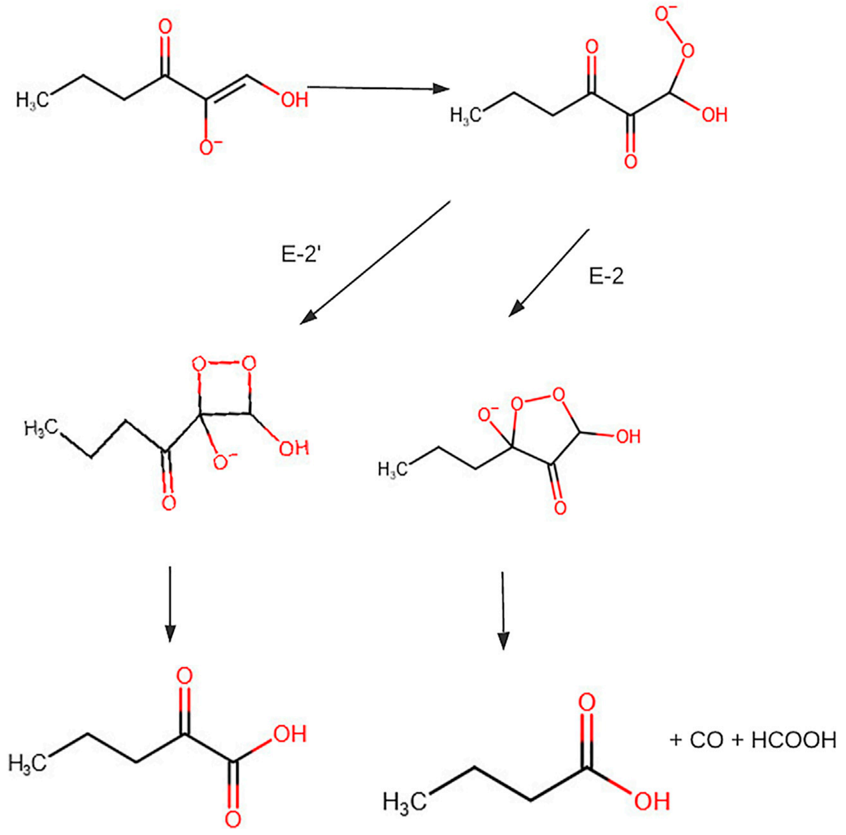

FIGURE 8
Conversion of S-methylthioadenosine to methionine. Figure redrawn after reference (Dai et al., 1999).

carboxylation phase reaction. The synthesis of cellular material from CO_2 proceeds with the reductive acetyl-CoA pathway involving pyruvate. The reactions responsible for this process were detected using radioactive compounds and enzymatic studies with *M. thermoacetotrophium*. The mechanism is the reduction of CO_2 to methanol in a bound form (Figure 6). The second CO_2 molecule is reduced to CO by CODH. The reducing force is provided by the H_2 activation by hydrogenases and transmitted by enzymes reacting with F420 (8-hydroxy-5-deazaflavin) or NADP (Nicotinamide adenine dinucleotide phosphate). As a result of the methyl-1x carbonylation, acetyl-X is formed, and the reductive carboxylation of acetyl-CoA by pyruvate synthase leads to pyruvate from which cell materials are formed *via* well-known pathways (Figure 6) (Schlegel, 2004).

4.3 CO microbiological production—Pathways and enzymes

The process of CO metabolism is much better described in the literature than the production, which is connected with researchers focus on CO utilization during fermentation process and bioethanol production (Abubackar et al., 2011). CO is biologically generated during the following pathways:

(i) The Wood-Ljungdahl (acetyl-CoA) pathway,
(ii) Conversion of S-methylthioadenosine to methionine (Dai et al., 1999),
(iii) Aromatic amino acid metabolism by bacteria (Hino and Tauchi, 1987),
(iv) Aldehyde decarbonylation by plants (Cheesbrough and Kolattukudy, 1984),
(v) Heme degradation by heme oxygenase (Tenhunen et al., 1969),
(vi) Homoacetate and acetate fermentation (Schlegel, 2004).

4.3.1 Wood-Ljungdahl pathway

The Wood–Ljungdahl pathway (Figure 7) is found in a broad range of phylogenetic classes and is used in both the oxidative and reductive processes. The pathway is used in the reductive direction for energy conservation and autotrophic carbon assimilation in acetogens. When methanogens grow on (H_2 + CO_2), they use the Wood–Ljungdahl pathway in the reductive direction (like acetogens) for CO_2 fixation (Ljungdahl, 1994). However, they conserve energy by the conversion of (H_2 + CO_2) to CH_4 (Stupperich et al., 1983).

Organisms using the reduction pathway of the acetyl-CoA cycle, referred to as the Wood-Ljungdahl pathway, reduce atmospheric CO_2 to CO through dehydrogenase (CODH), with Ni, Zn, Fe cofactors (Menon and Ragsdale, 1999) (Figure 7). The electron donor for this reaction is hydrogen. The CO combined with the dehydrogenase is linked to a methyl group carried by a corrinoid protein with a structure similar to vitamin B12. This protein takes methyl groups from

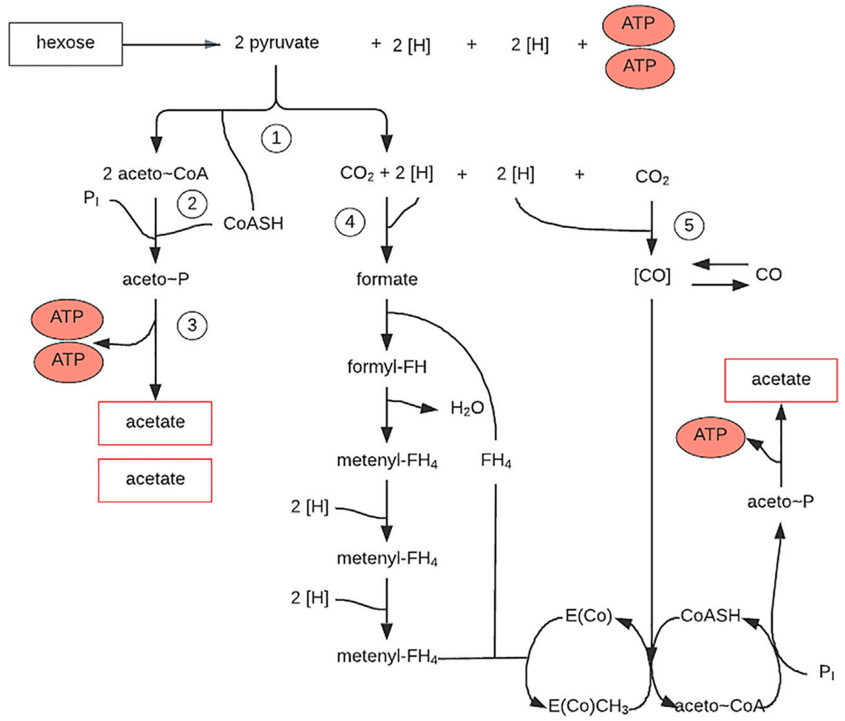

FIGURE 9
Acetate biosynthesis pathway from hexose (*via* the Acetyl-CoA pathway) in *Clostriudium thermoaceticum*. E (Co), corrinoid protein; FH4, tetrahydrofolic acid; (H), hydrogen equivalents in the form of $NADH_2$ or FdH_2; CO, exogenous carbon monoxide; (CO), bound carbon monoxide. Figure redrawn after reference (Schlegel, 2004).

tetrahydro-methanopterin and attaches to enzyme-bound CO. The acetyl group formed in the reaction is transferred to coenzyme A, which leads to the formation of acetyl coenzyme A (Ragsdale, 2008).

Unlike the Calvin cycle reducing the TCA pathway or the 3-hydroxypropionate cycle, the Wood-Ljungdahl pathway consists of two branches that require eight reducing equivalents and one ATP (adenosine triphosphate) to form acetyl-CoA from the two CO_2. ATP energy is recovered by phosphorylation at the substrate level during acetate formation, but net ATP is not obtained, requiring an anion driving force for net energy conservation (Diender et al., 2015).

4.3.2 Conversion of S-methylthioadenosine to methionine

Previous isotope tracer studies of purified E-2 and E-29 activity in extracts and experiments for CO production led to proposing a mechanism that could head to the formation of two different sets of products (Figure 8). The hydroperoxide radical or anion adds to C-2 or C-3. The addition to C-3 produces formate, CO, and butyrate. The addition to C-2 produces formate and 2-oxopentanoic acid. The metal ion affects the active site structure and thereby determines the point of addition of the hydroperoxide radical or anion and, consequently, the nature of the products (Dai et al., 1999).

4.3.3 Homoacetate fermentation

Some *Clostridium* bacteria transfer the hydrogen equivalents released in the early stages of substrate oxidation, converts CO_2 to acetate with the following formula:

$$8[H] + CO_2 \rightarrow CH_3 - COOH + 2H_2O \qquad (1)$$

The thermophilic bacteria *C. thermoaceticium* and the mesophilic *C. formicoaceticum* ferment glucose primarily into acetate. They metabolize hexose in the fructose bisphosphate pathway, producing nearly 3 moles of acetate for every mole of glucose used. A large proportion of the CO_2 generated during pyruvate decarboxylation must be rebound to hydrogen acceptor to achieve this. The formation of acetate from CO_2 and reducing equivalents (electrons) obtained in the initial oxidation reactions proceeds according to the diagram (Figure 9). Hexose is converted to pyruvate in the fructose bisphosphate pathway. Two enzymes—pyruvate oxidoreductase and ferrodoxin—are involved in forming acetate, CO_2, FdH_2 ($\alpha_2\beta_2$ enzyme), and ATP from pyruvate. CO_2 serves as a hydrogen acceptor. Partly it is reduced by formate dehydrogenase to formate, then to the methyl group of the third acetate molecule, and partly by CODH to CO, which is the acetate carboxyl group (Schlegel, 2004).

4.3.4 Acetate fermentation

CO_2 is reduced to CO (with CODH enzyme) in the acetate fermentation pathway, which finally gives the carboxyl group of acetate; methyltetrahydrofolate is carbonylated and acetyl-CoA and finally acetate is formed (Gottschalk, 1986). That reaction is, therefore, reversible under physiological conditions:

$$CO_2 + X - H_2 \leftrightarrow CO + X + H_2O \qquad (2)$$

As depicted in Figure 10, the pathway also allows outlining the routes used for acetate formation from $CH_3OH + CO_2$ and $H_2 + CO_2$. The strategy is to make CO from CO_2 and methyltetrahydrofolate from $H_2 + CO_2$ or methanol. Thus, part of the methanol has to be

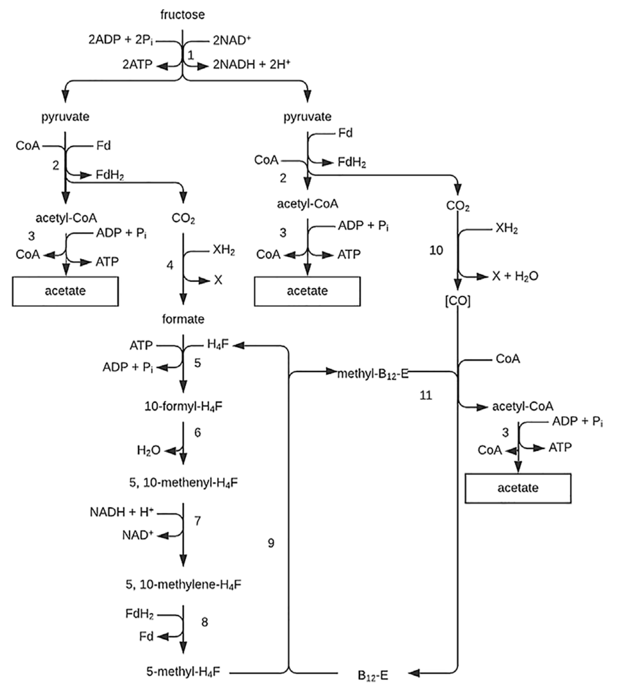

FIGURE 10
Pathway of the acetate fermentation. Figure redrawn after reference (Gottschalk, 1986). 1, Degradation of fructose *via* the Embden-Meyerhof-Parnas pathway; 2, pyruvate-ferredoxin oxidoreductase; 3, phosphotransacetylase plus acetate kinase; 4, formate dehydrogenase; 5, formyl-tetrahydrofolate synthetase; 6, methenyl-tetrahydrofolate cyclohydrolase; 7, methylene-tetrahydrofolate dehydrogenase; 8, methylene-tetrahydrofolate reductase; 9, tetrahydrofolate: B 12 methyltransferase; 10, CO dehydrogenase; 11, acetyl-CoA-synthesizing enzyme (probably ATP-requiring); (CO), enzyme-bound.

oxidized to reduce CO_2 to CO; methyltetrahydrofolate and CO finally yield acetate.

4.4 CO production during biological waste treatment processes

The growth in the human population combined with industrialization, urbanization, and improving living standards increases the amount of generated waste (Singh et al., 2014). It is estimated that global waste mass will increase to 3.4 billion tons in 2050 (Karim et al., 2019). Organic waste, such as kitchen and food waste, garden waste, agricultural and animal wastes, and sewage sludge, deserves special attention due to their link to greenhouse gas (GHG) emissions, odor, and sanitary and human health concerns. Grass, leaves, branches, and household food waste, collectively referred to as “bio-waste” (or “biowaste”), make up the largest share of municipal solid waste in low- and middle-income countries. Moreover, researchers and community organizers note that about one-third of the organic waste produced globally is food waste (Bellemare et al., 2017), and the amount is still increasing. Therefore, both the production and management of organic waste are perceived as an environmental problem globally.

An inherent element of organic waste management is the emission of air pollutants, which include GHG (the most important of which are CH_4, CO_2, and N_2O), ammonia, volatile organic compounds (VOCs), and hydrogen sulfide (Rincón et al., 2019). These gaseous emissions have been widely noted in the literature (Cao et al., 2019). On the other hand, production of CO during biological waste treatment processes—according to the bibliometric analysis—seems to be mostly ignored. We have been proposing that more attention should be paid to CO generation, fate, emissions, and its potential synergistic opportunities for a more sustainable development, which in case of CO—can change the term “pollutant.”

4.4.1 Composting process

In Europe, composting is one of the dominant bio-waste treatment options. Of the total of 48 million tonnes processed at ~4,250 plants, more than 30.5 million tonnes (>60%) were processed in 3,400 composting plants in 2019. Additionally, 4.4 million tonnes of bio-waste is integrated into composting and anaerobic digestion plants (European Compost Network e.V., 2019). Directing bio-waste to composting is the leading practice in almost all European countries (except Sweden and Denmark).

Data on the production (generation) of CO during the waste composting are minimal. There are few reports in the literature about CO sources, CO formation mechanisms, and optimal CO emissions conditions (Hellebrand, 1998; Hellebrand and Kalk, 2001; Haarstad et al., 2006; Hellebrand and Schade, 2008). The discovery of high CO concentrations (~100 ppm) during the biological decomposition of OM was surprising at the time. Back then, CO was known as an incomplete combustion product but not composting (Hellebrand, 1998). Nevertheless, follow-up studies have shown that biomass’s gradual decomposition leads to O_2 depletion and CO release (Arshadi and Gref, 2005). CO can reach significant levels when composting waste exceeding 1,000 ppm (Haarstad et al., 2006; Stegenta et al., 2018; 2019b). CO emissions are also a secondary source of GHG emissions from the composting process, especially related to such substrates as green waste, animal and municipal waste (Andersen et al., 2010a; Sánchez et al., 2015). Due to the health effects of CO on humans and the legal requirements for the hermetization of composting facilities, the process and the associated CO production may also pose a risk to composting plant workers directly involved in handling the process (Sobieraj et al., 2021).

CO generation has been observed during composting of green waste (Stegenta et al., 2019b), a mixture of green waste with dairy manure (Hellebrand and Kalk, 2001), organic waste (Haarstad et al., 2006), and during aerobic biostabilization of the municipal waste (Stegenta et al., 2018). The knowledge obtained so far has allowed for the formulation of two hypotheses on the mode of CO production. CO formation has both abiotic and biotic nature, which was determined not only based on observations of CO formation during the process but also during the analysis of samples of sterilized and non-sterilized material subjected to composting (Stegenta-Dąbrowska et al., 2019). Thus, CO production from waste composting is seen as a combination of physical processes dependent on temperature and O_2 concentration and related to microorganisms’ biological activity (Sánchez et al., 2015).

The most common observation in research on gas emissions from composting is that CO production increases immediately after the start of the process, both on a laboratory, pilot, and industrial-scales (Stegenta et al., 2018; Stegenta et al., 2019a), and it subsequently declines, often quite sharply (Stegenta et al., 2018). Modeling of CO production during the typical 14-day composting process showed, that its concentration can reach 3.2% (31,600 ppm) and 36.1% (360,000 ppm) for reactors with the daily release of accumulated gas and without ventilation, respectively (Sobieraj et al., 2021). High CO production in the initial stage of the process correlates with the temperature increase in the compost piles and shows the most significant increase in thermophilic conditions (Stegenta et al., 2019a). Interestingly, the temperature appears to be the driver of CO as well. For example, the second CO peak production was observed after as many as 100 process days was caused by the 80°C spikes (Andersen et al., 2010a). Higher CO concentrations were recorded mainly for sterile material compared to non-sterile samples (Stegenta-Dąbrowska et al., 2019). Due to the dependence of CO emissions on temperature, low production rates of CO are also observed in winter piles when the ambient temperature is low (<0°C, December—March in Europe) (Stegenta et al., 2019a).

The increased O_2 availability, together with the temperature increase, stimulates CO production (Phillip et al., 2011). This was also confirmed experimentally on a technical scale (Stegenta et al., 2018). During the aerobic biostabilization of municipal waste, higher levels of CO were recorded in perforated reactors, in which the oxidation of the waste was higher compared to the tightly sealed material. Based on the reports on the stimulating effect of temperature and O_2 on CO production, it was determined that the CO source's thermochemical processes have a dominant influence, and O_2 is playing a slightly lesser role (Stegenta-Dąbrowska et al., 2019). It also proves that the thermal degradation of OM in waste, resulting in CO production, may occur at a relatively low temperature, not exceeding 100°C (Stegenta-Dąbrowska et al., 2019).

Microorganisms are also assumed to be responsible for CO production, especially in aerobic conditions. Their influence as suppliers of substrates and accelerating CO production under aerobic conditions was proposed (Rich and King, 1999), specifically, the oxidation of fatty acids and free radicals' breakdown leading to humic substances. On the other hand, the mesophilic conditions (~40°C) may favor biogenic CO production (Stegenta-Dąbrowska et al., 2019). There are also reports of anaerobic CO production in unsterilized samples. Transient peaks of increased CO concentration were observed, resulting from a temporary O_2 depletion (Haarstad et al., 2006). The activity of methanogenic bacteria explained the production of CO under such conditions. However, the mechanisms of aerobic and anaerobic CO production are still subject to speculation (Stegenta-Dąbrowska et al., 2019).

The decrease in CO production observed in the later stages of the composting process is explained by achieving the maximum growth of microorganisms that consume the O_2 necessary for the thermochemical oxidation of CO (Stegenta-Dąbrowska et al., 2019). Faster reduction of O_2 was observed at higher temperatures (50°C–60°C), which proves the occurrence of optimal thermal conditions for microorganisms active in the composting process (Stegenta-Dąbrowska et al., 2019). These observations are additionally confirmed by the observed increase in CO_2 concentration, especially in non-sterilized material subjected to aerobic processes (Phillip et al., 2011).

The inverse correlation between CO and CO_2 concentration highlights the likely oxidation of CO by bacteria (Phillip et al., 2011). The highest concentrations of CO_2 occurred in a wide temperature range; during the experiment on non-sterile material (Stegenta-Dąbrowska et al., 2019), its maximum production was recorded at 40°C, consistent with the results of (Lee et al., 2012). On the other hand, in studies conducted by (Eklind et al., 2007), the highest emission occurred at higher temperatures, overlapping the previously mentioned minimum O_2 concentration (50°C–55°C). At 65°C, CO_2 was further produced, consistent with the range of activity of CO metabolizing bacteria (55°C–82°C) (Stegenta-Dąbrowska et al., 2019).

This sometimes conflicting evidence indicates the composting process's complexity and its dependence on various (mainly) biodegradable substances contained in the material. Moreover, during the composting process, CO becomes an energy source for anaerobic carboxydotrophic bacteria, which contributes to reducing CO concentration (Pomaranski and Tiquia-Arashiro, 2016). As proved by (Hellebrand and Kalk, 2001), the overlapping of both types of processes, CO oxidation and its consumption by microorganisms, causes variability in CO emissions and may lead to several CO release spikes during waste composting. The presence of biological determinants of CO formation was also confirmed in the case of non-sterilized material, specifically the importance of process time on net gas production (Stegenta-Dąbrowska et al., 2019). This factor may indirectly affect the growth kinetics of microorganisms and thus the formation or metabolism of CO.

The net CO emission rate depends on competitive processes of production and microbial oxidation, with each of these processes being mainly influenced by the process temperature and O_2 concentration. Due to the dual—biotic and abiotic—nature of CO production, the factors affecting its formation are also, among others, moisture content of composted material or the presence of other gases (Stegenta-Dąbrowska et al., 2019). Moreover, the CO production is influenced by the substrate's composition, including OM content (Phillip et al., 2011). However, the number of sources mentioning these variables is limited in the literature; there are also no experiments analyzing these factors.

The moisture content of the material was taken into account by (Hellebrand and Schade, 2008), according to which the CO production is dependent on the mutual interaction of O_2 and water content, and the decrease in CO production is probably the result of drying out of the decomposed material. This is in line with reports by (Schade, 1997), showing that high CO production in the early stages of the process is because, initially, the samples are wet, and the O_2 has not yet been consumed. The laboratory analyzes carried out by (Haarstad et al., 2006) showed that the addition of lime to aerobic processes causes a significant increase in the CO concentration of CO (average value of 101 and 486 ppm without and with the addition of lime, respectively). This is explained by the supply of a high load of OM and thus faster O_2 depletion, which is also confirmed by the overlap of the CO production peak with a strong O_2 decrease and an extended presence of CH_4. Moreover, in the same experiment, a strong correlation of CO with H_2S was noted during anaerobic degradation. This experiment thus confirms the above-mentioned hypothesis of CO production by methanogens (Rich and King, 1999).

4.4.2 Spatial distribution in composting waste pile

The subject of the spatial distribution of CO in composted waste is rarely undertaken by researchers. Its distribution in the material shows high variability, both in the cross-sections and longitudinal sections of the piles, and may depend on the scale of the process and its management method, the type of substrates, and environmental factors (Stegenta et al., 2019a; Stegenta et al., 2019b). Nevertheless, the increased CO concentration was observed in the entire cross-section of the material shortly after the pile was formed, and the maximum concentrations occurred earlier than for other gases (Stegenta et al., 2019b).

An O_2 gradient influences the spatial distribution of CO concentration in the compost pile—an increased CO concentration occurs in areas with high oxidation. This is due to the highest CO concentrations near the top of the stack, while the lowest CO content is characteristic of its lower part, in line with the passively aerated waste stacks/piles (Andersen et al., 2010b). Similarly, a higher CO concentration was recorded at the beginning and end of the pile prism, which may be associated with a larger contact surface of the material with ambient air (Stegenta et al., 2019b). Additionally, due to the material's anaerobic zones, an increase in the CO content was observed after the material was turned over (Hellebrand and Schade, 2008), i.e., a typical practice in industrial scale composting.

However, the reason for the apparent compost pile sections with high CO and O_2 concentrations is not clear. The high CO levels were present in the pile's surface layer, but the O_2 concentrations were low (Stegenta et al., 2019a; Stegenta et al., 2019b). Additionally, CO was not detectable as the oxidation increased. The O_2 reduction with the pile depth led to the formation of anaerobic conditions in its core (<2% O_2) during the composting of green waste and manure (López et al., 2016). This, in turn, favored the CO presence. CO concentration increased with the depth of sampling, reaching a maximum close to 800 ppmv at 80 cm depth. CO was also detected in the center of leaf and grass clippings piles (Hellebrand and Schade, 2008). This inverse CO dependence on O_2 also manifested itself indirectly in CO concentration changes depending on the wind direction (Andersen et al., 2010b). Higher CO levels were recorded on the west side for the east-to-west wind and not on the east side, where higher O_2 concentrations were observed.

The decomposition of CO in the composted mass of waste was also dependent on the temperature, and its increase caused an increase in the CO release rate (Phillip et al., 2011). Therefore, the optimal conditions for CO production are shaped by the thermal "chimney effect" in compost (Andersen et al., 2010b). In this way, areas with increased CO concentration overlap with thermophilic zones in the composted mass (Stegenta et al., 2019b). There was also an inverse correlation between the CO and CO_2 concentrations in a pile. The minimum CO concentration occurred as soon as CO_2 reached its maximum, and when CO is present at high concentrations, the level of the CO_2 decreases (Stegenta et al., 2019a). This is likely related to the CO consumption by microorganisms, which results in CO_2 production (Hellebrand, 1998).

4.4.3 Biomass and bio-waste storage and transportation

Dangerous CO levels and reduced O_2 concentrations were also identified during biomass and waste storage, e.g., wood pellets, forest residues, liquid pig manure, and dry grain (Whittle et al., 1994; Svedberg et al., 2004; He et al., 2012; Matulaitis et al., 2015). Laboratory analyses of gases emitted from the storage of various biomass types have shown that the CO concentration in reactors' headspace increases with time (Kuang et al., 2008). A faster accumulation rate was recorded at the beginning of trials, and after a few days, the CO emissions stabilized, following the first-order reaction kinetics.

Occupational accidents related to the maritime transport of wood pellets were researched (Svedberg et al., 2008). CO concentration in the sealed containers and shipping vessels can reach lethal levels ranging as high as 1,460–14,650 ppm and diffuse into adjacent spaces within the first week of wood pellets storage. The high CO production at the beginning of organic materials' storage is consistent with (Kuang et al., 2009) observations.

The ratio of headspace/reactor volume (H/R) is a significant determinant of the emission rate and CO concentration. At high H/R ratios, high peak emissions and reaction rates were reported. The net CO production depended on the O_2 concentrations, i.e., greater O_2 availability for pellets' oxidation results in the CO release. However, the higher H/R makes slows down the decomposition of biomass as excess air/O_2 utilization takes time. Other factors on CO emissions from waste and biomass storage, such as temperature and material moisture content, were the subject of additional research (He et al., 2012). The increase in temperature caused an increase in CO concentration up to 1,600 ppm. At lower moisture, the CO decreased at the expense of CO_2 production. For this reason, CO production is likely the result of a combination of chemical and biological processes.

4.4.4 Anaerobic processes

The CO emergence during the anaerobic digestion process is related to the activity of the CODH. It has been discovered in most of the methanogenic and acetogenic bacteria and used in catabolic and anabolic oxidoreductase reactions (Zeikus et al., 1985). High CODH levels have been observed in *Methanothrix soehngenii*, one of the major species responsible for acetate catabolizing in fermentation systems (Kohler and Zehnder, 1984) and in sulfate-reducing bacteria (Schauder et al., 1986). CO was produced in the presence of 80% H_2 and 20% CO_2 gas mixture by *M. thermoautotrophicum* (Conrad and Thauer, 1983) and *M. barkeri* by culturing both pure and enriched acetate cultures (Hickey, 1987). In the case of the second of these strains, CODH constituted approx. 50% of the soluble bacterial protein (Krzycki and Zeikus, 1984). CO is also an important component of the acetate-to-CH_4 conversion performed by *Methanosarcina* strain TM-1 and *M. acetivorans* (Nelson and Ferry, 1984). However, analyses carried out on methanogens lacking this enzyme indicated that they did not produce CO in the batch culture at a detectable level (Bott et al., 1985).

During the process of methanogenesis, the decomposition of acetate into bound carbonyl and methyl intermediates leads to the subsequent oxidation of the former with CODH to CO_2, with the simultaneous production of reducing equivalents used for the reduction of methyl coenzyme M (methyl-CoM) to CH_4 (Kohler and Zehnder, 1984). These processes excluded free CO as used carbonyl intermediates (Eikmanns and Thauer, 1984). CO equilibrates with a component of the carbonyl pathway (Nelson and Ferry, 1984), and the concentration of this component (carbonyl or bound CO, likely to metal) is directly related to the acetate concentration during its methanogenesis (Hickey et al., 1987). This conclusion was reached while observing a CO increase after adding acetate as a substrate during system equilibrium-focused work (Hickey et al., 1987). A similar trend was noted in another experiment when the acetate accumulation was associated with a proportional increase in CO gas (Hickey and Switzenbaum, 1991). In turn, approx. 54% of the energy available for the CH_4-to-acetate conversion was used to oxidize CO to CO_2 in fermentation chambers operating under mesophilic and thermophilic conditions (Hickey and Switzenbaum, 1990). To the contrary, the relationship between CO and acetate has not been noted by (Bae and McCarty, 1993). Together with (Hickey and Switzenbaum, 1990) they explain this by the different conditions of the anaerobic fermentation process and the CO production and consumption by various bacterial strains, which may result in the observed differences and the lack of apparent trends. The microbial flora is a complex system, and their mode of operation change over time, which may also translate into other pathways of compound degradation and the potential for CO production.

The increase in the CO concentration with the increase of H_2 concentrations in addition to the dependence of CO on acetate was observed in anaerobic fermentation (Bae and McCarty, 1993). The authors explain this possibility of CO production by methanogens using H_2 or acetate as an intermediate product of the metabolic pathway or in the form of an electron sink product. Additionally, the literature reported increasing the organic load in the process on CO and CH_4 concentrations. The CO concentration increased, resulting in a decrease in CH_4 (Hickey and Switzenbaum, 1990). A relationship between CO concentration and the accumulation of volatile fatty acids (VFAs) in the liquid-phase of fermentation has been reported by (Molina et al., 2009). An increased CO and H_2 levels in the gas-phase is a typical sign of organic overload (Huang et al., 2000). The fact of the increased CO and H_2 levels and the negligible presence of CH_4 is related to the imbalance between acidogenic and methanogenic processes (Ahring et al., 1995).

The importance of CODH and practical implications increased due to the latest findings on its biological mediation of water-gas shift bioreaction (BWGS) (Alfano and Cavazza, 2018). This process involves converting CO to H_2 according to:

$$CO + H_2O \rightarrow H_2 + CO_2 \tag{3}$$

Apart from using the critical enzyme CODH, reaction (3) is based on the activity of the dihydrogen-producing enzyme [NiFe]-hydrogenase (Kung and Drennan, 2011). H_2 production during the reaction (3) is assisted thermodynamically. WGS requires appropriate conditions such as low temperature and pressure and darkness (Bičáková and Straka, 2012). The use of CODH-producing microorganisms and the ability to convert CO to H_2 at room temperature and pressure make them a promising alternative to inorganic industrial processes (Alfano and Cavazza, 2018). The biological reaction of WGS may become a favored technology for biohydrogen production not only from an ecological but also an economic point of view (Rittmann et al., 2015). Due to the processes taking place at an ambient temperature and atmospheric pressure, they can be carried out locally, using available bio-waste materials. The production of H_2 from readily available biomass and bio-waste will reduce the costs of substrate transport and energy (Bičáková and Straka, 2012). Therefore, the controlling of CO production during biological OM decomposition is crucial.

5 Future directions and limitations

With reference to the above information about BWGS, it is necessary to analyze the possible future directions of the development of CO extraction from biological waste treatment processes and the factors that may limit them. The coupling of composting technology and CO production at industrial scale may face many obstacles for which no solutions are currently being developed.

First, there is a need for a thorough understanding of the mechanisms of CO production during aerobic organic waste treatment processes. The existing premises assume that it occurs in biotic way with the participation of microorganisms, but to date, no specific groups of bacteria that carry out this process have been indicated. This is important due to the fact that the researchers isolated aerobic strains capable of metabolizing CO and at the same time noticed the presence of anaerobic bacteria in compost piles (see Section 4.1). As this topic is currently not addressed by researchers, it is necessary to plan comprehensive basic research based on microbiology and molecular biology, taking into account not only isolation and identification of the bacterial species, potentially responsible for the CO production, but also the analysis of expression of CODH encoding gene at different composting conditions. Identification of specific groups of microorganisms that are able to produce CO in the composted mass of waste will allow to control and adjust the process to optimal conditions conducive to their development, while taking into account the quality of the final product. Additionally, due to the observations of high CO production at the beginning of composting and its subsequent decrease, analyzes of the variability of microorganisms during the process should also be carried out; it is possible that the production of CO takes place through the cooperation of various groups of bacteria, and the disappearance of some due to the occurrence of unfavourable conditions causes a decrease in the activity of the strains correlated with them. Indirectly, it can be also drawn on the knowledge acquired in the BMWGS processes; while analyzing this reaction, a problem related to gas-to-liquid mass transfer was discovered (Alfano and Cavazza, 2018). It has been proven that the activity potential of CO-metabolizing microorganisms in BMWGS reactors depends on the concentration of CO, and due to slow diffusion from the bulk gas into the pores of the catalyst, the reaction rate is significantly reduced, as the organisms have to wait for the next part of the substrate (Amos, 2004). It may also translate into the composting process, in which the delivery of the CO by specific strains for subsequent groups of microorganisms is too slow, which results in the gradual death of the latter.

What is more, learning about specific bacterial strains may also lead to the development of precise protocols and recommendations for conducting composting directed at CO production, regarding e.g., the use of a specific dose of microorganisms added to compost piles or bioreactors at the right phase in the processes of biological oxygen degradation of OM, as well as the method of their application. Such a solution can also lead to the development of another niche, dealing with the production and distribution of ready-made biopreparations dedicated to such composting processes, excluding groups that offset each other.

Taking into account the currently achieved CO concentrations from the composting process, close to 1,000 ppm on a technical scale, it is also necessary to intensify the production of this gas, so that its generation rate is valuable for further processing in order to obtain specific products on a semi-industrial or industrial scale. However, this intensification will be possible when all process conditions influencing the biological formation of CO are known; on their basis, it will be possible to develop a model that will take into account the most important variables of the process, including the effect of O_2 concentration and temperature. This is particularly important in the context of reports on the more efficient metabolism of CO by bacteria during BMWGS conducted at high temperatures, which results from increased gas diffusion rates (Alfano and Cavazza, 2018). Conducting the process with specific parameters adjusted to this scenario will allow to maximize the yield of CO.

As mentioned earlier, composting is one of the dominant organic waste treatment methods used in Europe; the number of currently operating plants of this type provides extensive technical facilities that could serve the purposes of the future development of infrastructure focused on CO production. However, it is necessary to develop solutions enabling the collection, transmission and storage of CO from the composting process, as well as specially dedicated bioreactors, enabling an effective process of OM degradation. These efforts to develop technologies for larger-scale work, however, entail high investment costs, which in turn highlights the need to finance pilot operations. However, in order to produce a valuable product, not only the availability of technological lines, but also the quality of the substrates fed to the composting process play an important role. Organic waste, covering a wide group of fractions of various origins, is a material with high variability, including seasonal one, consisting, among others, in different diet habits throughout the year (availability of vegetables and fruit, ending up in food waste) or resulting from different weather conditions (composition green waste from parks or gardens contains more fractions of leaves or grasses depending on the care treatments carried out during the year). Stable CO production from the composting process therefore needs to take into account this variability in order to produce a high amount of homogeneous product. This is related to the aforementioned modelling of CO production during the composting process; in the model of its intensification, it is necessary to reduce this variability to the basic properties, i.e., to take into account the influence of material moisture or OM content. In addition, in order to develop the technology of coupled composting and CO production, it is necessary to develop and implement an efficient system for collecting, storing and transporting the often dispersed waste stream to processing sites. Only with appropriate logistics will it be possible to continuously produce CO, competing with industrial inorganic processes.

As discussed earlier, the production of CO from composting processes is now recognized as a combination of biotic and abiotic processes but with an unknown ratio of both. While abiotic processes can be triggered by manipulating process parameters, controlling the activity of microorganisms is more challenging. One of the most serious obstacles to biological CO production during waste composting may be the effect of the CO itself on the microorganisms present in the composted mass. Due to the CO toxicity, there is a concentration barrier in the liquid phase that limits the growth of bacteria. This is based on the high affinity of CO to metalloenzymes that can completely block the catabolic activity of microorganisms (Alfano and Cavazza, 2018). For this reason, it is necessary to collaborate with omics data specialists and bioinformatics. Multidisciplinary collaborations can facilitate developing predictive models taking into account the variables influencing the metabolic processes of microorganisms. This, in turn, will allow to control and optimize the outcomes. In addition, the engineering of bacterial strains isolated from composted waste, which will adapt them to work with a gaseous substrate in a challenging environment, is also gaining importance in this context.

The current requirements for composting plants included in the BAT (best available technologies) reference indicate that these plants must implement procedures minimizing the impact of the process on the environment, mainly in terms of pollutants emitted to the atmosphere (Pinasseau et al., 2018). For this reason, one of the requirements is hermetization of compost halls, using negative pressure, limiting the leakage of unwanted substances outside. Taking into account the previously mentioned information on excessive CO production in bioreactors (both without ventilation and those opened to release process air), the safety aspect of composting plant employees becomes important. Having direct contact with composted waste, they are exposed to CO, the concentration of which significantly exceeds the acceptable generally limit values indicated by the WHO (Sobieraj et al., 2021). Therefore, it is necessary to develop personal protective equipment for workers currently working with the aerobic processing of organic waste in composting plants; however, it is also important to consider this problem in future facilities that interconnect composting plants with lines for further processing of the obtained CO, especially due to the intensification of this gas formation during the waste composting stage.

The coupling of composting and CO production processes may also face legal problems. The lack of regulations in this area, regarding the definition of the final product and the process itself, in relation to the applicable regulations, may become a barrier preventing the use of the produced CO and its circulation on the market. For this reason, it is important to conduct research in this area at the same time and involve other stakeholder groups, including representatives of the legislation.

It is also worth emphasizing that the potential for CO production from bio-waste composting is not negated but rather ignored. This is due to greater technology readiness of existing, competing methods such as syngas fermentation or BMWGS, which are still under development. It should be emphasized, however, that competing methods do exist, but in this aspect it is not only about economic efficiency, but about searching for new ways of circularity. Additionally, as with other technologies, biological processes based on microorganisms activity during composting occur at ambient temperature and pressure, lowering energy requirements and costs of CO production.

6 Conclusion

This review analyzed the literature on the subject of CO production during the processes of aerobic and anaerobic biological waste treatment, showing that the current state-of-the-art lacks comprehensive studies of the conditions under which CO is formed during composting. The mechanism of CO generation from this process is also unexplained. The impact of the type of substrate on the amount of CO emissions has not been investigated here; factors influencing CO formation and process parameters such as waste moisture, aeration, fragmentation, etc., are still unknown. Moreover, studies focused on identification of the bacteria responsible for CO production during composting has not been conducted and the link between the composting process and the activity of CODH enzyme, which may be the crucial element of this issue, is still unknown.

Due to the gaps in the literature, the current studies of CO emissions from the aerobic processes can lead to results that are burdened with high uncertainty. It is recommended to conduct comprehensive basic research on optimal parameters for CO production during bio-waste composting. Determining the impact of individual variables, such as aeration and temperature, will allow the development of a mathematical model to control and intensify CO production from this process. Further studies on the isolation and identification of the bacterial species, potentially responsible for the CO production are needed. It is also necessary to analyze the expression of CODH encoding gene at different composting conditions.

Author contributions

KS and AB contributed to conception and design of the study. KS and SS-D performed the literature search and data analysis. KS and SS-D wrote the first draft of the manuscript. All authors contributed to manuscript revision, read, and approved the submitted version.

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Conflict of interest

Publisher's note

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Appendix No. 3

Karolina Sobieraj Wrocław, 22.06.2023
(Name and surname) (Place and date)

Department of Applied Bioeconomy
Wrocław University of Environmental and Life Sciences
Wrocław, Poland
(Affiliation)

STATEMENT

I hereby declare that in the publication:

Karolina Sobieraj, Sylwia Stegenta-Dąbrowska, Gang Luo, Jacek A. Koziel, Andrzej Białowiec, 2023. *Biological treatment of biowaste as an innovative source of CO —The role of composting process*, Frontiers in Bioengineering and Biotechnology, 11, 1-16, doi: 10.3389/fbioe.2023.1126737

my contribution included:
developing the concept and design of the study. I performed the bibliometric analysis, the results of which are presented in sections *3.1 Bibliographic record on carbon monoxide,* and *3.2 Bibliographic record on composting and CO*. I have prepared the text of the entire article and Figures 1-3. During the review process, I made corrections to the manuscript text and prepared responses for the reviewers. I obtained funds for the publication of the article.

Signed by /
Podpisano przez:

Karolina Natalia
Sobieraj

Date / Data:
2023-06-22 21:04

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(Date and signature)

Appendix No. 3

Sylwia Stegenta-Dąbrowska
(Name and surname)

21.06.2023, Wrocław
(Place and date)

Department of Applied Bioeconomy
Wrocław University of Environmental and Life Sciences
Wrocław, Poland
(Affiliation)

STATEMENT

I hereby declare that in the publication:

Karolina Sobieraj, Sylwia Stegenta-Dąbrowska, Gang Luo, Jacek A. Koziel, Andrzej Białowiec, 2023. *Biological treatment of biowaste as an innovative source of CO —The role of composting process*, Frontiers in Bioengineering and Biotechnology, 11, 1-16, doi: 10.3389/fbioe.2023.1126737

my contribution included:
assistance in preparing the text of the manuscript included in section *4.2. CO microbiological consumption – pathways and enzymes* and *4.3 CO microbiological production—Pathways and enzymes*, including preparing Figures 4-10. During the review process of the article, I assisted in making corrections to the text of the manuscript and in preparing responses to the reviewers.

21.06.2023, Sylwia Stegenta-Dąbrowska
(Date and signature)

Appendix No. 3

Luo Gang
(Name and surname)

Shanghai, China, 2023/5/31
(Place and date)

Shanghai Key Laboratory of Atmospheric
Particle Pollution and Prevention (LAP3),
Department of Environmental Science and Engineering,
Fudan University, Shanghai, China
(Affiliation)

STATEMENT

I hereby declare that in the publication:

Karolina Sobieraj, Sylwia Stegenta-Dąbrowska, Gang Luo, Jacek A. Koziel, Andrzej Białowiec, 2023. *Biological treatment of biowaste as an innovative source of CO —The role of composting process*, Frontiers in Bioengineering and Biotechnology, 11, 1-16, doi: 10.3389/fbioe.2023.1126737

my contribution included: revision of the final version of the manuscript before submission and participation in the process of reviewing the article and related modifications to the manuscript.

Gang Luo 2023/5/31
(Date and signature)

USDA
United States Department of Agriculture

Research, Education, and Economics
Agricultural Research Service

Appendix No. 3

Jacek A. Koziel
(Name and surname)

Bushland, Texas, June 22, 2023.
(Place and date)

U.S. Department of Agriculture, Agricultural Research Service
Conservation and Production Research Laboratory
Bushland, Texas
(Affiliation)

STATEMENT

I hereby declare that in the publication:

Karolina Sobieraj, Sylwia Stegenta-Dąbrowska, Gang Luo, Jacek A. Koziel, Andrzej Białowiec, 2023. *Biological treatment of biowaste as an innovative source of CO —The role of composting process*, Frontiers in Bioengineering and Biotechnology, 11, 1-16, doi: 10.3389/fbioe.2023.1126737

my contribution included:

the revision of the manuscript, in particular, the preparation of the text included in the introduction part and the abstract. While working on the manuscript, I participated in English language editing.

JACEK KOZIEL Digitally signed by JACEK KOZIEL
Date: 2023.06.22 17:50:16 -05'00'

…………………………………..
(Date and signature)

Jacek Koziel, Ph.D., Research Leader
Livestock Nutrient Management Research Unit
USDA-ARS Conservation & Production Research Lab
Bushland, TX 79102
Phone: 806-356-5744
Email: jacek.koziel@usda.gov
Google Scholar – ORCID – Web of Science

Appendix No. 3

Andrzej Białowiec
(Name and surname)

Wrocław, 21.06.2023
(Place and date)

Department of Applied Bioeconomy
Wrocław University of Environmental and Life Sciences
Wrocław, Poland
(Affiliation)

STATEMENT

I hereby declare that in the publication:

Karolina Sobieraj, Sylwia Stegenta-Dąbrowska, Gang Luo, Jacek A. Koziel, Andrzej Białowiec, 2023. *Biological treatment of biowaste as an innovative source of CO —The role of composting process*, Frontiers in Bioengineering and Biotechnology, 11, 1-16, doi: 10.3389/fbioe.2023.1126737

my contribution included:
developing the concept and design of the study, including the idea of bibliometric analysis, the results of which were presented in section *3.1 Bibliographic record on carbon monoxide* and *3.2 Bibliographic record on composting and CO*. I participated in the revision of the manuscript. I was also the corresponding author.

Signed by /
Podpisano przez:

Andrzej Białowiec

Date / Data:
2023-06-21
08:19

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(Date and signature)

materials

MDPI

Article

Carbon Monoxide Production during Bio-Waste Composting under Different Temperature and Aeration Regimes

Karolina Sobieraj [1], Sylwia Stegenta-Dąbrowska [1], Christian Zafiu [2], Erwin Binner [2] and Andrzej Białowiec [1,3,*]

[1] Department of Applied Bioeconomy, Wrocław University of Environmental and Life Sciences, 37a Chełmońskiego Str., 51-630 Wrocław, Poland; karolina.sobieraj@upwr.edu.pl (K.S.); sylwia.stegenta-dabrowska@upwr.edu.pl (S.S.-D.)
[2] Department of Water-Atmosphere-Environment, Institute of Waste Management and Circularity, University of Natural Resources and Life Sciences, Vienna, Muthgasse 107, 1190 Vienna, Austria; christian.zafiu@boku.ac.at (C.Z.); erwin.binner@boku.ac.at (E.B.)
[3] Department of Agricultural and Biosystems Engineering, Iowa State University, 605 Bissell Road, Ames, IA 50011, USA
* Correspondence: andrzej.bialowiec@upwr.edu.pl; Tel.: +48-713-205-973

Abstract: Despite the development of biorefinery processes, the possibility of coupling the "conventional" composting process with the production of biochemicals is not taken into account. However, net carbon monoxide (CO) production has been observed during bio-waste composting. So far, O_2 concentration and temperature have been identified as the main variables influencing CO formation. This study aimed to investigate CO net production during bio-waste composting under controlled laboratory conditions by varying aeration rates and temperatures. A series of composting processes was carried out in conditions ranging from mesophilic to thermophilic (T = 35, 45, 55, and 65 °C) and an aeration rate of 2.7, 3.4, 4.8, and 7.8 $L \cdot h^{-1}$. Based on the findings of this study, suggestions for the improvement of CO production throughout the composting process have been developed for the first time. The highest concentrations of CO in each thermal variant was achieved with an O_2 deficit (aeration rate 2.7 $L \cdot h^{-1}$); additionally, CO levels increased with temperature, reaching ~300 ppm at 65 °C. The production of CO in mesophilic and thermophilic conditions draws attention to biological CO formation by microorganisms capable of producing the CODH enzyme. Further research on CO production efficiency in these thermal ranges is necessary with the characterization of the microbial community and analysis of the ability of the identified bacteria to produce the CODH enzyme and convert CO from CO_2.

Keywords: carbon monoxide dehydrogenase (CODH); bio-waste treatment; lab-scale composting; kinetics; oxygen deficit

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1. Introduction

The constantly growing problems related to the existing excessive use of raw materials and fossil fuels by humans, combined with excessive consumerism and waste generation, have resulted in an urgent need to find new ways to produce goods. An important role in this challenge is played by the use of secondary raw materials, still rich in useful compounds and substances, but directed imprudently to landfills or incineration plants. In this way, approaches that fit into the ideas of the circular economy and bioeconomy, based on constantly maintaining a high value of materials and products through their turnover in closed loops, are becoming more and more important. Continuously developed solutions together with a well-established system approach and the involvement of all relevant entities can result in an even more efficient use of valuable raw materials.

The area of interest of the bioeconomy includes organic materials, such as bio-waste, and green or agricultural waste. As substrates rich in organic carbon, they are currently mainly subjected to composting or anaerobic digestion processes; however, their potential is constantly recognized in other areas, such as biorefinery processes [1]. Despite the development of this field of study in recent years, so far, the possibility of coupling "conventional" waste-processing processes with the production of valuable biochemicals or biofuels by converting generated process gases has not been taken into account [2]. However, the bio-waste composting process, during which net carbon monoxide (CO) production has been observed, has the potential to couple these processes [3–8]. The benefits of using CO both as a product itself and as a substrate for subsequent conversions in the chemical industry have been extensively discussed in the literature [9–12]. Since the production of CO from biowaste is inevitable due to its high organic carbon content, the use of the generated CO appears to be an attractive solution to serve the objectives of the circular economy and the biorefinery approach. Although CO is commonly produced by the thermal gasification of bio-waste, this process requires energy to be supplied to dry the substrates [13]. Therefore, biowaste composting may become an economically and ecologically competitive method of obtaining CO. There are, however, no studies that specifically address this scenario to date.

So far, research on CO production from aerobic bio-waste processing is characterized by a high degree of uncertainty, largely based on sometimes contradictory reports on the optimal conditions for the formation of this gas. However, analyses conducted by a few researchers in the 1990s and early 2000s identified two main variables influencing CO formation: oxygen concentration and temperature [7,8,14–16]. Further studies conducted at the beginning of the second decade of the 21st century defined the production of CO from waste composting as a combination of a dual nature: abiotic and biotic processes [5,6,17,18].

CO production is stimulated by the increased availability of O_2; higher concentrations of CO were recorded after turning the material into a compost pile due to the aeration of areas where anaerobic conditions had previously developed [7]. In addition to the positive correlation to O_2 availability, CO generation is also temperature dependent. Based on studies conducted for soils, during which the soils produced CO during the day at a temperature of 30–40 °C and became a net CO sink at night (temperature < 30 °C), the researchers conjectured a hypothesis about the physicochemical sources of CO generation [19]. This hypothesis was later confirmed by analyses by Phillip et al. [6], where higher CO levels were found in sterilized samples of composts. An unambiguous indication of the nature of CO formation was, however, impossible due to the observed fluctuations in the concentration of this gas during waste composting. A high level of CO is characteristic of the initial phase of the process (a few hours [7,16] or even 10 min after starting the process, at a temperature of 35 °C [7]); then, the concentration of CO decreases and increases again after approx. 5–8 days (50 °C [3,7,16]). A similar trend was also observed during analyzes of CO production from wetlands [20]. Due to the increasing concentration of CO_2 occurring in parallel with the lowering of CO concentration, the gradual depletion of CO is associated with its microbial oxidation [7]. Although the first rapid increase in CO concentration is explained by the thermochemical processes of its generation, e.g., the abiotic degradation of fatty acids, polyphenols, and aromatic acids, the next peak was defined as the biotic [8]. As reported by Haarstad et al. [8] and Rich and King [20], CO production during composting is linked to methanogenesis and the activity of methanogens since the strong peak of CO concentration reached even 2022 ppm (0.2%) at a very low level of O_2.

An important aspect of adjusting the bio-waste composting process to CO production and subsequent coupling of this gas conversion in biorefinery processes is to increase the concentration of generated CO. Only finding the optimal conditions of the composting process for CO release will allow for an increase in its formation, which will translate into the technological application of these processes. Since it is known that a combination of abiotic and biotic processes (that can occur in parallel) are happening to stimulate or compete with each other, the issue of CO production during the bio-waste composting process should be treated holistically under the process conditions that are most conducive to its generation. This study aims to investigate CO production potential during bio-waste composting under controlled laboratory conditions by varying aeration rates and temperatures. For this purpose, a series of composting processes were carried out in conditions ranging from mesophilic to thermophilic (T = 35, 45, 55, and 65 °C) and aeration rates from 2.7 to 7.8 $L \cdot h^{-1}$. Daily measurements of CO concentration were used to determine the kinetic parameters of the decrease in CO concentration to find optimal conditions for its generation.

2. Materials and Methods

2.1. Materials

Bio-waste from the composting plant of Lobau, Vienna (Austria), collected from green and less densely populated areas of the city of Vienna, was used for composting on a laboratory scale. Waste material consisted of plant-based waste collected separately from bio-waste bins, i.e., vegetables and windfall fruit, leaves, tree and shrub cuttings, lawn clippings, and wilted flowers. The bio-waste was previously shredded, sifted, and screened in the mechanical treatment unit of the Lobau facility, and combined with chopped branches. Bio-waste samples were collected each time a new series of the composting process was started from a freshly formed waste pile (1–2 days old). A fresh waste sample of approx. 50 kg was collected manually with a shovel using the quartering method into plastic trays. After transporting them to the laboratory, they were again shredded to obtain a homogeneous particle size (elimination of larger pieces of wood blocking the material in the bioreactor). Substrate samples before the process were characterized (Section 2.5).

2.2. Bio-Waste Composting

For bio-waste composting on a laboratory scale, 12 adapted glass desiccators (bioreactors, Vienna, Austria) with a volume of ~7 L each were used. The working space of the bioreactor was divided into three areas: the upper part (headspace); the middle part, where bio-waste was placed (composting chamber); and the lower part, separated from the middle part by a perforated plastic screen for leachate collection (Figure 1). The bioreactors were equipped with covers with one closed valve and exhaust air outlet; after cooling, the exhaust air was collected in gas collection bags. A gas-tight connection of the cover with the body of the reactor was ensured by applying Vaseline on the edge of the bioreactor. A port with a screw cap in the middle part of the bioreactors allowed for the manual insertion of a thermocouple into the composted material to measure the temperature of the waste. Air supply was adjusted individually (from 2.7 to 7.8 $L \cdot h^{-1}$) for each bioreactor and was inserted at bottom part of the reactor by a hose connection and flow controller.

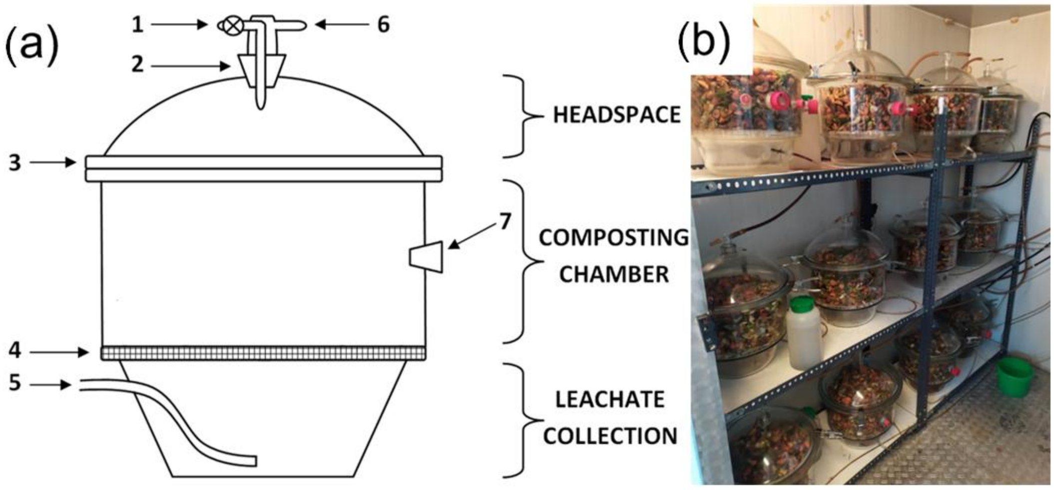

Figure 1. Bioreactors for laboratory-scale composting: (**a**) scheme of the bioreactor (based on [21]), 1—closed valve, 2—covers screw cup, 3—cover edge, 4—perforated plastic screen, 5—aeration hose, 6—exhaust air outlet, 7—screw cup for temperature measurements; (**b**) bioreactors in the climate chamber.

Bioreactors were weighted (accuracy 0.01 g, initial weight) and then placed in a climate chamber in rows of 4 bioreactors on one level (shelf, Figure 1b). To avoid the thermal 'shelf effect' (different ambient temperatures), variant repetitions in triplicates were analyzed vertically. Depending on the experiment variant, the climate chamber was set at 35, 45, 55, and 65 °C (Table 1). Since the CO concentration is highest in the initial phase of the process [3,4], one composting cycle lasted 14 days. After 7 days, the bio-waste was removed, manually mixed in a cuvette for aeration, and placed back in the bioreactor (compost turning).

Table 1. Experimental design for laboratory composting.

Composting Series #	Compost Substrates	Duration of the Process, Days	Temperature, °C	Aeration Rate, $L \cdot h^{-1}$
1	Bio-waste (green waste and vegetables) mixed with chopped branches	14	35	2.7
				3.4
				4.8
				7.8
2			45	2.7
				3.4
				4.8
				7.8
3			55	2.7
				3.4
				4.8
				7.8
4			65	2.7
				3.4
				4.8
				7.8

2.3. Measurements of Process Gases Concentration and Temperature

CO concentration (ppm) measurements were conducted every 24 h with the first measurement 24 h after placing the bioreactors in the climatic chamber and starting the process. The Polytector III G999 gas concentration analyzer with a measuring range of 0–1000 CO ppm (GfG Gesellschaft für Gerätebau mbH, Dortmund, Germany) was manually connected to silicone tubes that were connected to the gas bags collecting the process air from each of the bioreactors separately. The measurement was carried out until the concentration values stabilized (approx. 1 min). Since CO concentration was reported as co-dependent with O_2 and CO_2 levels, O_2 concentration (%) was measured in parallel using the same method and analyzer. After each CO and O_2 measurement, the analyzer was disconnected for a short pause to return to ambient levels (CO~0 ppm, O_2~20.2%, CO_2 ~0%). CO_2 concentration (%) was analyzed every 24 h by an infrared gas analyzer (type GMA 052, GfG Gesellschaft für Gerätebau mbH, Dortmund, Germany) connected to the gas bags (Figure 2). During the measurement series, the port with a screw cap in each bioreactor was opened, the thermocouple (Testo 925, Testo SE & Co. KGaA, Titisee-Neustadt, Germany) was inserted into the material (~15 cm depth) and the compost temperature was measured (±0.5 °C). On each level of the shelf, a 1 L plastic container filled with water was placed and the temperature was measured with a thermocouple (Testo 110, Testo SE & Co. KGaA, Titisee-Neustadt, Germany) to determine the ambient temperature (±0.2 °C).

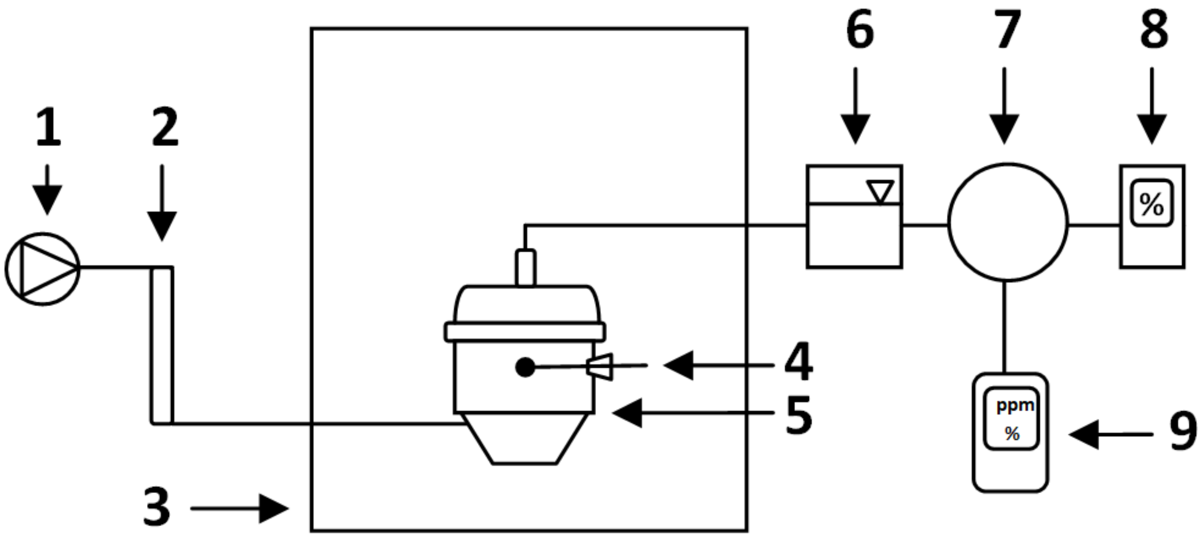

Figure 2. Laboratory-scale composting system (based on [21]): 1—air supply, 2—flowmeter, 3—climate chamber, 4—thermocouple, 5—bioreactor, 6—cooling system, 7—puffer bag, 8—IR gas analyzer (CO_2, % *v*/*v*), 9—gas concentration analyzer (CO, ppm; O_2, % *v*/*v*).

2.4. Bio-Waste Samples Collection

After this process, each of the bioreactors was weighed (accuracy 0.1 g). Then, bio-waste from 3 bioreactors with the same aeration rate variant was placed in a plastic hutch and mixed manually with a metal shovel. The homogenized material's properties were then analyzed (Section 2.5). Leachates from each bioreactor were collected separately with a pipette and placed into a plastic vessel and weighed (accuracy 0.01 g).

2.5. Bio-Waste and Compost Characterization

Substrates and compost samples were characterized according to methods published elsewhere [22,23]. Analyses of water content (WC), pH, electrical conductivity (EC), loss on ignition (LOI), total organic carbon (TOC), total nitrogen (TN), carbon/nitrogen ratio (C/N), respiration activity AT_4, ammonium nitrogen (NH_4-N) and nitrate nitrogen (NO_3-N) content were carried out.

2.6. Calculations

The TOC, TN, C/N, NH_4-N, and NO_3-N content values of substrates and composts were calculated according to equations presented elsewhere [24].

The initial properties of the bio-waste and the final compost samples were compared to determine the process's efficiency.

The kinetics of CO concentration decrease, during composting, were analyzed using linear and nonlinear least-squares regression. The models of the 0th-order (linear) and 1st-order (nonlinear) reactions were used for the analysis (Equations (1) and (2)):

$$C_{CO} = a \cdot t \tag{1}$$

where:

C_{CO}—CO concentration at time t, ppm;

a—regression coefficient indicating the decrease in CO concentration during the experiment, ppm·d^{-1});

t—time, days (d).

$$C_{CO} = C_{COmax} \cdot e^{-k \cdot t} \tag{2}$$

where:

C_{COmax}—maximum CO concentration, ppm;

k—decrease in CO concentration rate constant , $days^{-1}$ (d^{-1});

t—time, days (d).

2.7. Statistical Analyses

All data were analyzed using Statistica StatSoft Inc., TIBCO Software Inc, and the analysis involved estimating the measurements' mean, standard deviation, analyzing variance (CO concentration vs. process temperature and aeration), and correlation analysis (CO concentration vs. CO_2, O_2 concentrations, ambient temperature, and compost temperature for first 7 days of composting). Parametric tests (unequal-variance analysis and Tukey's post hoc test at the significance level $\alpha = 0.05$) were used to compare the differences between variants.

3. Results

3.1. Bio-Waste Initial Properties

The bio-waste, which was collected every ~2 weeks for the composting process, had similar TOC (33.0–36.9% d.m.), TN (1.3–1.6% d.m.) and C/N (23–28, Table 2) across the study period. The water content of the samples also did not differ much for substrates processed at 35 °C, 45 °C and 55 °C (~54%); only the bio-waste composted at 65 °C was characterized by higher moisture (61%). A similar trend was observed for organic matter content. The highest LOI was achieved by bio-waste processed at the highest temperature (71.0% d.m.), followed by substrates from the 35 °C variant (69.2% d.m.) and similar values for 45 °C and 55 °C samples (67.5 and 64.2% d.m., respectively). Bio-waste was acidic in most cases (pH equal to 5.6, 6.5, and 6.8 for 45 °C, 55 °C and 65 °C, respectively); the exception was waste composted at 35 °C, where the pH was 7.5. Electrical conductivity reached a similar level for all samples (~3 mS·cm^{-1}), with the highest value exceeding 4 mS·cm^{-1} for substrates processed at 45 °C. The largest differences between the substrates were observed in the case of NH_4-N and NO_3-N content. The ammonium nitrogen content ranged from 140.46 to >800 mg·kg d.m.$^{-1}$, with the highest values recorded for substrates at 45 °C and 55 °C (850.4 and 689.3 mg·kg d.m.$^{-1}$, respectively). The NO_3-N content was generally lower with a maximum of 169.6 mg·kg d.m.$^{-1}$ (in the 65 °C variant).

Table 2. Properties of substrates for the composting process in different temperature variants (average ± std. dev.); d.m.—dry matter.

	Substrates for the Composting Process			
Properties ± std. dev.	35 °C	45 °C	55 °C	65 °C
pH	7.48 ± 0.07	5.60 ± 0.01	6.51 ± 0.11	6.75 ± 0.09
EC, mS·cm^{-1}	3.53 ± 0.03	4.06 ± 0.05	3.09 ± 0.06	3.51 ± 0.04
TOC, % d.m.	35.66 ± 0.07	33.81 ± 0.47	33.03 ± 0.12	36.94 ± 0.83
TN, % d.m.	1.26 ± 0.02	1.50 ± 0.01	1.44 ± 0.03	1.62 ± 0.03
C/N	28	24	23	23
Water content, %	54.40 ± 0.30	55.70 ± 0.89	54.28 ± 0.32	61.00 ± 0.23
LOI, % d.m.	69.19 ± 0.26	67.48 ± 0.44	64.15 ± 0.30	71.01 ± 0.37
AT_4, mg O_2·g d.m.$^{-1}$	37.6 ± 0.2	64.0 ± 2.8	61.7 ± 1.2	71.1 ± 10.0
NH_4-N, mg·kg d.m.$^{-1}$	140.46 ± 3.02	850.40 ± 13.76	689.28 ± 44.56	248.08 ± 1.63
NO_3-N, mg·kg d.m.$^{-1}$	58.22 ± 6.10	82.90 ± 6.36	30.42 ± 12.69	169.62 ± 26.65

The highest respiratory activity was found in substrates directed to the process at 65 °C (71.1 mg O_2·g d.m.$^{-1}$). Slightly lower, similar levels of AT_4 were found for biowaste processed at 45 °C and 55 °C (~60 mg O_2·g d.m.$^{-1}$); a low index was observed for substrates in the variant with the lowest temperature (37.6 mg O_2·g d.m.$^{-1}$).

3.2. Composts Properties

When analyzing the properties of the composts after 2 weeks of the process, it was seen that the water content and organic matter content (LOI) reached a similar level for the samples composted within the same temperature, even if the aeration rate was different (Figures 3 and 4). Generally, the highest values of both of these indicators were characteristic of the lowest aeration rate (2.7 L·h^{-1}) except for the 65 °C variant, where LOI was higher for bio-waste aerated with 3.4 L·h^{-1}. Similarly to the substrates, the TOC and TN values of the compost samples were similar for each temperature variant. TOC in composts was similar to that in substrates, while TN was slightly higher than in bio-waste samples (>30% d.m. and ~2% d.m. for TOC and TN, respectively, Figures 3 and 4, Table S1); hence, the C/N ratio for the composts decreased compared to the input values (<20). After the process, the pH of the bio-waste changed from acidic to alkaline; the only exception was compost processed at 45 °C with the lowest level of aeration (pH, in this case, was 5.3). As before, also after the process, the samples from the 35 °C temperature variant were characterized by the highest pH (>8.4). The highest EC was found for composts processed at 45 °C (ranging from 3.5 to 5.0 mS·cm^{-1} for the highest to lowest aeration variants, respectively, Table S1). For the remaining temperature variants, the EC decreased compared to the values recorded for the substrates and did not exceed 3 mS·cm^{-1}.

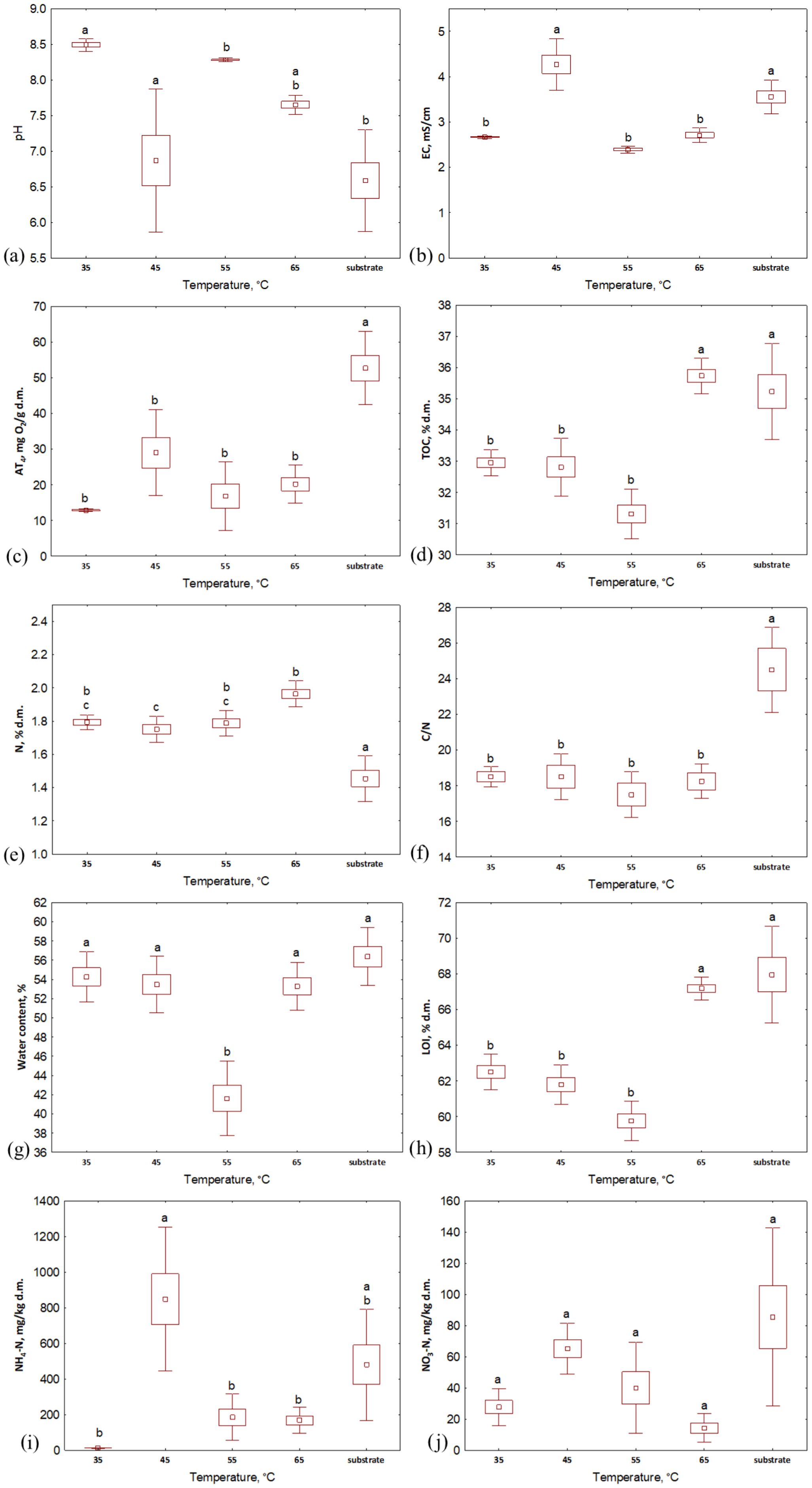
(a)
pH
9.0
8.5
8.0
7.5
7.0
6.5
6.0
5.5
a
a
b
a
b
b
35
45
55
65
substrate
Temperature, °C
(b)
EC, mS/cm
5
4
3
2
1
0
b
a
b
b
a
Temperature, °C
(c)
AT_4, mg O_2/g d.m.
70
60
50
40
30
20
10
0
b
b
b
b
a
Temperature, °C
(d)
TOC, % d.m.
38
37
36
35
34
33
32
31
30
b
b
b
a
a
Temperature, °C
(e)
N, % d.m.
2.4
2.2
2.0
1.8
1.6
1.4
1.2
1.0
b
c
c
b
c
b
a
Temperature, °C
(f)
C/N
28
26
24
22
20
18
16
14
b
b
b
b
a
Temperature, °C
(g)
Water content, %
62
60
58
56
54
52
50
48
46
44
42
40
38
36
a
a
b
a
a
Temperature, °C
(h)
LOI, % d.m.
72
70
68
66
64
62
60
58
b
b
b
a
a
Temperature, °C
(i)
NH_4-N, mg/kg d.m.
1400
1200
1000
800
600
400
200
0
b
a
b
b
a
b
Temperature, °C
(j)
NO_3-N, mg/kg d.m.
160
140
120
100
80
60
40
20
0
a
a
a
a
a
Temperature, °C

Figure 3. Properties of substrate and composts after 14 days of the composting process in different temperature variants: (**a**) pH; (**b**) EC, mS·cm^{-1}; (**c**) AT_4, mg O_2·g d.m.$^{-1}$; (**d**) TOC, % d.m.; (**e**) TN, % d.m.; (**f**) C/N; (**g**) water content, %; (**h**) LOI, % d.m.; (**i**) NH_4-N, mg·kg d.m.$^{-1}$; (**j**) NO_3-N, mg·kg d.m.$^{-1}$; letters (a, b, c) indicate the homogeneity group according to Tukey's test at a significance level $p < 0.05$.

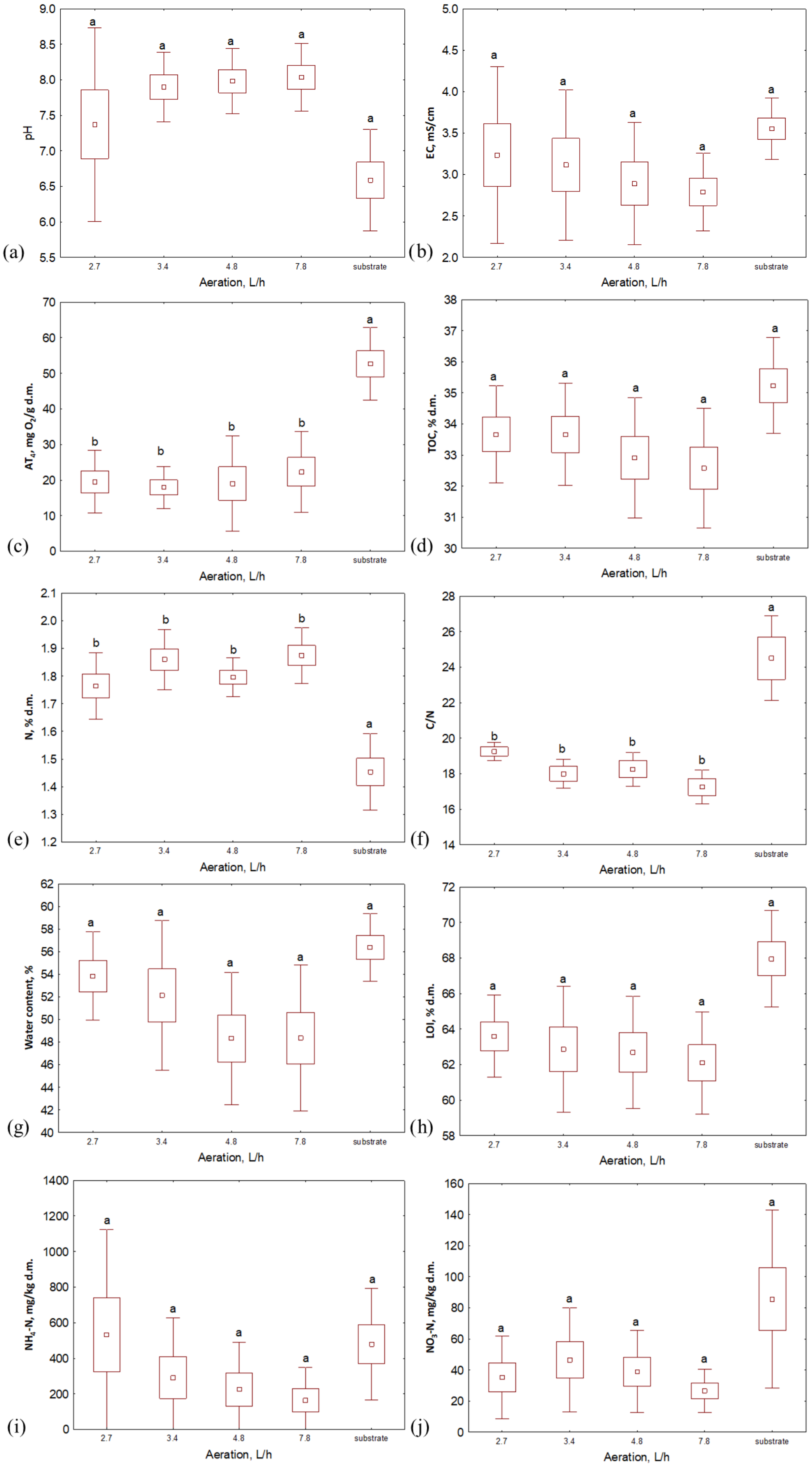
(a)
pH
9.0
8.5
8.0
7.5
7.0
6.5
6.0
5.5
a
a
a
a
a
2.7
3.4
4.8
7.8
substrate
Aeration, L/h
(b)
EC, mS/cm
5.0
4.5
4.0
3.5
3.0
2.5
2.0
a
a
a
a
a
2.7
3.4
4.8
7.8
substrate
Aeration, L/h
(c)
AT_4, mg O_2/g d.m.
70
60
50
40
30
20
10
0
b
b
b
b
a
2.7
3.4
4.8
7.8
substrate
Aeration, L/h
(d)
TOC, % d.m.
38
37
36
35
34
33
32
31
30
a
a
a
a
a
2.7
3.4
4.8
7.8
substrate
Aeration, L/h
(e)
N, % d.m.
2.1
2.0
1.9
1.8
1.7
1.6
1.5
1.4
1.3
1.2
b
b
b
b
a
2.7
3.4
4.8
7.8
substrate
Aeration, L/h
(f)
C/N
28
26
24
22
20
18
16
14
b
b
b
b
a
2.7
3.4
4.8
7.8
substrate
Aeration, L/h
(g)
Water content, %
62
60
58
56
54
52
50
48
46
44
42
40
a
a
a
a
a
2.7
3.4
4.8
7.8
substrate
Aeration, L/h
(h)
LOI, % d.m.
72
70
68
66
64
62
60
58
a
a
a
a
a
2.7
3.4
4.8
7.8
substrate
Aeration, L/h
(i)
NH_4-N, mg/kg d.m.
1400
1200
1000
800
600
400
200
0
a
a
a
a
a
2.7
3.4
4.8
7.8
substrate
Aeration, L/h
(j)
NO_3-N, mg/kg d.m.
160
140
120
100
80
60
40
20
0
a
a
a
a
a
2.7
3.4
4.8
7.8
substrate
Aeration, L/h

Figure 4. Properties of substrate and composts after 14 days of the composting process in different aeration rate variants: (**a**) pH; (**b**) EC, mS·cm^{-1}; (**c**) AT_4, mg O_2·g d.m.$^{-1}$; (**d**) TOC, % d.m.; (**e**) TN, % d.m.; (**f**) C/N; (**g**) water content, %; (**h**) LOI, % d.m.; (**i**) NH_4-N, mg·kg d.m.$^{-1}$; (**j**) NO_3-N, mg·kg d.m.$^{-1}$; letters (a, bindicate the homogeneity group according to Tukey′s test at a significance level $p < 0.05$.

The largest variability between compost samples was noted for NH_4-N and NO_3-N content. In terms of temperature variants, the lowest NO_3-N values were obtained by composts processed at 65 °C (from 3.3 to 25.2 mg·kg d.m.$^{-1}$, Figures 3 and 4, Table S1), but when considering this indicator′s value with respect to the aeration variants, no clear trend was observed. The highest NO_3-N content was characteristic of aeration of 3.4 L·h^{-1} at 35 °C and 55 °C (38.4 and 86.6 mg·kg d.m.$^{-1}$, respectively), while for 45 °C and 65 °C, it was characteristic for bioreactors aerated with 4.8 L·h^{-1} (81.0 and 25.2 mg·kg d.m.$^{-1}$, respectively). In turn, NH_4-N content reached the lowest level for composts processed at the lowest temperature (<15 mg·kg d.m.$^{-1}$); higher values were recorded for composts processed at 45 °C, 55 °C and 65 °C with maxima at aeration rates of 2.7 L·h^{-1} (1461.1, 390.9 and 265.4 mg·kg d.m.$^{-1}$, respectively). These results indicate the most substantial nitrification in the case of the variant with the lowest temperature. This is consistent with observations reported in the literature. The optimal conditions for nitrification are the mesophilic temperatures (20–35 °C) and a pH from 7.5 to 8.0 [25,26], which was noted in the 35 °C variant in this experiment.

The lowest respiratory activity after 2 weeks of composting was characteristic of the material processed at 35 °C; samples from each aeration variant reached a similar AT_4 value here (~13 mg O_2·g d.m.$^{-1}$). These composts can be considered stabilized since AT_4 is <20 mg O_2·g d.m.$^{-1}$ [27]. For the other temperatures, only four samples did not exceed the required threshold (compost at 55 °C aerated with 3.4, 4.8, and 7.8 L·h^{-1} and at 65 °C with an aeration of 4.8 L·h^{-1}). However, it is worth mentioning that the initial AT_4 values for substrates depended on the thermal variant. In this way, bio-waste that was processed at 35 °C, with the lowest respiratory activity index, exhibited the lowest final value, while for bio-waste in variants at 45–65 °C, that initially reached an AT_4 > 60 mg O_2·g d.m.$^{-1}$, the activity of microorganisms during the 14 days of the process did not decrease to the limit value.

Despite the use of different temperatures and aeration rates, each of the variants′ physical and chemical properties changed in ways typical to waste composting (Table 3). After 14 days of the process, the pH of the composts increased relative to the initial values of bio-waste; this increase, which was probably related to the degradation of organic and volatile fatty acids [28], ranged from approx. 11% at the highest temperature to >30% for material processed at 45 °C. An increase in the final values was also noted for TN. The highest increase was achieved at 35 °C (>38%), while the lowest at 45 °C (minimum 9.92%). This is consistent with the observations of other researchers who associated such a trend with the activity of nitrogen-fixing bacteria [29]. The remaining parameters were characterized by a decrease in value after 14 days of the process. For AT_4, TOC and LOI, the decrease in the ranges from 32.91 to 85.02%, from 2.55 to 9.66 and from 5.15 to 11.70%, respectively, was related to the degradation of organic compounds by microorganisms; after metabolizing easily degradable compounds, the activity of microorganisms declined [30]. Due to the processes of mineralization and the gradual stabilization of waste [31], the C/N ratio was decreased from ~17% (the lowest aeration rate for temperatures of 45 °C, 55 °C and 65 °C) to >32% (in the variant with lowest temperature). Lower ECs for composts than substrates (from 0.49% to 27.25%) confirmed the gradual stabilization of processed waste [32]. Single exceptions to the general trend were noted for aeration rates of 2.7 and 3.4 L·h^{-1} at 35 °C (water content), 45 °C (pH, EC, water content and NH_4-N content), 55 °C (NO_3-N content) and 65 °C (NH_4-N content, Table 3).

Table 3. Relative process efficiency in different temperature variants (%).

Process Temperature, °C	Aeration Rate, $L\cdot h^{-1}$	pH	EC	AT_4	TOC	TN	C/N	Water Content	LOI	NH_4-N	NO_3-N
35	2.7	12.47	−24.54	−65.38	−6.17	38.93	−32.14	3.27	−8.21	−90.94	−51.23
	3.4	12.47	−25.25	−65.65	−6.87	43.77	−35.71	1.65	−8.89	−89.95	−33.10
	4.8	13.87	−24.54	−65.65	−8.56	39.05	−32.14	−5.27	−9.83	−92.11	−55.96
	7.2	15.08	−23.40	−66.18	−8.70	46.42	−35.71	−0.73	−11.70	−93.21	−66.07
45	2.7	−6.59	21.20	−78.99	−6.02	9.92	−16.67	1.01	−6.37	74.25	−12.06
	3.4	31.17	11.64	−51.58	−4.22	17.24	−20.83	0.59	−7.68	−2.08	−32.01
	4.8	32.06	−0.49	−27.23	−9.66	22.50	−29.17	−7.49	−10.22	−22.49	−4.45
	7.2	32.50	−13.60	−32.91	−8.29	18.12	−25.00	−10.23	−9.39	−45.47	−44.19
55	2.7	27.59	−19.68	−46.19	−3.02	19.07	−17.39	−12.36	−5.15	−43.29	−7.94
	3.4	27.44	−22.90	−76.23	−4.06	28.45	−26.09	−23.68	−8.93	−80.02	184.66
	4.8	26.83	−26.45	−85.02	−5.16	20.02	−21.74	−28.44	−6.15	−79.62	−21.03
	7.2	27.36	−22.90	−80.00	−8.54	29.52	−30.43	−28.84	−7.18	−89.53	−30.12
65	2.7	11.64	−18.97	−63.33	−2.55	20.23	−17.39	−9.44	−5.87	6.97	−94.06
	3.4	11.56	−17.83	−69.03	−2.71	25.04	−21.74	−8.80	−4.67	−21.63	−98.09
	4.8	14.68	−26.96	−75.93	−3.06	15.45	−17.39	−15.98	−4.84	−61.60	−85.16
	7.2	15.72	−27.25	−52.55	−4.80	24.82	−26.09	−16.96	−6.18	−53.74	−89.25

During the composting of biowaste, the largest total weight loss was observed for an aeration of 4.8 $L\cdot h^{-1}$ in each temperature variant (Table 4). With an increase in process temperature, mass loss increased (from 13.64% to 16.20% for temperatures of 35–65 °C). Moreover, mass loss in the form of leachate for each aeration rate was the highest at 55 °C. The more efficient the air supply, the larger the percentage weight loss in the leachate (16.47–20.10% for 2.7–7.8 $L\cdot h^{-1}$ aeration).

Table 4. The bio-waste weight loss during composting under different process conditions.

Aeration Rate, $L\cdot h^{-1}$	Weight Loss, %	35 °C	45 °C	55 °C	65 °C
2.7	Total	11.44 ± 1.05	4.90 ± 2.50	7.48 ± 0.82	9.94 ± 0.96
	As leachate	5.95 ± 1.03	0.99 ± 1.71	16.47 ± 1.65	9.20 ± 2.98
3.4	Total	11.66 ± 1.16	8.09 ± 3.87	12.51 ± 0.85	12.04 ± 2.02
	As leachate	6.18 ± 0.35	2.88 ± 2.50	18.84 ± 2.47	5.41 ± 4.69
4.8	Total	13.64 ± 0.41	15.83 ± 9.10	16.15 ± 0.09	16.20 ± 0.37
	As leachate	5.32 ± 0.83	7.43 ± 7.21	20.03 ± 0.82	12.31 ± 1.18
7.8	Total	13.59 ± 0.76	12.88 ± 8.07	13.26 ± 1.27	16.14 ± 2.94
	As leachate	5.80 ± 1.86	4.71 ± 4.93	20.10 ± 2.88	14.61 ± 4.09

3.3. CO Concentrations

The average CO concentration reached the lowest values during composting at 35 °C, regardless of the aeration rate used (<100 ppm, Figure 5). In this thermal variant, CO also reached its minimum the fastest (for most repetitions in ~4–6 days of the process). As the process temperature increased, the CO concentration became less stable and the variations between daily measurements for repetitions increased (see Figure 5a,d). During bio-waste composting at 35 °C, the highest CO concentration was recorded for an aeration of 2.7 $L\cdot h^{-1}$ (up to 89 ppm); in this variant, these values reached the minimum the latest (day 9 of the process compared to day 5 for variants with aerations of 4.8 $L\cdot h^{-1}$ and 7.8 $L\cdot h^{-1}$, Figure 5a). The most stabilized CO concentration was observed when compost was aerated with 4.8 $L\cdot h^{-1}$ (the lowest variations).

Among all tested variants, the highest average initial CO concentration was observed in the composting process conducted at 45 °C with an aeration rate at 3.4 $L\cdot h^{-1}$ (>130 ppm, Figure 5b). However, in the case of the 2.7 $L\cdot h^{-1}$ and 7.8 $L\cdot h^{-1}$ aeration rate variants, the elevated CO concentrations (~40 ppm) were maintained until the 14th day of the process; for the highest aeration rate, these values were lower on the first day of composting, but remained at a slightly higher level on the last day (average 17 vs. 14 ppm CO for 7.8 $L \cdot h^{-1}$ and 2.7 $L \cdot h^{-1}$, respectively).

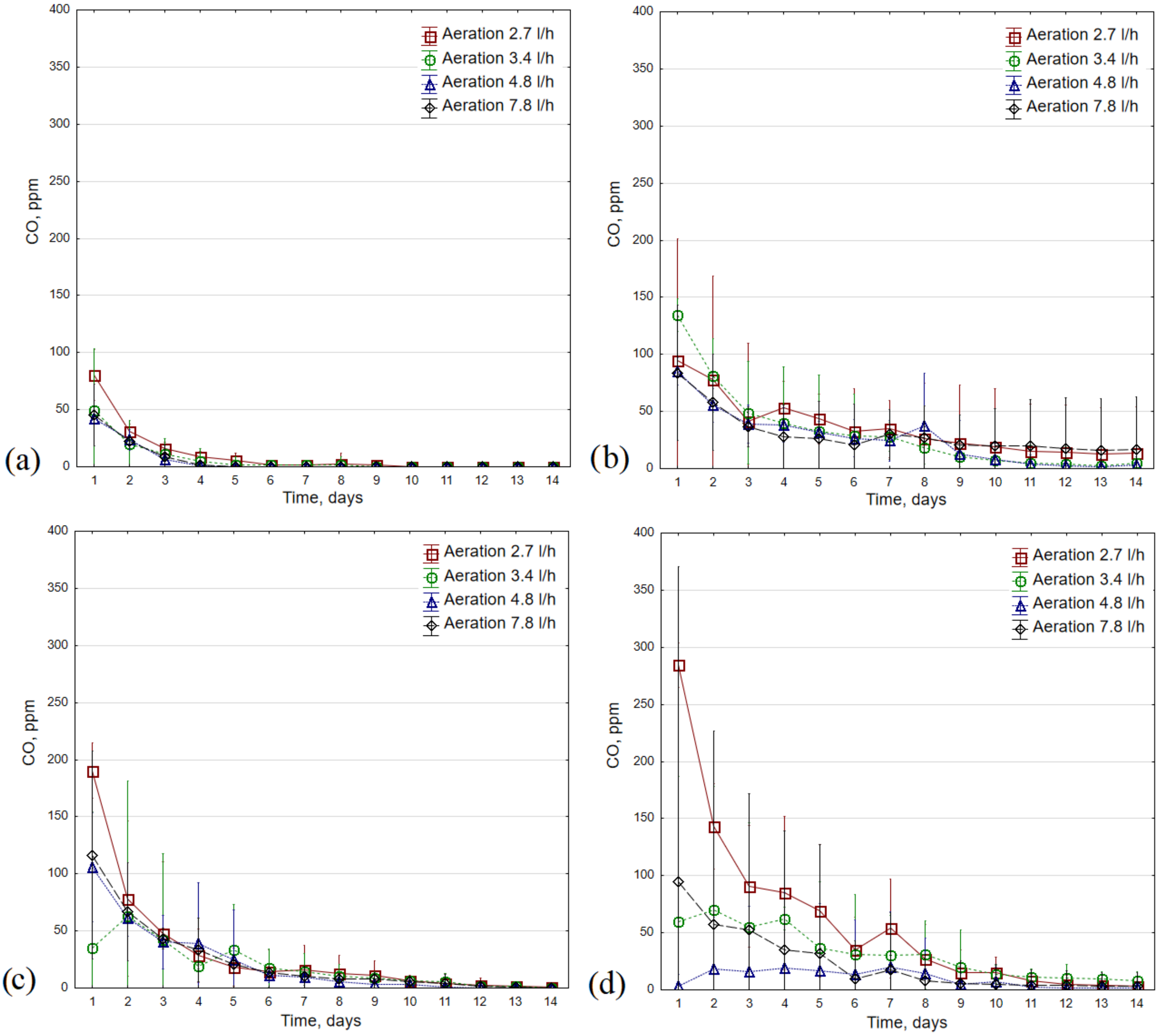

Figure 5. CO concentration average values (± standard deviation) during 14 days of the composting process in different temperature variants: (**a**) 35 °C; (**b**) 45 °C; (**c**) 55 °C; (**d**) 65 °C.

The highest average CO concentration on the first day of the composting process at 55 °C was characteristic of the variant with the lowest aeration rate, followed by variants with 7.8 $L \cdot h^{-1}$, 4.8 $L \cdot h^{-1}$ and 3.4 $L \cdot h^{-1}$ (190, 116, 106 and 35 ppm, respectively, Figure 5c). Compared to the process at 45 °C, the average CO concentration decreased faster, reaching several ppm in the second week. The largest differences between aeration rate variants were found during composting at 65 °C (Figure 5d). For the lowest aeration rate, the average CO concentration on the first day of the process was close to 300 ppm, while for the 4.8 $L \cdot h^{-1}$ variant, it was 3 ppm, and for 3.4 $L \cdot h^{-1}$ and 7.8 $L \cdot h^{-1}$, it did not exceed 100 ppm. The CO level was most stable at an aeration rate of 3.4 $L \cdot h^{-1}$; in the last three days of composting, it was 8–11 ppm, while for the experiment with the highest initial CO levels, it was 3–5 ppm (options 3.4 and 2.7 L·h^{-1}, respectively).

This study confirmed that temperature and aeration level affect CO concentrations but only at low temperatures and aeration rates (35 °C and 2,7 L·h^{-1}; Figure 6). Higher temperatures (>35 °C) and aeration rates (>3.4 L·h^{-1}) did not influence CO production during the composting process (no statistically significant differences).

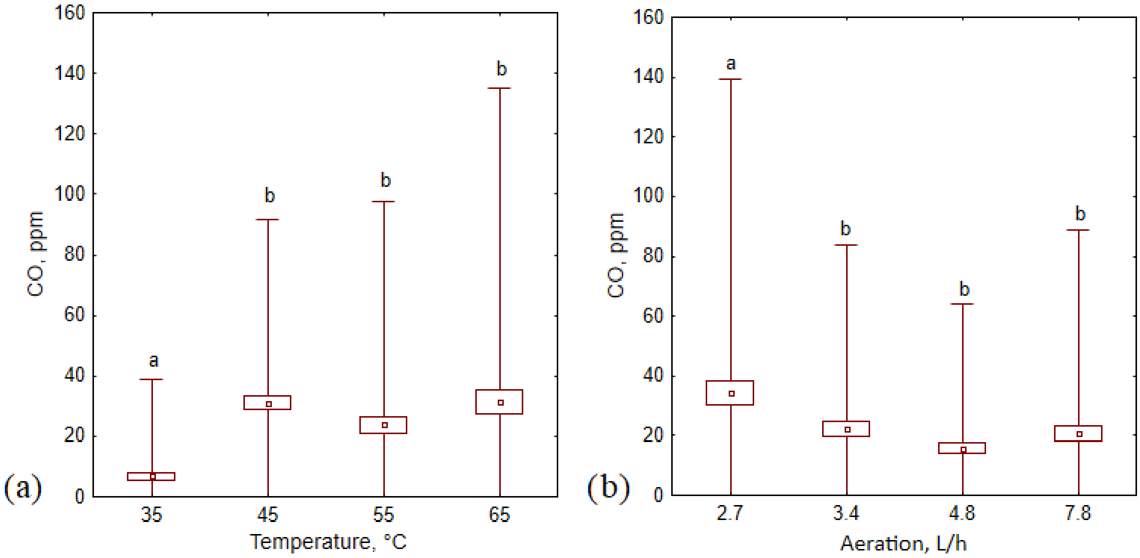

Figure 6. Average CO concentration (± standard deviation) during 14 days of the composting process in (**a**) different temperature variants, and (**b**) different aeration variants; letters (a, b) indicate the homogeneity group according to Tukey's test at a significance level $p < 0.05$.

A statistically significant correlation between the CO concentration and all other investigated variables (concentrations of CO_2 and O_2 process gases, ambient and compost temperatures) for the first 7 days of the process was observed only in the case of the thermal variant at 35 °C (Table 4). CO concentration was inversely correlated with O_2 concentration (Pearson correlation coefficient r = −0.47), while there was a positive correlation between CO level and compost temperature, ambient temperature, and CO_2 concentration (strongest for the first, r = 0.6, r = 0.2 and r =0.4 for compost temperature, ambient temperature, and CO_2 concentration, respectively). A positive correlation between CO and CO_2 concentrations was characteristic only for this variant of the process. For the temperatures of 45 °C and 55 °C, there was a negative relationship between CO and CO_2 levels, which was stronger for 55 °C (r = −0.6). Apart from the variant at 35 °C, the ambient temperature played a statistically significant role only at 65 °C; r was higher than that of the lowest temperature (0.3 vs. 0.2 for 65 °C and 35 °C, respectively).

Table 4. Correlation analysis between CO and CO_2, O_2 concentrations, ambient temperature, and compost temperature for thermal variants of the composting process (first 7 days); * shows values with Pearson r correlation coefficients that were statistically significant with $p < 0.05$.

	The Composting Variant, °C	**Ambient Temperature, °C**	**Compost Temperature, °C**	**CO_2, %**	**O_2, %**
CO, ppm	35	0.22 *	0.59 *	0.40 *	−0.47 *
	45	−0.03	−0.24 *	−0.03	0.09
	55	−0.11	−0.56 *	−0.53 *	0.55 *
	65	0.31 *	0.16	0.22 *	−0.11

3.4. CO Production Kinetics

For most cases, the decrease in CO concentration during composting was proceeded by the 1st-order reaction; only compost processed at 65 °C and aerated at 4.8 $L\cdot h^{-1}$ was consistent with the 0th-order reaction (Table 5).

Table 5. CO production kinetics during composting under different temperatures and aeration rates.

Process T, °C	Aeration, $L\cdot h^{-1}$	Reaction Order	$C_{CO\ max}$, ppm	k, d^{-1}	a = k· $C_{CO\ max}$, ppm· d^{-1}
35	2.7	1st-order	185.3 ± 21.4	0.846 ± 0.025	156.5 ± 15.6
	3.4	1st-order	109.5 ± 52.5	0.822 ± 0.185	90.4 ± 43.1
	4.8	1st-order	101.0 ± 19.0	0.834 ± 0.160	86.2 ± 32.5
	7.8	1st-order	120.1 ± 77.7	0.850 ± 0.335	119.4 ± 120.5
45	2.7	1st-order	103.1 ± 44.0	0.185 ± 0.053	18.5 ± 8.8
	3.4	1st-order	214.6 ± 84.7	0.453 ± 0.301	114.2 ± 116.2
	4.8	1st-order	95.6 ± 12.4	0.218 ± 0.041	21.1 ± 6.5
	7.8	1st-order	80.2 ± 18.2	0.179 ± 0.092	14.9 ± 10.2
55	2.7	1st-order	471.9 ± 283.4	0.816 ± 0.496	478.8 ± 530.0
	3.4	1st-order	168.8 ± 84.4	0.338 ± 0.088	61.9 ± 46.1
	4.8	1st-order	153.0 ± 19.3	0.410 ± 0.051	62.2 ± 5.0
	7.8	1st-order	183.9 ± 87.3	0.434 ± 0.157	89.0 ± 61.6
65	2.7	1st-order	403.6 ± 43.1	0.422 ± 0.090	172.8 ± 56.2
	3.4	1st-order	127.5 ± 89.1	0.183 ± 0.111	29.9 ± 27.6
	4.8	0th-order	–	–	0.09 ± 0.1
	7.8	1st-order	161.9 ± 152.2	0.670 ± 0.620	92.0 ± 73.2

Except for the 45 °C temperature variant, the trend for C_{COmax} was similar for the other thermal conditions: the highest values in the range of 185.3 ppm (35 °C) to 471.9 ppm (55 °C) were recorded for aeration rates of 2.7 $L\cdot h^{-1}$, while the lowest C_{COmax} was characteristic of an aeration of 4.8 $L\cdot h^{-1}$ (with a minimum of 32.5 ppm, Table 5, Figures S1 and S2). The highest average C_{COmax} among all aeration variants, equal to 244.4 ppm, was recorded for the 55 °C variant.

Similarly to the CO concentration and C_{COmax} values (characterized by higher deviations for successive temperature variants), the constant rate k within one thermal variant varied more with the increasing temperature of the composting process (Figures S3 and S4). However, in none of the analyzed cases did the constant k exceed 0.9 d^{-1}. The most similar values between the different reactor aeration variants were observed for the temperature of 35 °C. Under these process conditions, the CO concentration decreased the fastest ($k > 0.8\ d^{-1}$). The lowest k value was recorded during composting at 65 °C with an aeration of 4.8 $L\cdot h^{-1}$ ($k = 0.085\ d^{-1}$), although the lowest average k was characteristic of the process carried out at 45 °C ($k = 0.259\ d^{-1}$). In addition, there was no trend in the reaction rate decrease in CO concentration between the aeration variants. The highest k was recorded for different aerations depending on the composting process temperature: 7.8 $L\cdot h^{-1}$ (35 °C variant and 65 °C, k = 0.850 and 0.670 d^{-1}, respectively), 3.4 $L\cdot h^{-1}$ (45 °C variant, $k = 0.453\ d^{-1}$) and 2.7 $L\cdot h^{-1}$ (55 °C variant, $k = 0.816\ d^{-1}$).

The analysis of the average reaction rate indicated that the highest daily CO concentration for most thermal variants was achieved with the lowest aeration rate (the exception was the 45 °C variant, for which the coefficient reached the highest value of 114.2 $ppm\cdot d^{-1}$ with an aeration rate of 3.4 $L\cdot h^{-1}$). The average reaction rate ranged from >100 $ppm\cdot d^{-1}$ up to 478.8 $ppm\cdot d^{-1}$ (variant 55 °C, aeration 2.7 $L\cdot h^{-1}$).

4. Discussion

The conducted research proved that CO concentration varies depending on the temperature of the process and the level of aeration. Observations made for individual composting variants, however, highlight two CO production environments: at 35 °C and 65 °C, with simultaneous oxygen deficit.

Despite the forced, constant temperature level throughout the composting process, the phase of CO production reported earlier by the researchers was also noted during the experiment. For each of the analyzed thermal variants, the CO level was high at the beginning of the process, and then gradually decreased after about 7 days. This finding is consistent with the trend observed for composting various fractions of organic waste, including animal dung, leaves, grass, sewage sludge with bio-waste, green waste, and livestock waste [3,7,16]. The stimulation of CO production through the low aeration of composted waste was also consistent for all temperature variants, as it was also associated with the highest C_{COmax}. Oxygen deficits were favorable for CO release at lower-than-optimal aeration (variant 2.7 $L \cdot h^{-1}$), indicating anaerobic processes as a probable source of CO production for each temperature variant. As mentioned earlier, Haarstad et al. [8] and Rich and King [20] came to similar conclusions in their research. During aerobic processing of municipal solid waste, the CO concentration even exceeded 2000 ppm, which the authors explained by the activity of methanogens at intermittent O_2 loading [8]. In turn, a closer association with anaerobic biotic H_2 generation was observed for the production of CO by wetland peats [20].

The intensification of net CO production associated with the presence of anaerobic conditions was also confirmed by the highest CO concentrations obtained during composting at 65 °C. This suggests a connection with the biological nature of CO formation, based on the activity of anaerobic microorganisms capable of producing the carbon monoxide dehydrogenase (CODH) enzyme. This enzyme catalyzes the reversible oxidation of CO to CO_2 in the water–gas shift reaction [33] and thus it is responsible for both the production and conversion of CO. Potential microorganisms inhabiting the compost that produce oxygen-tolerant CODH are, e.g., *Desulfovibrio vulgaris* and *Carboxydothermus hydrogenoformans* [34]. In addition, this enzyme can be reactivated after a temporary occurrence of conditions with increased oxygen concentration [33,35]. Thus, turning the material after 7 days of the process could interrupt the biological production of CO, but would restart when O_2 is depleted again.

The association of the high CO concentrations in the 65 °C thermal variant observed in this experiment with the activity of CODH-producing bacteria is based on the characteristics of the bacterial species capable of converting CO. The thermophilic CODH-producing strains discovered so far are more numerous than the mesophilic species [36]. The conditions prevailing in the temperature variant of 65 °C in the experiment carried out here were therefore optimal for a number of anaerobic bacterial strains for which the production of CODH was proven [37]. Oxygen deficit (2.7 $L \cdot h^{-1}$ aeration) together with high temperature in the climatic chamber could lead to the development of bacterial groups such as methanogenic, carboxydotrophic, hydrogenogenic, and acetogenic microorganisms, with potential representatives for which the optimum temperature is 65 °C: others *Methanothermobacter thermautotrophicus*, *Thermoanaerobacter kivui*, *Carboxydothermus pertinax*, *Carboxydothermus islandicus*, *Calderihabitans maritimus* KKC1, among others [37]. It has also been proven that with increasing temperature, CODH activity increases and is associated with a greater yield of CO_2 to CO conversion [38] and, thus, in the high-temperature variant of this experiment, the expression of the CODH gene in the bacteria colonizing the composted waste could occur.

The biological production in this temperature variant may also be the reason why it was characterized by the greatest randomness, and the measured CO concentrations differed significantly from each other (high standard deviation for samples at 65 °C). Different bacterial strains, characterized by different efficiencies of CO production, could have appeared in individual reactors [39]. In turn, the analysis of the kinetics of the decrease in CO concentration during composting showed that although C_{COmax} was the highest for an aeration of 2.7 $L \cdot h^{-1}$ in each temperature variant, the reaction rate constant k for this level of aeration was highest at 55 °C, not 65 °C. This can be explained by the doubling time of CODH-producing bacteria, for example, for the *Methanothermobacter thermautotrophicus* strain, developing optimally at 65 °C, its doubling time is reported as extremely slow, reaching up to 200 h [37].

However, it is not only the thermophilic conditions in the experiment that indicate the biological production of CO during composting in oxygen-deficient areas. As proved by the analysis of variance, the lowest of the analyzed temperatures (35 °C) had a significant impact on the concentration of CO. Additionally, the correlation analysis showed that both for the 35 °C and 65 °C variants, the CO concentration was negatively correlated with the availability of oxygen, which can also be explained by the activity of CODH-producing anaerobes. In addition to species developing at temperatures >65 °C, this group also includes mesophiles, and the optimum point of their activity is in the thermal range of 30–37 °C. The strains known so far that function in the environment with CO include *Methanosarcina barkeri*, *Methanosarcina acetivorans*, *Alkalibaculum bacchi*, and *Butyribacterium methylotrophicum* (with an optimum reached at 37 °C) or *Acetobacterium woodii*, *Rhodospirillum rubrum* and *Clostridium drakei* (with an optimum at 30 °C) [37]. It should be emphasized that although the bacterial strains discussed above have been analyzed for CO conversion using CODH, there are no studies that analyze the ability of the same bacteria to carry out the reverse process: net CO production using the same enzyme. The release of a small amount of CO during laboratory analyses at the end of the 20th century, noted in the case of *Moorella thermoacetica* and *Methanothermobacter thermautotrophicus*, did not lead to the continuation of research [40].

Although the composting process in individual temperature conditions (35, 45, 55, 65 °C) was carried out separately, each of the analyzed variants takes place in a real, traditional composting process. Starting from the mesophilic phase, when the temperature of the material increases from 35 °C to 45 °C, the decomposition of organic matter in the composted waste generates heat and the pile or bioreactor becomes thermophilic (55–65 °C and above) [41]. Combining this information with the results obtained in this study, it can therefore be assumed that CO production under oxygen-deficit conditions follows changes in the microbial community in the waste, which are caused by process temperature phases. In this way, CO can be produced by CODH-producing bacteria; first by mesophilic species growing at 35 °C, and then by thermophiles as the process temperature increases (65 °C). Such a trend is consistent with the observations of other researchers who noted the second peak of CO production when the temperature of the material after previous cooling increased again to thermophilic conditions [7,16].

As mentioned earlier, waste composting is currently not seen as a technology with the potential to be coupled with biorefinery processes. However, the observations made during this research may lead to the formulation of recommendations for the composting process focused on CO production. According to the results obtained, CO production is significantly affected by low aeration (<3.4 $L \cdot h^{-1}$) and low temperature (<45 °C). From a practical point of view, composting aimed at generating a large net CO could therefore be carried out in economically effective conditions, based on the low efficiency of aeration systems. The composting process system would change; when controlling the thermal conditions in the pile or bioreactor, it would be advantageous to extend the mesophilic phase with temperatures close to 35 °C and not exceeding 45 °C. Then, in order to hygienize the material and simultaneously generate CO in thermophilic conditions, the material would be exposed to a high temperature (65 °C). Such an artificially imposed system, however, requires prior analysis in controlled laboratory conditions, and then during pilot composting processes on a semi-technical or technical scale in order to assess the quality of the final product of the process.

Additional requirements for a plant that utilizes bio-waste for the production of CO are of course strict safety considerations due to the toxicity of the gas and developments towards the capture and purification of CO. Although CO is a key reactant in many processes, which can lead to the production of many valuable chemical compounds, its use is limited by the need to obtain a high purity of gas stream [9]. Obtaining CO in a concentration exceeding 99 mol% therefore requires an energy-efficient separation process. Despite significant research in developing new CO separation technologies in recent years, this problem still remains unresolved, and developed methods such as cryogenic distillation, absorption, membrane or adsorptive separation still face a lot of challenges [9]. Among the weaknesses of these solutions, there are the unsolvable problems regarding CO and N_2 separation based on the similar boiling points of these compounds during cryogenic distillation or low recovery rates with insufficient CO concentration feeds for adsorption. In turn, despite the elimination of these problems in the case of methods based on absorption, environmental and safety aspects appear, such as the disposal of spent and volatile solvents [9]. Due to the need of using CO separation for industrial purposes, the problem of high costs is not without significance. It can be based on capital expenditures, such as the purchase of a cooling utility for cryogenic distillation, as well as operating costs, including the use of pretreatment to remove impurities (adsorption and cryogenic distillation) or a multistage process to yield in higher purity (membrane separation) [42].

In the context of the discussed problem, it is also important to take into account the scale of the composting process and the feedstock available for the process. Only in the European Union does composting annually process 42 million tons of bio-waste (59% of the total stream). However, by 2035, the number of tons is projected to increase by another 40 million tons per year. Thus, from 3800 composting plants in 2022, the number of installations will increase to approx. 7600 [43]. Therefore, the existing and future infrastructure, as well as the increasing supply of substrates, are favorable conditions for the development of this niche in the circular economy.

5. Conclusions and Future Research Recommendations

Based on the findings of this study, suggestions for improving CO production throughout the composting process have been developed for the first time. The conducted research proved that the production of CO during bio-waste composting on a laboratory scale depends on the aeration rate and the process temperature. The highest concentrations of CO in each thermal variant was achieved with an oxygen deficit (aeration 2.7 $L \cdot h^{-1}$). Additionally, CO levels increased with temperature, reaching concentrations of ~300 ppm at 65 °C. The production of CO in mesophilic and thermophilic conditions (35 °C and 65 °C variants) highlights the biological nature of CO formation by microorganisms capable of producing the CODH enzyme. For this reason and taking into account the high standard deviations between CO concentrations in the variant with the highest process temperature, further research is necessary on the efficiency of obtaining CO in these thermal ranges, possibly in specially dedicated bioreactors. It is also necessary to characterize the microbial community involved in the process under these process conditions and to analyze the ability of the identified bacteria to produce the CODH enzyme, and to analyze the direction of CO and CO_2 conversion.

Supplementary Materials: The following supporting information can be found within this article: Figure S1: Average C_{COmax} (± standard deviation) during 14 days of the composting process in different temperature variants; letters (a, b) indicate the homogeneity group according to Tukey’s test at a significance level $p < 0.05$; Figure S2: Average C_{COmax} (± standard deviation) during 14 days of the composting process in different aeration rates variants; letters (a, b) indicate the homogeneity group according to Tukey’s test at a significance level $p < 0.05$; Figure S3: Average constant rate k (± standard deviation) during 14 days of the composting process in different temperature variants; letters (a, b) indicate the homogeneity group according to Tukey’s test at a significance level $p < 0.05$; Figure S4: Average constant rate k (± standard deviation) during 14 days of the composting process in different aeration rate variants; letters (a, b) indicate the homogeneity group according to Tukey’s test at a significance level $p < 0.05$; Table S1: Properties of composts from different temperature variants ± std. dev., d.m.–dry matter; Table S2: Properties of substrates and composts after 14 days of the composting process in different temperature and aeration rate variants (raw data).

Author Contributions: Conceptualization, K.S. and A.B.; methodology, K.S. and C.Z.; formal analysis, K.S. and E.B.; investigation, K.S., S.S.-D. and E.B.; resources, K.S.; data curation, K.S. and S.S.-D.; writing—original draft preparation, K.S.; writing—review and editing, S.S.-D., C.Z., E.B. and A.B.; visualization, K.S. and S.S.-D.; supervision, C.Z. and A.B.; project administration, K.S.; funding acquisition, K.S. All authors have read and agreed to the published version of the manuscript.

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Conflicts of Interest: The authors declare no conflicts of interest.

List of Abbreviations

EC	electrical conductivity
TOC	total organic carbon
TN	total nitrogen
LOI	loss on ignition
AT_4	respiratory activity
NH_4-N	ammonium nitrogen content
NO_3-N	nitrate nitrogen content

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Appendix No. 3

Karolina Sobieraj Wrocław, 22.06.2023
(Name and surname) (Place and date)

Department of Applied Bioeconomy
Wrocław University of Environmental and Life Sciences
Wrocław, Poland
(Affiliation)

STATEMENT

I hereby declare that in the publication:

Karolina Sobieraj, Sylwia Stegenta-Dąbrowska, Christian Zafiu, Erwin Binner, Andrzej Białowiec, 2023. *Carbon monoxide production during biowaste composting under different temperature and aeration regimes*, Materials (accepted for the publication)

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my contribution included:
preparation of a graphic abstract of the article. I prepared the text of the entire article, including the introduction part, description of the methodology, materials, description of the results with discussion and conclusions. I prepared the figures and tables included in the manuscript. I conducted statistical analyses, the results of which are presented in Figure 6, Tables 4 and 5. I obtained funds for the publication of the article.

Signed by /
Podpisano przez:

Karolina Natalia
Sobieraj

Date / Data:
2023-06-22 21:04

………………………………..

(Date and signature)

Appendix No. 3

Sylwia Stegenta-Dąbrowska
(Name and surname)

Wrocław, 21.06.2023
(Place and date)

Department of Applied Bioeconomy
Wrocław University of Environmental and Life Sciences
Wrocław, Poland
(Affiliation)

STATEMENT

I hereby declare that in the publication:

Karolina Sobieraj, Sylwia Stegenta-Dąbrowska, Christian Zafiu, Erwin Binner, Andrzej Białowiec, 2023. *Carbon monoxide production during biowaste composting under different temperature and aeration regimes*, Materials (accepted for the publication)

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my contribution included:
help in the idea of the graphic abstract of the article. I performed statistical analyses, the results of which are shown in Figures 3 and 4, and calculations, the results of which are presented in Table 3.

21.06.2023 Sylwia Stegenta-Dąbrowska
(Date and signature)

Appendix No. 3

Christian Zafiu ... 20.6.2023

(Name and surname) (Place and date)

Department of Water-Atmosphere-Environment
Institute of Waste Management and Circularity (ABF-BOKU)
University of Natural Resources and Life Sciences (BOKU)
Vienna, Austria
(Affiliation)

STATEMENT

I hereby declare that in the publications:

Karolina Sobieraj, Sylwia Stegenta-Dąbrowska, Christian Zafiu, Erwin Binner, Andrzej Białowiec, 2023. *Carbon monoxide production during biowaste composting under different temperature and aeration regimes*, Materials (under review)

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my contribution included:
the revision of the final version of the manuscript before submitting. While working on the manuscript, I participated in English language editing. I edited Figure 1.

20.6.2023 ...
(Date and signature)

UNIVERSITÄT FÜR BODENKULTUR WIEN
Department für Wasser-Atmosphäre-Umwelt
Institut für Abfall- und Kreislaufwirtschaft
Muthgasse 107, 1190 Wien, Tel:(01)47654-813[illegible]
E-Mail: abf@boku.ac.at

Appendix No. 3

Erwin Binner
(Name and surname)

Vienna, 20.6.23
....
(Place and date)

Department of Water-Atmosphere-Environment
Institute of Waste Management and Circularity (ABF-BOKU)
University of Natural Resources and Life Sciences (BOKU)
Vienna, Austria
(Affiliation)

STATEMENT

I hereby declare that in the publication:

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my contribution included:
the revision of the final version of the manuscript before submitting. I performed analyzes of AT_4 respiratory activity of substrates and composts samples, the results of which are presented in Table 2 and Figures 3 and 4.

20.6.23
....
(Date and signature)

Appendix No. 3

Andrzej Białowiec Wrocław, 21.06.2023
(Name and surname) (Place and date)

Department of Applied Bioeconomy
Wrocław University of Environmental and Life Sciences
Wrocław, Poland
(Affiliation)

STATEMENT

I hereby declare that in the publication:

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my contribution included:
developing the concept and design of the study, including the range of temperatures and aeration regimes during composting process under the laboratory conditions. I participated in the revision of the manuscript. I was also the corresponding author.

Signed by /
Podpisano przez:
Andrzej Białowiec
Date / Data:
2023-06-21
08:20
……………………………………
(Date and signature)

Isolation and identification of CO-producing microorganisms from compost

Karolina Sobieraj1)*, Daria Derkacz2), Anna Krasowska2), Andrzej Białowiec1,3)

1) Wrocław University of Environmental and Life Sciences, Department of Applied Bioeconomy, 37a Chełmońskiego Str., 51-630 Wrocław, Poland, e-mail: karolina.sobieraj@upwr.edu.pl, andrzej.bialowiec@upwr.edu.pl

2) University of Wrocław, Faculty of Biotechnology, Department of Biotransformation, F. Joliot-Curie 14a Street, 50-383 Wroclaw, Poland, e-mail: daria.derkacz@uwr.edu.pl, anna.krasowska@uwr.edu.pl

3) Iowa State University, Department of Agricultural and Biosystems Engineering, 605 Bissell Road, Ames, IA 50011, USA

*To whom correspondence should be addressed: Karolina Sobieraj

e-mail: karolina.sobieraj@upwr.edu.pl, tel: +48 71 320 5973

Abstract:

Carbon monoxide (CO) formation has been observed during composting of various fractions of organic waste. It was reported that this production can be biotic, associated with the activity of microorganisms. However, there are no sources considering the microbial communities producing CO production in compost. This preliminary research aimed to isolate and identify microorganisms potentially responsible for the CO production in compost collected from two areas of the biowaste pile: with low (118 ppm) and high CO concentration (785 ppm). Study proved that all isolates were bacterial strains with the majority of rod-shaped Gram-positive bacteria. Both places can be inhabited by the same bacterial strains, e.g. *Bacillus licheniformis* and *Paenibacillus lactis*. The most common were *Bacillus* (*B. licheniformis, B. haynesii, B. paralicheniformis,* and *B. thermolactis*). After incubation of isolates in sealed bioreactors for 4 days, the highest CO levels in the headspace were recorded for *B. paralicheniformis* (>1000 ppm), *B. licheniformis* (>800 ppm), and *G. thermodenitrificans* (~600 ppm). High CO concentrations were accompanied by low O_2 (<6%) and high CO_2 levels (>8%). It is recommended to analyze the expression of the gene encoding CODH to confirm or exclude the ability of the identified strains to convert CO_2 to CO.

Keywords: carbon monoxide; carbon monoxide dehydrogenase (CODH); composting; biowaste; aerobic; *Bacillus paralicheniformis*; *Bacillus licheniformis*

1. Introduction

Numerous previous studies have proven that compost is a habitat for many types of microorganisms with a wide spectrum of interacting species, including bacteria, actinobacteria, and fungi **(Liu *et al.*, 2011)**. They play a key role in the decomposition of organic matter in waste, providing enzymes to the composted matrix **(Meng *et al.*, 2019)**. As a result, the decomposition of different types of compounds, including proteins, lipids, carbohydrates, cellulose, and lignin, becomes possible **(Ren *et al.*, 2016)**. Along with the change of the phases of the composting process from mesophilic conditions, through thermophilic, lowering the thermal conditions again during the cooling phase and finally maturation of the compost, microbial communities also change **(Zhao *et al.*, 2018)**, but it is always their effective activity that ensures the success of the entire process and the quality of final product.

In the last decade, microorganisms have become a key element in modern bio-based production systems **(Chen and Nielsen, 2016)**. This trend is related to replacing fossil fuels and derivatives and turning waste and biomass into valuable raw materials **(Mansouri *et al.*, 2017)**. Green building blocks of this upstream operations are gaining more and more importance on the market, being the result of academic and industrial developments, supported at the same time by governments funding **(Harmsen *et al.*, 2014)**. The growing advantage of microorganisms in the production area of the bio-based market is based on their ability to produce fuels, materials, and chemicals from renewable sources **(Diender *et al.*, 2021)**. One such valuable compound that can be obtained and converted in a biological way is certainly carbon monoxide (CO), the production of which has been observed during composting of various fractions of organic waste **(Hellebrand, 1998; Hellebrand and Kalk, 2001; Phillip *et al.*, 2011; Stegenta *et al.*, 2018, 2019; Stegenta-Dąbrowska *et al.*, 2019)**.

Analysis of the sterilized and non-sterilized organic waste in laboratory composting conditions allowed to determine the nature of CO production related to this process; according to research conducted by Stegenta et. al. **(Stegenta-Dąbrowska *et al.*, 2019)** this production takes place at two levels: biotic and abiotic, and the first type is associated with the activity of microorganisms under mesophilic conditions. However, in the literature on the subject, mainly information about the metabolism of CO by bacteria occurring in natural environments, such as soil, sediments, and seawater can be found **(Weber and King, 2007; Nguyen *et al.*, 2013)**. At the same time, there are only few sources considering microbial communities responsible for CO production, which is related to the greater involvement of researchers in bioethanol production and fermentation process **(Abubackar *et al.*, 2011)**. What is important, is that aerobic and anaerobic bacteria capable of functioning in an environment with increased CO concentration (>1%), using this gas as a carbon and energy source, use CO dehydrogenase (CODH) for CO oxidation **(King and Weber, 2007)**. This enzyme is reported as active in both directions, also catalyzing the production of CO **(Lazarus *et al.*, 2009)**, according to the reaction **(Volbeda and Fontecilla-Camps, 2004)**:

$$\mathbf{CO + OH^- \leftrightarrow CO_2 + 2e^- + H^+} \qquad (1)$$

Thus, there is a premise that the microorganisms that produce CO during organic waste composting may be the same that have been shown to metabolize this gas before.

As CO_2 is the most common C_1 compound and greenhouse gas emitted from compost piles, its conversion with the help of the CODH enzyme to industrially useful CO seems to be justified. Since it is known that CO can be produced and metabolized by biological processes during composting, it becomes important to understand the microbial species responsible for these processes, which can be seen as an initial step in the new way of biochemicals production and biological carbon capture system (BCCS) technology.

The aim of the study was to isolate and identify microorganisms present in the composted waste collected from two areas of the compost pile: with low and high CO production. The isolated strains were incubated in bioreactors, where the concentration of produced CO, CO_2 and O_2 was controlled. Bacteria producing high concentrations of CO (>800 ppm) have been identified.

2. Materials and methods

2.1.Biowaste sampling procedure

2.1.1. Biowaste composting

The composting process was carried out in April 2021 on a technical scale in the BEST-EKO Ltd. composting plant, Silesian Voivodship, Poland, in a closed hall with the ambient temperature ~25°C. The mixture of biowaste and sewage sludge was formed into a pile with dimensions of 70 x 6.5 x 2.5 m (length x width x height). The analyzed pile was placed in the back of the hall (close to the second entry gate); on the right side it was adjacent to another pile of similar dimensions, created from the same type of waste, while on its left side there was a passage for machines and vehicles supporting the process. Detailed description of the composing plant may be found elsewhere (Stegenta *et al.*, 2019).

2.1.2. Measurements of process gases concentration and temperature in the pile

The concentration of process gases (CO in ppm, CO_2 and O_2 in %) and temperature (°C) within the composting pile were measured according to the method implemented during earlier studies (Stegenta *et al.*, 2018, 2019). Briefly, gas samples were collected from 28 points at four cross sections at 1/5, 2/5, 3/5, and 4/5 of the length of the pile and at three heights – 0.5, 1.0 and 1.5 m (approx 1.50 m horizontally from the surface) with one deep measurement (pile core) at height 1.0 m (Figure 1). The measurements were performed using a long stainless steel probe with holes at the end, in which a silicone tube was placed, connected to the electrochemical analyzer Kigaz 300, Kimo Instruments, Chevry-Cossigny, France, and a thermocouple. The measuring system was sealed to separate it from the outside air. The measurement was carried out until the gas concentrations were stabilized (~5 min) with intervals between sampling for returning of indicated values to atmospheric levels.

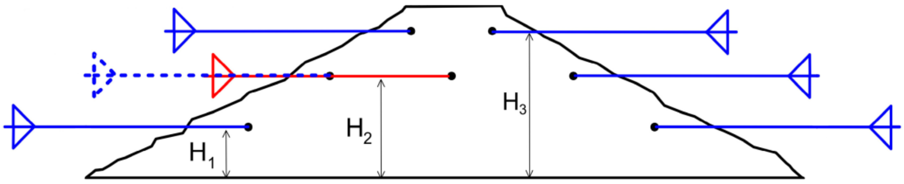


Figure 1. Cross section of a compost pile with the location of gas and temperature sampling points (shallow measurements – blue and deep – red), based on **(Stegenta *et al.*, 2019)**

The concentration of CO (ppm), CO_2, and O_2 (%) and temperature (°C) at measurement points in the tested pile are shown in Supplementary Material 1.

2.1.2. Samples collection

Samples of raw substrates (biowaste and sewage sludge) were collected from their storage point using the quartering method until the final sample weight was reduced to about 15 kg **(Jędrczak and Szpadt, 2006)**. The samples were transported in closed sealed plastic containers to the laboratory on the same day.

In order to isolate and identify microorganisms potentially responsible for CO production during composting, samples from areas with the lowest recorded CO concentration (left side of the pile, 2/5 of the length, height 0.5 m, 118 ppm of CO, T=61,6°C,) and the highest CO concentration (right side of the pile, 4/5 of the length, height 0.5 m, 785 ppm of CO, T=50,1°C) in the composting pile were taken. These places were previously marked with plastic markers. The material was carefully collected with a manual spatula and immediately placed in triplicates in sterilized plastic containers (material for analysis; marked as A, B, C for material with low CO production potential and D, E, F for compost samples with high CO level) and additionally in a sealed string bags (material marked as CO/L and CO/H for places with low and high CO production, respectively), and then transported to the laboratory in a portable refrigerator at ~5°C. The collected samples were stored at -20°C before starting microbiological analyzes.

2.2.Characteristics of composted biowaste

To build the analyzed compost pile, biowaste was used and delivered to the facility in the spring season of 2021 (April, Central Europe), including grass, leaves, and branches, which were combined in a 4:1 ratio (v/v) with sewage sludge coming from the "Boguszowice" wastewater treatment plant, Silesian Voivodship, Poland. At the time of measurement and sampling, the mix was composted for 7 days. The characterization of the substrates (mixture of biowaste) and sewage sludge, i.e. pH, electrical conductivity, moisture, loss on ignition (LOI), was carried out in accordance with the relevant standards **(Polish Committee for Standardization, 2011a, 2011b, 2012, 2022)**.

Total carbon (TC) and total nitrogen (TN) were determined using dry combustion method at 900°C in Elementar Vario Max Analyser, Frankfurt, Germany. Approx. 200 mg (±0.001 g) of biowaste and sewage sludge samples dried in 105°C and milled to <0.5 mm were used in duplicates. Total inorganic carbon (TIC) was determined for the same samples after LOI analysis. Total organic carbon content (TOC) was calculated as the difference between TC and TIC.

For NH_4-N and NO_3-N analysis, 100 g (±0,1 g) of fresh biowaste and sewage sludge materials with 1000 ml of deionized water was shaken for 2 hours with an overhead shaker. Then the solution was filtered with Macherey-Nagel filter papers, type MN 614 1/4. The content of NH_4 was measured in triplicates using DR 5000 UV-Vis Spectrophotometer, Hach, London, UK, at 655 nm. First, 13 g of sodium salicylate ($C_7H_5O_3Na$), 13 g of sodium citrate ($C_6H_5O_7Na_3 \cdot 2H_2O$), and 0.097 g nitroprusside sodium ($Na_2Fe(CN)_5NO \cdot 2H_2O$) were dissolved in 100 ml deionized water (Reagent 1). Then Reagent 2 was prepared by dissolving in 100 ml of deionized water 3.2 g of NaOH and 0.2 g of dichloroisocyanuric acid ($C_3N_3Cl_2O_3Na$). The ammonium standard solution was obtained using 0.4717 g $(NH_4)_2SO_4$ dried for 2 hours at 105°C filled up to 1000 ml with deionized water. To prepare the calibration curve, the standard ammonia solutions with concentrations of 0.2-2.0 mg $NH_4\text{-}N \cdot l^{-1}$ with the 0.2 mg $NH_4\text{-}N \cdot l^{-1}$ interval were used. For analyses dilutions of sample eluations (1:25, 1:100, and 1:200 for biowaste, compost from low and high production of CO areas, and sewage sludge, respectively) were mixed with 2 ml of Reagent 1 and 2 ml of Reagent 2 in a 25 ml volumetric flask, filled up to 25 ml with deionized water. After 1 hour the NH_4-N content was determined.

The content of NO_3-N was measured in triplicates using DR 5000 UV-Vis Spectrophotometer, Hach, London, UK, at 324 nm. First, the acid solution was obtained using H_3PO_4 p.a. 1.71 and H_2SO_4 p.a. 95-97% with a 1:1 ratio (v/v, Reagent 1). Then dimethylphenol solution was prepared by adding 1.2 g of 2.6 dimethylphenol to the 1 l of deionized H_2O (Reagent 2). The standard solution of 50 mg $NO_3\text{-}N\cdot l^{-1}$ was obtained from 0.3609 g KNO_3 dried at 105°C filled up with 1000 ml of deionized H_2O. The testing platform was calibrated using the nitrate standards prepared to the following concentrations: 5, 10, 15, 20, and 25 ppm NO_3-N. Firstly, the blind extinction was measured for 1 ml of sample with 8 ml of Reagent 1 and 1 ml of H_2O. To prepare samples for measurement, 1 ml of samples dilutions was combined with 8 ml of Reagent 1 and 1 ml of Reagent 2; after 10 minutes the absorption was determined again using the photometric method.

2.3.Identification of microorganisms in compost samples

2.3.1. Used chemicals

Culture media, chemicals, and reagents used in this study were obtained from the following sources: yeast extract, peptone (manufacturer: BD; distributor: Life Technologies; Warsaw, Poland); D-glucose, LB Broth (Miller), agar (manufacturer: BioShop; distributor: Epro Science, Puck, Poland); sodium chloride (NaCl) (Stanlab, Lublin, Poland).

2.3.2. Microorganisms isolation procedure

From compost samples collected from places with low (plastic containers A, B, C) and high (D, E, F) CO production, 1 g of material was taken into sterile phalcon, and then flooded with physiological fluid (PF; 0.9% NaCl, v=30 mL) and incubated for 30 minutes at room temperature while shaking (450 rpm). A series of dilutions of all samples was then made (dilutions 10^{-2}, 10^{-3}, 10^{-4}, 10^{-5} and 10^{-6}) and each was plated in YPD (1% peptone, 1% yeast extract, 2% glucose, and 2% agar) and LB agar plates. The A-F samples were incubated for 24 h at 25°C (YPD, fungi), 37°C (LB, bacteria) and additionally at 62°C (A-C samples) and 50°C (D-F samples). For the part of the plates, where no growth was noted, incubation was continued under the same conditions for another day, and after this time, in the absence of observation again – the plates were discarded.

In the case of plates on which the growth of microorganisms was noted, in order to achieve pure cultures the mass was seeded on fresh media (YPD or LB, respectively) and incubated in analogous to the previous conditions (24 h at temperatures 28, 37, 50 or 62°C or for the next 24 hours in the absence or poor growth). In each case, photographic documentation of obtained cultures was performed after 24-hour incubation of the plates in appropriate conditions. The isolated pure cultures were visualized using Zeiss Axio Imager A2 equipped with a Zeiss Axiocam 503 mono microscope camera and a Zeiss HBO100 mercury lamp (Oberkochen, Germany), marking them with successively increasing numbers for individual samples (i.e. A1, A2, etc.)

Strains were selected for further studies on CO release, taking into account two aspects:

(1) shape and growth resembling the reference strain, selected on the basis of a literature review (*Moorella thermoacetica*, formerly: *Clostridium thermoaceticum*, releasing CO into the environment) and other bacteria of the genus *Clostridium*,

(2) shapes of bacteria that are unusual for the environment from outside the compost: long, tangled, with septic lines, and with an unusual colony color (e.g. orange).

As a result of the selection, 15 strains (7 for samples A-C and 8 for samples D-F) were selected. In order to protect the isolated colonies, pre-cultures were maintained in a suitable liquid medium (LB/YPD) and cultures were carried out for 24 or 48 h under appropriate conditions, analogous to the method of culturing microorganisms on a solid medium.

2.3.3. DNA Isolation for identification of microorganisms from compost samples

In order to assign strains isolated from compost to species, DNA was isolated from grown pre-cultures using a commercial kit for DNA isolation (Bacterial & Yeast Genomic DNA Purification Kit, EurX, Gdansk, Poland), following the manufacturer's protocols. In order to identify whether the obtained gDNA belongs to bacteria or fungi, PCR reactions were performed that multiplied 16S rRNA fragments (bacteria) or ITS (fungi). The PCR reaction mixture consisted of 12.5 µL iProof HF Master Mix (Bio-Rad, Hercules, CA, USA), 0.5 µL Primer_F, 0.5 µL Primer_R, 2 µL gDNA solution (diluted 10x), and 9.5 µL H_2O_{dd}. PCR assays were carried out using two different pairs of primers: for fungi: ITS1 (5'-TCCGTAGGTGAACCTGCGG-3') and ITS4 (5'-TCCTCCGCTTATTGATATGC-3'); for bacteria: BACT_27F (5'-AGAGTTTGATCCTGGCTCAG-3') and BAC1492R (5'-GGTTACCTTGTTACGACTT-3'). PCR was performed using A C100 Touch Thermal Cycler (BioRad, Hercules, CA, USA). The following reaction was performed for control templates (for fungi: gDNA from *Candida albicans* CAF2-1; for bacteria: gDNA from *E. coli* DH5α) in order to verify the obtained products for isolated strains. The reaction protocol is shown in Table 1.

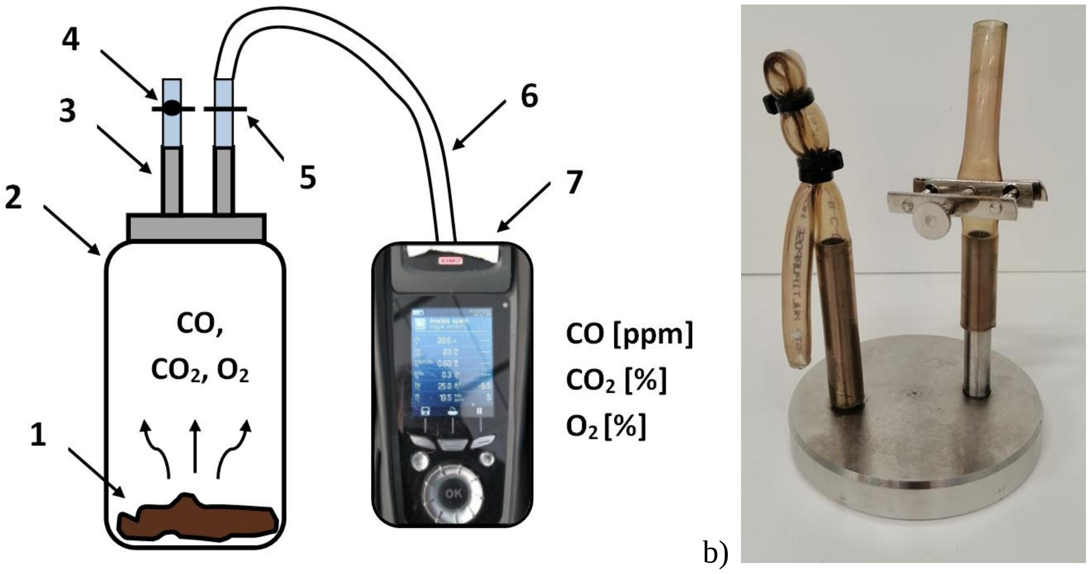
Table 1. The PCR protocol

Step	**ITS (fungi)**		**16S rRNA (bacteria)**	
	Temperature, °C	**Time, s**	**Temperature, °C**	**Time, s**
Initial denaturation	98	10	94	4
Denaturation	98	1	94	1
Annealing	51	1	52	1 800
Extension	72	1 800	72	2
Final extension	72	10	72	5
Cooling	4	∞	4	∞

The results of the PCR were electrophoresized using 1% (w/v) agarose gel and visualized using FastGene Blue/Green GelPic LED Box (Nippon Genetics Europe GmbH, Düren, Germany). Bands were excised and purified from the agarose gel using the Agarose out kit, (EurX, Gdansk, Poland). PCR products were sequenced using the Sanger method (Microsynth Seqlab GmbH, Göttingen, Germany).

2.4. CO production by isolated strains of bacteria on a laboratory scale

In order to verify the CO production by isolated strains of bacteria in liquid media, selected strains indicated in Table 1 were pre-cultured in LB or YPD (50°C, 24 h, 150 rpm). Then, the 50 mL fresh LB or YPD media were inoculated with isolated strains, where starting $OD_{600} = 0.1$. Cultures were performed for 96 hours, at 50 (for D5, D6, D9, E5, E6, E8, F4, and F5 strains) or 62 °C (for A2, B6, B8, C7) in previously sterilized (121 °C, 20 minutes) glass bioreactors (working volume: 1 L) (Figure 2). The bioreactors were equipped with metal tight caps with two measuring nozzles, on which short silicon tubes were placed; the airflow in one of them was blocked by the use of two hose clamps, the other was closed with a Hoffman clamp, the removal of which enabled the connection of a gas concentration analyzer Kigaz 300, Kimo Instruments, Chevry-Cossigny, France, and the measurement of process gas concentrations (CO, CO_2, O_2) **(Stegenta-Dąbrowska *et al.*, 2019)**.



a) b)

Figure 2. Diagram of process gases measuring system; a) bioreactor with gas sampling system, 1 – compost sample with bacteria culture, 2 – glass vessel, 3 – metal cap with two nozzles, 4 – hose clamp, 5 – Hoffman clamp, 6 – silicon tube, 7 – gas concentration analyzer; b) photo of metal cap with connectors for gas sampling

In order to determine the CO production by an isolated strain of bacteria on solid media (compost) selected strains were pre-cultured in propper media as described previously. Pre-cultures were washed twice with PF (4500 rpm, 5 minutes), resuspended in PF and 50 mL of PF was incoluted to obtain a starting OD_{600} = 0.1. Then 40 g of compost (previously pasteurized: 100 °C, 1 hour and cooled to room temperature) was inoculated with starting inoculum of bacteria and then cultured for 96 hours at 50 or 62 °C. Strains marked as B3, C2 and C5 due to poor growth in the liquid medium were grown on 40 g of Koch pasteurized compost (100°C, 1.5 h) by collecting the freshly grown mass from LB plates and adjusting the inoculum to a starting OD_{600} = 0.1.

After 4 days, the concentration of process gases was measured by connecting the gas concentration analyzer to the silicon tube and opening the Hoffman clamp; an internal pump of the analyzer created a negative pressure analyzer, allowing each time to take a gas sample from the headspace of the bioreactors and to indicate the concentrations of CO (ppm), CO_2 and O_2 (%).

On the basis of the obtained gas concentrations in preliminary measurements, the strains were selected for further analysis, guided by the following aspects:

(1) The strain produced a low concentration of O_2 (<10%) with a simultaneous high concentration of CO_2 (>10%) – samples D9, F5, C7,

(2) The strain produced a higher concentration of CO in the liquid medium than other strains – sample E8, A2.

Additionally, due to relatively low values of CO concentration in liquid media (<30 ppm), further cultures of selected strains, except for strain E6, were established only on a solid medium (40 g of pasteurized compost); for each variant, adequate negative controls were used using pasteurized compost in an autoclaved bioreactor (1h, 100°C). Measurements of the process gas concentrations were carried out again after 4 days using the same methodology, and due to the exceeding of the measuring range of the analyzer (CO concentration >2000 ppm), new cultures were established with a two-fold predominance of the inoculum (40 mL of inoculum with OD_{600}=0.1 for 20 g of autoclaved compost). Measurements of gas concentrations for such cultures were carried out in three replications, and in the case of strains E8, F5, and E6 – in four.

2.5.Calculations

The total organic content (TOC) of the composted waste was calculated according to the equation:

$$\mathrm{TOC} = \left(\mathrm{TC} - \frac{\mathrm{TIC\,.\,LOI}}{100}\right).\frac{100}{(100-\mathrm{RW})} \tag{2}$$

where:
TOC – total organic carbon, % DM (dry matter),
TC – total carbon, % DM,
TIC – total inorganic carbon, % DM,
LOI – loss on ignition, % DM,
RW – residual water content, %.

Total nitrogen content (TN) in the composted waste was calculated based on to the equation (3):

$$\mathrm{TN} = \mathrm{N}\,.\frac{100}{(100-\mathrm{RW})} \tag{3}$$

where:
TN – total nitrogen, % DM,
N – N-value from Variomax device,
RW – residual water content, %.

NH_4-N content in the composted waste was calculated according to the equation:

$$\mathrm{NH_4\text{-}N} = \frac{0.78 \cdot \mathrm{c} \cdot \mathrm{Dil.}\cdot\ .\ 10000\ .100}{2.5 \cdot 1000 \cdot (100-\mathrm{WC})} \tag{4}$$

where:
NH_4-N - ammonia nitrogen content, mg·(kg DM)$^{-1}$,
0,78 – factor for calculating NH_4 to NH4-N,
c_{NH4} – concentration of NH_4 in the filtrate, mg·l^{-1},
Dil. – dilution factor,
WC – water content, %.

The content of NO_3 in the composted waste was calculated using the following formula:

$$\mathrm{NO_3\text{-}N} = \frac{0.23 \cdot \mathrm{c} \cdot \mathrm{Dil.}\cdot 10000\ .\ 100}{2.5\ \cdot 1000 \cdot (100-\mathrm{WC})} \tag{5}$$

where:

NO_3-N – nitrate nitrogen content, mg·(kg DM)$^{-1}$,

0.23 – factor for calculating NO_3 to NO_3-N,

c_{NO3} – concentration of NO_3 in the filtrate, mg·l^{-1},

Dil. – dilution factor,

WC – water content, %.

2.6.Statistical analyses

All data were analysed using Statistica StatSoft Inc., TIBCO Software Inc, i.e., estimating the measurements mean, standard deviation, conducting the correlation analyses between concentrations of process gasess: CO and CO_2, O_2.

3. Results

3.1.Composting material characterization

Among the substrates used in the composting process on a technical scale, sewage sludge was characterized by almost twice higher moisture than biowaste; higher pH, LOI, TOC, TN and the 3-fold lower C/N ratio than in biowaste were also obtained for sewage sludge sample (Table 2). This material was also characterized by a 20-fold higher level of NH_4-N and twice as high a content of NO_3-N (4209.7 vs. 211.0 and 128.5 vs. 64.6 mg·kg DM^{-1}, respectively). At the same time, biowaste and sewage sludge obtained similar conductivity (approx. 2 mS·cm^{-1}).

The material taken from the area of the pile with lower CO production (CO/L) compared to the CO/H sample was characterized by lower water content (43.7 and 52.8%, respectively) and higher organic matter content (LOI of 62.9% DM). However, both samples achieved similar levels of TOC and TN (~30.0 and ~1.2% DM, respectively). The material from the high CO production site had higher pH and conductivity, but both samples were still in the acid range (6.02 and 5.56 for CO/H and CO/L, respectively). What differed the materials collected from the two areas of the pile, was the level of NH_4-N and NH_3-N, which was higher in both cases for the CO/H sample. Both materials had C/N ratios in the range on the optimum values **(Li *et al.*, 2013)**.

Table 2. Characterization of substrates and compost samples, average ± standard deviation

Material	pH	EC	TOC	TN	C/N	Water content	LOI	NH_4-N	NO_3-N

		mS·cm^{-1}	**% DM**			**%**	**% DM**	**mg·kg DM^{-1}**	
Biowaste	5.53 ± 0.02	2.06 ± 0.11	29.6 ± 0.1	1.05 ± 0.01	28	41,8 ± 3,93	57.2 ± 0.1	211.0 ± 1.2	64.6 ± 35.7
Sewage sludge	7.67 ± 0.05	2.08 ± 0.07	37.7 ± 0.1	4.27 ± 0.02	9	78,4 ± 0,32	65.4 ± 0.0	4209.7 ± 129.6	128.5 ± 42.9
CO/L	5.56 ± 0.03	2.60 ± 0.06	32.9 ± 0.1	1.14 ± 0.01	29	43,7 ± 0,22	62.9 ± 0.5	718.7 ± 49.4	40.7 ± 0.0
CO/H	6.02 ± 0.10	3.00 ± 0.05	30.0 ± 0.1	1.30 ± 0.01	23	52,8 ± 0,53	55.8 ± 0.5	926.8 ± 36.4	50.2 ± 17.1

3.2.Isolated microorganisms from compost samples

After the initial incubation of material collected from places with low and high CO production (samples A, B, C, and D, E, and F, respectively), colonies or cell mass appeared in 37 plates. Of the A-B samples, this growth was observed in 17 of them (9 in LB and 8 in YPD agar plates). Most of the colonies appeared under thermophilic conditions (9 plates incubated at 62°C), while 5 of them grew at 37°C and 3 at 28°C. A similar situation was noted for material marked as D-F; of the 20 plates where colonies or cell mass were observed, 55% were incubated at 50°C and 11 plates contained LB medium. Photographic documentation of the plates on which the growth of microorganisms was observed along with the marking of the dilution used, incubation temperature, and the type of medium is shown in Supplementary Material 2. The photos also marked the places from which the material for reduction inoculations was taken.

Incubation of the mass to obtain pure cultures was again more effective for samples taken from pile areas with high CO production. As a result of the reduction inoculation, in the case of material D-F, the growth of microorganisms was observed on 30 plates, while for samples A-C – on 19. Analyzing the incubation temperatures, a more frequent appearance of colonies was again observed in thermophilic conditions compared to control conditions (28 and 37°C). For both samples A-C and D-F, approximately 60% of the microorganisms grew at 62 or 50°C, respectively. As for the type of media used in incubation, for the material taken from a place with low CO production, LB agar plates (11 out of 19 plates) had a slight advantage; for samples D-F, an equal ratio between LB and YPD medium was observed (15 plates for both types).

Most of the identified strains were rod-shaped bacteria, characterized by mucoid (strains B1, D2, E6, E9), slightly mucoid (A2, B5, E8), or non-mucoid growth (B6, Figure 2). Some of them, such as B6, E8, and F1, had spores. Among the bacteria growing at 62°C, most of the identified strains had long or very long cells, differing in colony colors. This group included, among others, morphologically similar strains B8 and C5 with septa; for the former, the colonies were orange, while for the latter whitish. In the group of bacteria A-C, spherical-shaped strains B2 and B7 were also found.

Bacteria isolated from the area of composting pile with increased CO concentration (samples D-F) were characterized by a more diverse shape. Cells resembling the letter 'v' (D1, D8), 'i' (D6, D9) and bottle-like (E5) were observed here, as well as the diplobacilli (E7, F2).

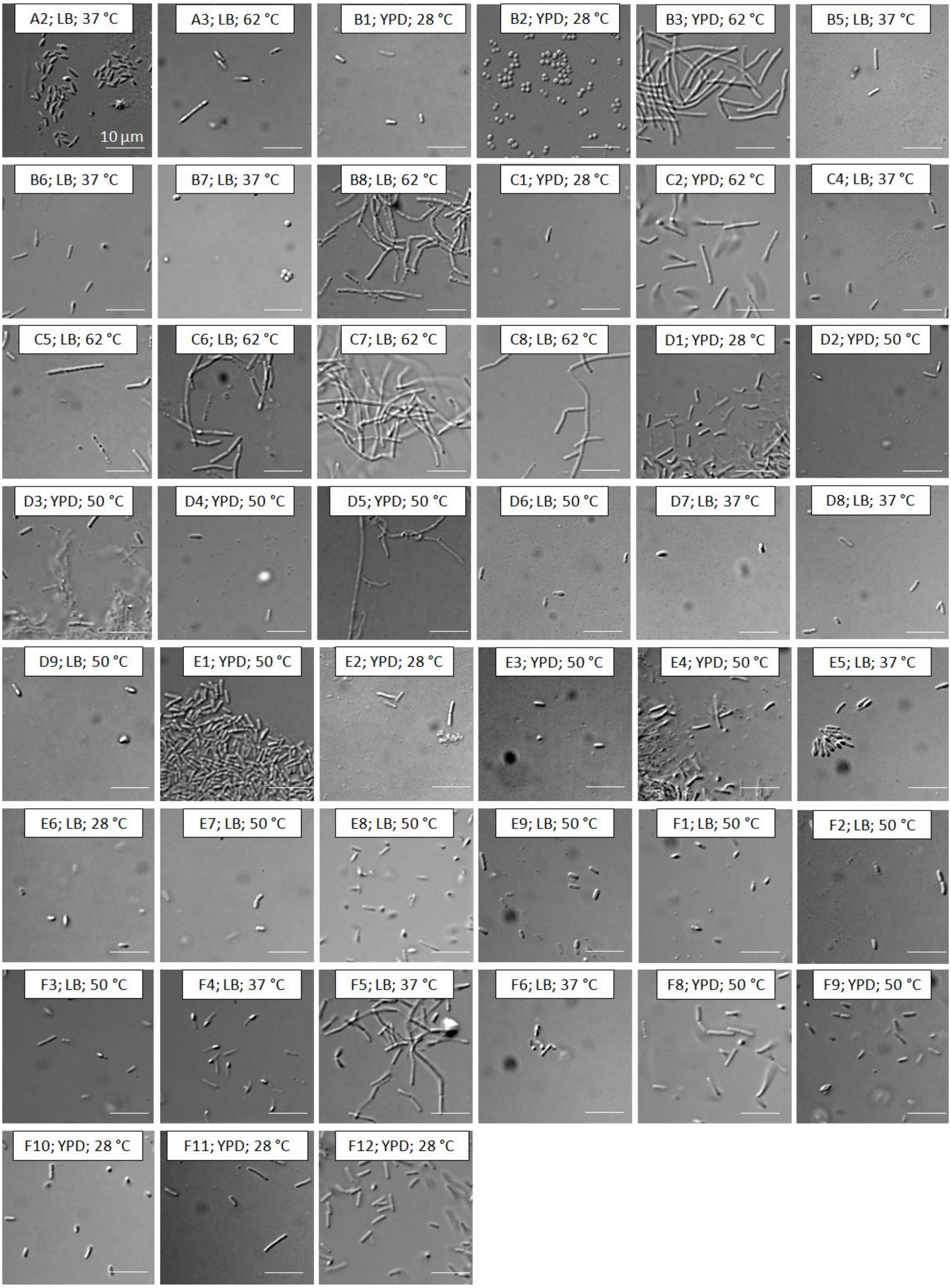


Figure 3. Visualization of isolated pure cultures (magnification 100x, scale 10 µm); photos labels show name of the sample, culture medium and incubation temperature used

From the isolated strains, on the basis of previously defined criteria (see section 2.3.2), those for which the determination to the species was carried out were selected (Table 3, Supplementary Material 3).

Table 3. Characteristics of strains selected for DNA sequencing; the asterisk marks the strains that were preserved by taking a large amount of cell mass from a freshly seeded plate, transferring the cell mass to 500 µL PF and adding 250 µL 50% glycerol

Strain	**Culture temperature, °C**	**Culture medium**
A2	62	LB
B3*	62	YPD
B6	37	LB
B8	62	LB
C2*	62	YPD
C5*	62	LB
C7	62	LB
D5	50	YPD
D6	50	LB
D9	50	LB
E5	50	LB
E6	50	LB
E8	50	LB
F4	50	LB
F5	50	LB

3.3.Identification of microorganisms from compost samples

For all of the selected isolates an partial 16S rDNA sequence was obtained. For one isolate DNA extraction had to be repeated after failure of initial 16S rDNA amplification attempt (sample D5). A total of 2 isolates possessed a 16S rDNA sequence with 100% similarity to characterized bacterial species; a 6 of them exhibited a ~99% similarity, 3 isolates ~98% and 5 of them ~97%. For sample F5 the analysis showed two possible strains. An analysis of 16S rDNA detemined that 93.7% of identified isolates belonged to group of gram-positive bacteria; only one gram-negative bacteria was found in B3 sample (Table 4).

Table 4. Identified bacteria strains from isolates; the asterisk marks the strains that were preserved by taking a large amount of cell mass from a freshly seeded plate, transferring the cell mass to 500 μL PF and adding 250 μL 50% glycerol

Strain	**Identified bacteria**	**Group**
A2	*Bacillus licheniformis*	Gram-positive
B3	*Sediminibacterium lactis*	Gram-negative
B6	*Paenibacillus lactis*	Gram-positive
B8	*Geobacillus stearothermophilus*	Gram-positive
C2	*Weizmannia coagulans*	Gram-positive
C5	*Bacillus licheniformis*	Gram-positive
C7	*Geobacillus thermodenitrificans*	Gram-positive
D5	*Streptomyces thermoviolaceus*	Gram-positive
D6	*Paenibacillus lactis*	Gram-positive
D9	*Bacillus haynesii*	Gram-positive
E5	*Paenibacillus lactis*	Gram-positive
E6	*Bacillus paralicheniformis*	Gram-positive
E8	*Bacillus licheniformis*	Gram-positive
F4	*Paenibacillus barengoltzii*	Gram-positive
F5	*Caldibacillus kokeshiiformis*	Gram-positive
	Bacillus thermolactis	

The analysis indicated that the same species of bacteria may be found in areas with lower and higher CO production in the compost pile. An example is *Bacillus licheniformis,* which was the most common bacterium found in isolates; it was identified in 3 samples A2, C5 and E8. A similar commonality between the material from the two pile sites was shown by the strain *Paenibacillus lactis,* which was identified in B6 and D6.

3.4.CO net production by isolated and identified strains of bacteria on a laboratory scale

3.4.1. Initial incubation

After 4 days of initial incubation of isolated strains, CO concentration in the headspace of the reactor varied from 0 to 891 ppm, with high results only when using SSF medium (Table 5). The only strain that produced CO in a liquid medium was *Bacillus licheniformis* (sample A2), producing CO at a concentration of 21 ppm in the headspace of the bioreactor. Increased CO production (>800 ppm) was always accompanied by high CO_2 concentrations (≥11.0%) and decreased O_2 levels (<10.0%). The exception was *Geobacillus thermodenitrificans* (sample C7); in the case of the bioreactor inoculated with this bacteria species, no CO production occurred under conditions of low oxygenation and high CO_2 concentration (6.3 and 13.4%, respectively). Milder anaerobic conditions were also present in the reactor inoculated with the aforementioned *Bacillus licheniformis*. CO production at the level of 21 ppm occurred at the concentration of 16.2% O_2 and 4.8% CO_2.

Table 5. Net production of process gases after initial incubation of isolated strains

Strain	**Culture medium**	**Identified bacteria**	**Temperature, °C**	**CO, ppm**	**O_2, %**	**CO_2, %**
A2	Liquid	*Bacillus licheniformis*	62	21	16.2	4.8
B3	SSF	*Sediminibacterium lactis*		745	8.7	12.0
B6	Liquid	*Paenibacillus lactis*	37	0	16.6	3.9
B8	Liquid	*Geobacillus stearothermophilus*	62	0	20.3	0.5
C2	SSF	*Weizmannia coagulans*		891	8.4	11.1
C5	SSF	*Bacillus licheniformis*		822	9.3	11.0
C7	Liquid	*Geobacillus thermodenitrificans*		0	6.3	13.4
D5	Liquid	*Streptomyces thermoviolaceus*	50	7	19.0	1.5

D6	Liquid	*Paenibacillus lactis*		0	20.0	0.4
D9	Liquid	*Bacillus haynesii*		5	7.2	12.4
E5	Liquid	*Paenibacillus lactis*		2	13.0	7.8
E6	Liquid	*Bacillus paralicheniformis*		0	20.8	0.2
E8	Liquid	*Bacillus licheniformis*		27	6.5	13.0
F4	Liquid	*Paenibacillus barengoltzii*		4	20.2	0.7
F5	Liquid	*Caldibacillus kokeshiiformis / Bacillus thermolactis*		4	16.0	4.2

For strains D-F, incubated at 50°C, lower CO production was observed. The concentration of this gas ranged from 0 to 27 ppm, with the highest values of 27, 7, and 5 ppm, respectively, recorded for *Bacillus licheniformis* (sample E8), *Streptomyces thermoviolaceus* (D5), and *Bacillus haynesii* (D9). For strains E8 and D9 again elevated CO concentration was present in the headspace of reactors with high CO_2 (>12.0%) and low O_2 (<8.0%) levels. The situation was different with the D5 strain; higher than in most samples CO concentration of 7 ppm was accompanied by a high level of oxygenation (19.0%).

Strains B3, C2, and C5, despite producing high concentrations of CO, were excluded from further analysis, because of their poor growth on both solid and liquid media which unabled the proper inoculation of compost material.

3.4.2. Production of process gases by selected strains

The concentration of CO produced by the selected strains was always higher in the cultures grown on the autoclaved medium. Its value ranged from ~30 to 175 ppm (autoclaved compost) and from 66 to 1 072 ppm (pasteurized compost, Table 6). The highest net CO production was recorded for *Bacillus paralicheniformis* (sample E6, 1 072 ppm), *Bacillus licheniformis* (sample A2, 823 ppm), and *Geobacillus thermodenitrificans* (sample C7, 599 ppm). In each of the analyzed cases, the concentration of CO was characterized by high variability (high standard deviation was noted, among others, for samples E6 or C7). For each of the strains producing the highest amounts of CO, a decrease in the concentration of O_2 in the headspace of the bioreactor (3.7, 6.0 and 5.3% for samples E6, C7 and A2, respectively) and a high concentration of CO_2 (range from 8.3 to 14.4% for these three samples) were also characteristic.

Table 6. Net production of process gases after incubation of isolated strains (average ± standard deviation); P – pasteurization, A – autoclaving

Strain	**Identified bacteria**	**Culture conditions**	**Temperature, °C**	**CO, ppm**	**O_2, %**	**CO_2, %**
Control	–	P	62	403 ± 346	7.7 ± 1.9	12.3 ± 1.7
		A		334 ± 268	13.3 ± 7.1	7.1 ± 6.6
A2	*Bacillus licheniformis*	P		823 ± 152	5.3 ± 1.7	14.4 ± 1.8
		A		137 ± 77	15.0 ± 7.8	5.5 ± 7.2
C7	*Geobacillus*	P		599 ± 493	6.0 ± 0.7	14.2 ± 0.6

	thermodenitrificans	A		175 ± 128	15.0 ± 7.7	5.6 ± 7.2
Control	–	P	50	569 ± 622	4.5 ± 1.1	15.3 ± 1.1
		A		152 ± 148	7.4 ± 5.7	12.6 ± 5.4
E6	*Bacillus paralicheniformis*	P		1072 ± 960	3.7 ± 0.4	8.3 ± 10.8
		A		53 ± 70	4.3 ± 0.1	15.5 ± 0.1
E8	*Bacillus licheniformis*	P		66 ± 70	5.5 ± 6.0	13.8 ± 6.0
		A		74 ± 65	4.1 ± 0.8	15.8 ± 0.8
F5	*Caldibacillus kokeshiiformis / Bacillus thermolactis*	P		316 ± 89	4.8 ± 3.2	15.1 ± 3.3
		A		33 ± 21	8.2 ± 7.9	12.0 ± 7.4

Correlation analysis between the concentration of CO (ppm) and the concentration of O_2 (%) and CO_2 (%) indicated a statistically significant relationship only in the case of *Bacillus licheniformis* (A2) and *Geobacillus thermodenitrificans* (C7); for both of them, it occurred using an autoclaved medium (Supplementary Material 4). For both strains, there was a strong negative correlation between CO concentration and O_2 concentration (r=-1.0000 and -0.9975 for A2 and C7, respectively) and a strong positive correlation with CO_2 concentration (r=1.0000 and 0.9971 for the same strains, respectively).

4. Discussion

Although the mechanism and effectiveness of composting depend mainly on the microorganisms present in the waste directed to this process, the enormity and variety of their types and species do not allow for a complete characterization of the bacterial community in the composted material. Despite many attempts to describe it, undertaken by scientists around the world, there are many gaps in knowledge regarding the coordinated action of bacteria during the biological decomposition of organic matter. One niche, which still leaves many questions, is the CO production by bacterial strains involved in composting.

The bacterial strains identified in this study were previously reported to be present in the composted waste. Depending on the substrates used in the process, the authors indicated such strains as *Bacillus kokeshiiformis* (marine animal resources compost **(Poudel *et al.,* 2014)**), *Geobacillus thermodenitrificans* (manure compost **(Charbonneau *et al.,* 2012; Daas *et al.,* 2018)**), *Bacillus licheniformis* and *Bacillus paralicheniformis* (agricultural waste, chicken manure, spent mushroom composts **(Kleyn and Wetzler, 1981; Sivakumar *et al.,* 2016; Kazeem *et al.,* 2021; Zalma and El-Sharoud, 2021)**). Also, the morphology of the identified strains is consistent with the observations of other researchers, including their shape (most of them were rod-shaped bacteria **(Poudel *et al.,* 2014; Daas *et al.,* 2018)**) and the color of the strains (e.g. *Bacillus licheniformis* identified in isolate C5 forming whitish colonies **(Kazeem *et al.,* 2021)**).

The most dominant bacterial species in compost in the studies presented here was *Bacillus licheniformis*; it occurred both in places with low and high CO production in the pile (samples A2, C5 and E8). This is in line with the observations of \ **(Kleyn and Wetzler, 1981)**, who studied microbial species in spent mushroom compost and the dust generated during its dumping. In both cases, *B. licheniformis* was the most common bacterial isolate. **(Zalma and El-Sharoud, 2021)**, also agree, calling *Bacillus* 'the most prevailing bacteria genus' in compost feedstock. They explain this situation by the ability of this species to produce spores, which enable it to survive under harsh environmental conditions. **(Sivakumar *et al.*, 2016)**, investigating a similar substrate (compost obtained from a mushroom company), are of the same opinion. They associated the presence of only *B. licheniformis* with a high temperature in the composted material and, at the same time, with the ability of this species to produce spores. Both of these aspects were observed in this study; the isolate marked as E8, which was identified as *B. licheniformis*, was spore-forming and the presence of this species was noted in both 50 and 62°C incubation samples. This indicates that heat-resistant spores contributed to its durability and translated into its widespread use. The frequent occurrence of *B. licheniformis* in isolates may also be related to the ability of this strain to break down biological materials such as cellulose, hemicellulose, and lignin **(Zalma and El-Sharoud, 2021)**. Settling of this species in the compost pile may therefore be the result of the type of substrates used in the process, i.e. green waste, containing, among others, branches or wood, providing *B. licheniformis* with nutrients.

The strains listed above have previously been reported in composts; however, they were not subject to analysis of process gas emissions during waste composting, including their share in net CO production in the compost pile. In addition, no sources dealing with the production of the CODH enzyme by any bacterial strains isolated from compost can be found in the literature. The aforementioned *B. licheniformis* has been analyzed for its ability to produce many of the most important enzymes for commercial use, but to the broad spectrum occupied by β-lactamase, α-amylase, alkaline protease, keratinase, chitinase, xylase, β-mannanase, α-amylase, endoglucanase **(Ghani *et al.*, 2013; Seo *et al.*, 2013; Zafar *et al.*, 2016; Muras *et al.*, 2021)**, the authors did not include experiments verifying its ability to produce CODH. This would be particularly important considering the fact that this species is widespread in the composted material, decomposes green waste most often directed to this process **(Zalma and El-Sharoud, 2021)**, and due to its characteristics (significant amount and variety of produced enzymes, their resistance to high temperature and broad range of pH **(Muras *et al.*, 2021)**), could play an important role in biorefinery systems aimed at CO production from biowaste, based on its biotechnological potential **(Kumar *et al.*, 2013)**.

Here, for the first time, the results of process gas production by bacterial strains with a high potential for CO production isolated from compost are presented. Due to the lack of analogous literature data allowing to compare the obtained concentrations of CO, $CO_{2,}$ and O_2, it became necessary to search for information on the relationship between CO metabolism and representatives of bacteria of the same genera. Thermophilic Bacilli, also discovered in the samples analyzed here (samples A2, C5, D9, E6, E8, F5) became of great importance in this context. A strain of *B. schlegelii* isolated from settling ponds of sugar factories was reported to be able to grow with CO as the sole energy and carbon source **(Krüger and Meyer, 1984)**. It is important that the optimum temperature for its growth was 65°C, and 50°C was the limit value below which it did not grow, i.e. the conditions were analogous to those prevailing in the places where samples were taken for the analyzes described here. In turn, during the research of **(Engel *et al.*, 1972)** on the bacterial production of CO from heme compound, *B. cereus* turned out to be the only strain capable of releasing this gas by metabolizing erythrocytes, hemoglobin, bilirubin, hematin, myoglobin, cytochrome c, iron hemato-, copper hemato-, and protoporphyrin **(Engel *et al.*, 1972)**. Another connection can be found between bacteria of the genus Streptomyces. In this study, their representative *Streptomyces thermoviolaceus* was reported in isolate D5. In turn, during the research of **(Gadkari *et al.*, 1990)** its relative *Streptomyces thermoautotrophicus*, isolated from burning charcoal pile, has been proven to be able to grow with CO from various sources (including wood combustion or car exhaust). Again, the optimal conditions for the growth of these bacteria coincided with the conditions prevailing in the area of the pile with lower CO production (>60°C). This is confirmed by the reports of **(King and Weber, 2007)**, according to which these bacteria prefer other organic sources of energy and carbon, but are also able to develop at a CO concentration exceeding 10%. Since CODH is known to be a bi-directional enzyme and the above strains have been identified as being able to metabolize and/or produce CO, it is possible that their activity is based on the use of this enzyme and thus are also able to perform reverse processes. In addition, due to the similarities resulting from the classification to the same genera, it is likely that the strains of *B. licheniformis*, *B. paralicheniformis*, *B. thermolactis*, or *S. thermoviolaceus* identified here are responsible for the higher CO concentrations recorded during their incubation in bioreactors.

The theory of CO production by isolated bacterial strains using the CODH enzyme is based on the observed relationships between CO, CO_2, and O_2 concentrations. Characteristic for the headspace of bioreactors with the highest net CO production was that the O_2 level there did not exceed 6%, while the CO_2 concentration remained high (8.3-14.4%). This was the case for each of the most effective CO 'producers' inoculated into pasteurized compost – *B. licheniformis, B. paralicheniformis,* and *G. thermodenitrificans* (strains E6, A2 and C7, respectively). High CO_2 levels in SSF cultures with simultaneous high CO concentrations may be due to CODH potentially metabolizing CO to CO_2. The bacteria producing this enzyme, capable of using CO as a source of carbon or energy, were provided with a substrate, thus driving the conversion of this gas. Thus, for samples A2 and C7, where the CO_2 concentration exceeds 14%, the CO content was lower (823 and 599 ppm, respectively) than for sample E6 (1072 ppm CO and 8.3% CO_2). A similar situation can be observed for *the B. thermolactis* strain (sample F5); while growing these bacteria on autoclaved compost, the CO concentration was about 10 times lower than when incubated on pasteurized material, but the relationship between CO production and CO_2 and O_2 concentrations was analogous to the E6, A2, and C7 strains. High levels of CO_2 may indicate that the conversion of CO to CO_2 has begun in both cases. However, it is worth emphasizing this trend that for strains with effective net CO production on pasteurized material, significantly lower CO concentrations were recorded when they were incubated on autoclaved compost. This may be related to the breakdown of key substances necessary for specific bacterial strains to develop, function, and/or produce CO. Such a situation, apart from the already mentioned strain *B. thermolactis* in sample F5, was also potentially reported for *B. paralicheniformis* (sample E6).

The different rates of the mentioned potential conversion of CO to CO_2 may be due to the differences between the CODH produced by different bacterial strains. This was confirmed by **(Kang *et al.*, 2021, 612)**, who proved that the mutant strain co-overexpressing CODH they constructed showed a 3.1 higher CO oxidation rate compared to the *Eubacterium limosum*. This may also be explained by the different structure of this enzyme in each of the bacterial strains; **(Ragsdale *et al.*, 1983)** observed differences in the size of the small subunit of CODH between *Clostridium thermoaceticum* and *Acetobacterium woodii*.

However, it should be remembered that bacterial cultures were incubated for 4 days, and the measurement of gas concentration in the headspace of bioreactors took place in the last 24 hours. It is therefore possible that the incubated bacterial strains may have produced more CO at the beginning of their growth, and then, after reaching the optimum, start converting it to CO_2. This idea may be justified in the case of thermophilic Bacilli discovered in the analyzed isolates. The reported time of their growth in the presence of CO and the temperature of 62°C was only 3 h **(Krüger and Meyer, 1984)**. In turn, **(Maness and Weaver, 2002)** reported an extremely fast rate of CO metabolism using CODH at 50°C. According to the authors, 87% of the dissolved CO was absorbed by *Rubrivivax gelatinosus* bacteria in 10 seconds. **(Parkin *et al.*, 2007)**, described the enzymatic bidirectional conversion of CO into CO_2 as fast, occurring at a frequency of 40,000 s^{-1}. Combining this information, it can be assumed that the strains identified in these studies could also start converting CO to CO_2 quite quickly after their rapid growth.

The aspect influencing the recorded concentrations of process gases and the effectiveness of individual strains in the net CO production could also be the isolation of microorganisms from each other. The cooperation of various bacterial strains is a well-known element affecting the effective biodegradation of various components of organic matter. During incubation in bioreactors, the individual strains could not cooperate with each other, which affected their activity. In the context of the research conducted here, special attention should be paid to *B. licheniformis*, for which interactions with other bacterial species as well as with algae have been reported. Positive effects of such combined systems have been observed not only during composting **(Nakasaki *et al.*, 1996; Zhang *et al.*, 2021)**, but also in the rhizosphere **(Ansari and Ahmad, 2019)** and during wastewater treatment **(Liang *et al.*, 2013)**. It has been proven that *B. licheniformis* is able to form mixed biofilm with *Pseudomonas fluorescens* **(Ansari and Ahmad, 2019)** and promotes the growth of *Chlorella vulgaris* **(Liang *et al.*, 2013)**, increasing vegetative growth, photosynthetic parameters of plants and the effectiveness of NH_4^+ removal from 1% to 29%. In turn, during composting, *B. licheniformis* played an important role in the initial phase of the process, during which it prevented the pH fall and thus stimulated the growth of other thermophilic bacteria **(Nakasaki *et al.*, 1996)**. This is in line with research conducted by **(Zhang *et al.*, 2021)**, who observed changes in the bacterial community due to the presence of *Bacillus*; along with them, the cellulose and hemicellulose decomposers *Atopostipes, Chryseolinea* and *Flavitalea* also appeared, increasing the efficiency of decomposing organic matter. This documented effective cooperation of *B. licheniformis* with other bacterial strains may also indirectly translate into CO production; during their separate incubation, the synergy effect may not have occurred, and the strains producing CO under normal conditions in the compost pile may not have reached the optimal conditions for the release of this gas.

What cannot be overlooked is the fact that in the control samples, incubated at both 50°C and 62°C, high CO concentrations were observed, reaching 569 and 403 ppm in the pasteurized material, respectively. Although pasteurization is recognized as one of the best ways to partially sterilize the material and is commonly used in the industry, its application may result in microbial spores still being present in the compost and the nutrients in the compost not being completely decomposed. On the other hand, lower concentrations of CO in control samples after autoclaving (152 and 334 ppm at 50°C and 62°C, respectively) suggest that under the influence of high temperature and pressure inorganic substances, potentially releasing CO into the headspace of the bioreactor, were degraded.

5. Conclusions

Research on the isolation and identification of microorganisms potentially responsible for CO production in the compost pile proved that all isolates were bacterial strains. Most of them were rod-shaped Gram-positive bacteria. Studies have shown that both places can be inhabited by the same bacterial strains, including *Bacillus licheniformis* and *Paenibacillus lactis*. The most common genus in the compost samples was *Bacillus* with representatives, such as *B. licheniformis*, *B. haynesii*, *B. paralicheniformis* and *B. thermolactis*. During the incubation of isolated bacteria, the highest CO concentrations were recorded in the headspace of bioreactors inoculated with *B. paralicheniformis* (>1000 ppm), *B. licheniformis* (>800 ppm), and *G. thermodenitrificans* (~600 ppm). High CO concentrations were accompanied by low O_2 levels (<6%) and high CO_2 levels (>8%).

The potential explanation of CO production by isolated bacterial strains based on their ability to produce CODH requires further research on a laboratory scale. It is necessary to analyze the expression of the gene responsible for encoding this enzyme to check ability of the identified strains to convert CO_2 to CO.

6. Statements

Originality-Significance Statement

According to the Web of Science collection, there are only 39 manuscripts in the area of carbon monoxide production during composting (keywords: “carbon monoxide” and “composting”). To our knowledge, the bacteria producing high concentrations of CO (>1,000 ppm) from compost have been identified for the first time. Although CO formation has been observed during composting of various fractions of organic waste and it has been reported as a biotic, studies characterizing the microbial communities responsible for CO production in compost cannot be found in the literature.

Availability of data and materials

All data generated or analysed during this study are included in this published article and its supplementary information files.

Competing interests

The authors declare that they have no competing interests.

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Authors' contributions

Conceptualization, K.S. and A.B.; methodology, K.S., D.D., A.K. and A.B.; investigation, K.S. and D.D.; resources, K.S.; data curation, K.S. and D.D.; writing—original draft preparation, K.S.; writing—review and editing, D.D., A.K. and A.B.; visualization, K.S. and D.D.; supervision, A.K. and A.B.; funding acquisition, K.S. All authors have read and agreed to the published version of the manuscript.

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Table and Figure Legends

Figure 1. Cross section of a compost pile with the location of gas and temperature sampling points (shallow measurements – blue and deep – red), based on (Stegenta et al., 2019)

Figure 2. Diagram of process gases measuring system; a) bioreactor with gas sampling system, 1 – compost sample with bacteria culture, 2 – glass vessel, 3 – metal cap with two nozzles, 4 – hose clamp, 5 – Hoffman clamp, 6 – silicon tube, 7 – gas concentration analyzer; b) photo of metal cap with connectors for gas sampling

Figure 3. Visualization of isolated pure cultures (magnification 100x, scale 10 μm); photos labels show name of the sample, culture medium and incubation temperature used

Table 1. The PCR protocol

Table 2. Characterization of substrates and compost samples, average ± standard deviation

Table 3. Characteristics of strains selected for DNA sequencing; the asterisk marks the strains that were preserved by taking a large amount of cell mass from a freshly seeded plate, transferring the cell mass to 500 µL PF and adding 250 µL 50% glycerol

Table 4. Identified bacteria strains from isolates; the asterisk marks the strains that were preserved by taking a large amount of cell mass from a freshly seeded plate, transferring the cell mass to 500 µL PF and adding 250 µL 50% glycerol

Table 5. Net production of process gases after initial incubation of isolated strains

Table 6. Net production of process gases after incubation of isolated strains (average ± standard deviation); P – pasteurization, A – autoclaving

Supplementary Materials:

File name: Supplementary Material 1

File format: .xlsx

Title of data: The concentration of CO (ppm), CO_2, and O_2 (%) and temperature (°C) at measurement points in the tested pile

Description of data: Excel file with three sheets: 1) Readme, 2) Left side of the pile, 3) Right side of the pile. File shows composting process gases measurement procedure, research conditions, composting pile characterization and measured concentrations of CO (ppm), CO_2, and O_2 (%) and temperature (°C).

File name: Supplementary Material 2

File format: .docx

Title of data: Photographic documentation of the plates on which the growth of microorganisms was observed during initial incubation along with the marking of the dilution, type of the medium (LB or YPD) and incubation temperature used

Description of data: Word file with photographic documentation for compost material taken from area of the pile with low CO production (17 photos) and compost material taken from area of the pile with high CO production (21 photos).

File name: Supplementary Material 3

File format: .docx

Title of data: Table 1. Characteristics of strains selected for DNA sequencing; the asterisk marks the strains that were preserved by taking a large amount of cell mass from a freshly seeded plate, transferring the cell mass to 500 µL PF and adding 250 µL 50% glycerol

Description of data: Table showing photos of growth on a solid and liquid medium for strains A2, B3, B6, B8, C2, C5, C7, D5, D6, D9, E5, E6, E8, F4, F5 with culture temperature and medium (LB/YPD) used.

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Appendix No. 3

Karolina Sobieraj
(Name and surname)

Wrocław, 22.06.2023
(Place and date)

Department of Applied Bioeconomy
Wrocław University of Environmental and Life Sciences
Wrocław, Poland
(Affiliation)

STATEMENT

I hereby declare that in the publication:

Karolina Sobieraj, Daria Derkacz, Anna Krasowska, Andrzej Białowiec, 2023. *Isolation and identification of CO-producing microorganisms from compost*, Environmental Microbiology Reports (under review)

my contribution included:
preparation of a graphic abstract of the article. I prepared the text of the entire article, including the introduction part, description of the methodology, materials, and description of the results with discussion and conclusions. I prepared the figures and tables presented in the article, excluding Figure 3. I obtained funds for the research and publication of the article. I was also the corresponding author.

Signed by /
Podpisano przez:

Karolina Natalia
Sobieraj

Date / Data:
2023-06-22 21:05

……………………………………..
(Date and signature)

Appendix No. 3

Daria Derkacz
(Name and surname)

Wrocław, 20.06.2023 r.
(Place and date)

Faculty of Biotechnology
Department of Biotransformation
University of Wrocław
Wroclaw, Poland
(Affiliation)

STATEMENT

I hereby declare that in the publication:

Karolina Sobieraj, Daria Derkacz, Anna Krasowska, Andrzej Białowiec, 2023. *Isolation and identification of CO-producing microorganisms from compost*, Environmental Microbiology Reports (under review)

my contribution included:

the revision of the manuscript, in particular parts regarding the isolation and identification of microorganisms in compost samples, such as sections: *2.3.1. Used chemicals*, *2.3.2. Microorganisms isolation procedure*, *2.3.3. DNA Isolation for identification of microorganisms from compost samples*, and *2.4. CO production by isolated strains of bacteria on a laboratory scale*.

I prepared a visualization of isolated pure cultures presented in Figure 3, photographic documentation of the plates on which the growth of microorganisms was observed during initial incubation (Supplementary material 2), and characteristics of strains selected for DNA sequencing (Supplementary material 3).

20.06.2023r. Daria Derkacz
(Date and signature)

Appendix No. 3

Anna Krasowska Wrocław, 16.06.2023
(Name and surname) (Place and date)

Faculty of Biotechnology
Department of Biotransformation
University of Wrocław
Wroclaw, Poland
(Affiliation)

STATEMENT

I hereby declare that in the publication:

Karolina Sobieraj, Daria Derkacz, Anna Krasowska, Andrzej Białowiec, 2023. *Isolation and identification of CO-producing microorganisms from compost*, Environmental Microbiology Reports (under review)

my contribution included:
developing the concept and design of the study, in particular, the methodology of isolation and identification of microorganisms from compost samples. I participated in the revision of the manuscript.

Signed by /
Podpisano przez:

Anna Janina
Krasowska
Uniwersytet
Wrocławski

Date / Data:
2023-06-16 14:47

………………………………..
(Date and signature)

Appendix No. 3

Andrzej Białowiec
(Name and surname)

Wrocław, 21.06.2023
(Place and date)

Department of Applied Bioeconomy
Wrocław University of Environmental and Life Sciences
Wrocław, Poland
(Affiliation)

STATEMENT

I hereby declare that in the publication:

Karolina Sobieraj, Daria Derkacz, Anna Krasowska, Andrzej Białowiec, 2023. *Isolation and identification of CO-producing microorganisms from compost*, Environmental Microbiology Reports (under review)

my contribution included:
developing the concept and design of the study, in particular, the methodology of measurements and sampling during technical scale composting. I participated in the revision of the manuscript. While working on the article, I took part in English language editing.

Signed by /
Podpisano przez:

Andrzej Białowiec

Date / Data:
2023-06-21
08:20

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(Date and signature)

energies

Article

Modeling of CO Accumulation in the Headspace of the Bioreactor during Organic Waste Composting

Karolina Sobieraj [1], Sylwia Stegenta-Dąbrowska [1], Jacek A. Koziel [2] and Andrzej Białowiec [1,2,*]

[1] Department of Applied Bioeconomy, Wrocław University of Environmental and Life Sciences, 37a Chełmońskiego Str., 51-630 Wrocław, Poland; karolina.sobieraj@upwr.edu.pl (K.S.); sylwia.stegenta@upwr.edu.pl (S.S.-D.)

[2] Department of Agricultural and Biosystems Engineering, Iowa State University, 605 Bissell Road, Ames, IA 50011, USA; koziel@iastate.edu

* Correspondence: andrzej.bialowiec@upwr.edu.pl; Tel.: +48-71-320-5973

Abstract: Advanced technologies call for composting indoors for minimized impact on the surrounding environment. However, enclosing compost piles inside halls may cause the accumulation of toxic pollutants, including carbon monoxide (CO). Thus, there is a need to assess the occupational risk to workers that can be exposed to CO concentrations > 300 ppm at the initial stage of the process. The objectives were to (1) develop a model of CO accumulation in the headspace of the bioreactor during organic waste composting and (2) assess the impact of headspace ventilation of enclosed compost. The maximum allowable CO level inside the bioreactor headspace for potential short-term occupational exposure up to 10 min was 100 ppm. The composting was modeled in the horizontal static reactor over 14 days in seven scenarios, differing in the ratio of headspace-to-waste volumes (H:W) (4:1, 3:1, 2:1, 1:1, 1:2, 1:3, 1:4). Headspace CO concentration exceeded 100 ppm in each variant with the maximum value of 36.1% without ventilation and 3.2% with the daily release of accumulated CO. The airflow necessary to maintain CO < 100 ppmv should be at least 7.15 $m^3 \cdot (h \cdot Mg\ w.m.)^{-1}$. The H:W > 4:1 and the height of compost pile < 1 m were less susceptible to CO accumulation.

Keywords: carbon monoxide; composting; emission modeling; bioreactors; employees safety; occupational health and safety

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1. Introduction

Large-scale composting of organic waste, including sewage sludge and agricultural waste, has become a widely used method [1]. Composting takes place both outdoors and indoors in composting halls, where the organic waste is formed into long piles, as well as in closed reactors, also known as in-vessel systems [2]. Enclosing compost piles inside halls is considered the best available technology (BAT) [3]. However, this technology can pose a risk of exposure of employees and nearby residents to gaseous emissions. Carbon monoxide (CO) is rarely reported in the context of composting.

A variety of gaseous pollutants are generated during the decomposition of organic waste, such as volatile organic compounds (VOCs), odors, bioaerosols (bacteria and their endotoxins, protozoan parasites, allergic fungi), and dust [4–6]. The amount and type of pollution generated may vary depending on the feedstock, the composting technology, and in the case of an outdoor process, on atmospheric conditions [7]. Toxic substances may be released during each of the routine operations carried out in the composting plant, starting with the receipt of fresh material, sorting, shredding, composting, turning, compost maturation, and transport [8–11]. Thus, composting plant workers are subjected to various occupational risks, including inhalation risk depending on the tasks [12]. Therefore, mitigation of gaseous and dust emissions should be considered to improve employees' occupational health and safety and well-being [9].

CO is proven to be generated in compost, and this gas is one with the deadliest potential via inhalation. There is no research on CO emissions from composting waste and its harmfulness in the context of composting plant workers' occupational safety, except for the several studies identifying the presence of CO in the composting process gas. There are few high-quality methodological epidemiologic studies of long-term occupational exposure to CO [13].

CO is formed due to the biological decomposition of organic matter and CO_2, CH_4, H_2, N-containing compounds, volatile organic compounds (VOC), or H_2S [14]. CO emissions have been observed during composting of green waste [15], green waste with manure [16], organic waste [14], and municipal waste. In addition, our research carried out at the green waste with sewage sludge composting plant proved that CO accumulates in the composted material, exceeding the concentration of 300 ppm [17,18].

Due to the lack of taste, color, and smell, CO is called the "silent killer" [19]. Its high toxicity to the human body results from a 200× higher affinity for hemoglobin compared to oxygen [20]. When inhaled, it forms carboxyhemoglobin (COHb), causing cell hypoxia and, consequently, even death [21]. CO poisoning's initial symptoms are difficult to diagnose, often mistakenly attributed to influenza, food poisoning, gastritis, enteritis, or fatigue [22,23]. Acute CO poisoning is accompanied by upper respiratory tract infection, shortness of breath, lethargy, hallucinations, dizziness and headache, blurred vision, vomiting and diarrhea, as well as urinary incontinence, and gait and memory disorders [19]. However, long-term human exposure to CO can cause atherosclerosis, arterial disease and oxidative stress and manifest as angina, myocardial infarction, and reduced exercise capacity [13,24]. Chronic exposure to CO can impair cognitive function and gradually develop into mental symptoms [22,23].

According to the EU, the BAT Reference Document for Waste Treatment calls for the hermitization of composting plants [3]. While this directive can lower the ambient environment impact, the enclosed composting process can result in unwanted consequences such as toxic pollutant accumulation. CO is heavier than air, and therefore it can accumulate quickly even in well-ventilated closed areas [19]. The emissions of CO and other pollutants can exceed threshold values and occupational risk to compost plant workers. The immediately dangerous to life and health (IDLH) threshold is set at 1200 ppm (0.12%), while the ceiling threshold that should never be exceeded during 10 h workdays is 200 ppm. The chronic permissible exposure limit (PEL) for 8 h workdays is set at 50 ppm by the U.S. Occupational Health and Safety Administration [25].

Our previous measurements of CO inside compost piles show typical concentrations close to 100 ppm, sometimes exceeding 200 or 300 ppm, especially at the initial stage of the process [18]. It is also worth considering that the plant workers with significant physical activity are exposed to even greater risks of inhaling CO due to the increased frequency and depth of breathing. Assuming a CO concentration of 100 ppm, an average worker will experience a slight headache. After exposure to 200–300 ppm for 5–6 h, the headache becomes pronounced, and symptoms include nausea, general fatigue, and dizziness [26]. Exposure to concentrations close to 400 ppm CO for 3 h is life-threatening.

Although previous studies have shown that CO accumulates to high concentrations during waste composting inside piles [17,18,27], there is still no information about the occupational hazard of composting plant workers in the context of CO inhalation exposure. There are no sources in the literature about possible CO levels in composting plants, and no models have been developed predicting CO concentration depending on various parameters of the composting process. Furthermore, plant managers need to have practical information to mitigate the risks. These include adjusting the airflow of ventilation air, continuous air quality monitoring, personal exposure monitoring, and compost management (feedstock quality, compost pile size, frequency of turnovers). Here, for the first time, we present a tool that allows optimizing the composting process in terms of emission of harmful and dangerous gas—CO. Thus, we fill the gap left by other researchers, enabling not only to improve the process itself, but above all to ensure the safety of employees involved in the biological treatment of biowaste in closed bioreactors.

The objectives of this study were to (1) develop a model of CO accumulation in the headspace of the bioreactor during organic waste composting and (2) assess the impact of ventilation in compost headspace. A 100 ppm CO limit threshold value for up to 10 min was set to perform daily bioreactor maintenance. The composting process was modeled in the horizontal static reactor over 14 days. Seven different process scenarios were considered with the decreasing headspace-to-waste volumes (H:W) ratio in the reactor and the ventilation rate. Thus, we propose conducting CO accumulation modeling during organic waste composting for the first time. The result will inform further field trials and the development of recommendations for composting plant workers and managers.

2. Materials and Methods

2.1. Experimental Setup

The experimental setup is presented in Figure 1. The organic waste was a 1:1:1 mix (by mass) of dairy cattle manure, grass clippings, and pine sawdust. The experiments were performed in triplicates at 10, 25, 30, 37, 40, 50, 60, and 70 °C. The tests were carried out in 1 L reactors according to [28] at a constant setpoint temperature in the climatic chamber POL-EKO, model ST-3, Wodzisław Śląski, Poland. A detailed description of the methodology used is provided elsewhere [29]. The kinetics data for CO production rates during composting of organic waste were also reported elsewhere [29].

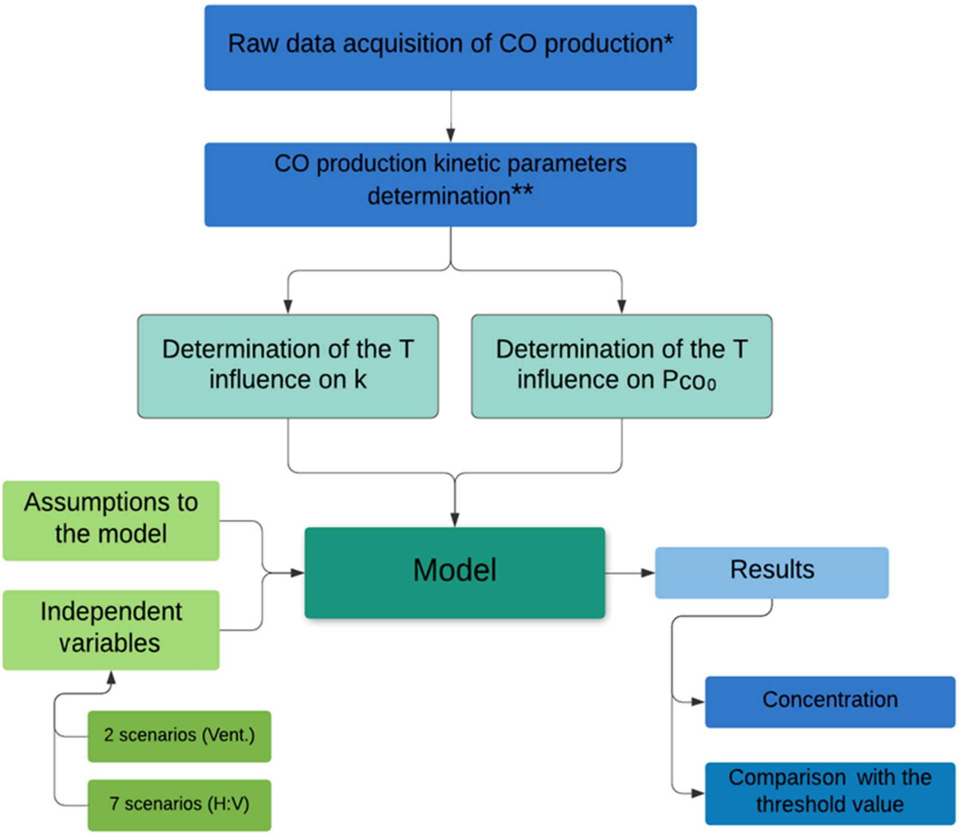

Figure 1. Model outline for prediction of CO accumulation during composting of biowaste under different scenarios of compost headspace ventilation and temperature; * data published in [27], ** estimated using raw data in [29].

2.2. Model of CO Accumulation: Inputs

All details of inputs used in model calculations presented in Supplementary Material (“Inputs” sheet) are as follows:

- the duration of the composting, d
- bulk density of the organic waste, kg·m^{-3}
- the volume of the bioreactor, m^3
- the volume of organic waste in the bioreactor, m^3
- the volume of the headspace (above the organic waste), m^3
- organic waste dry matter in reactor, kg
- CO concentration threshold value defined as a maximum desired CO level inside the bioreactor headspace for potential short-term occupational exposure, ppm
- the daily mean organic waste temperature during composting, °C.

2.2.1. Waste Characteristics

The bulk density of 460 kg·m^{-3} was assumed based on our extensive research focused on a large municipal-scale composting of biowaste and sewage sludge [17]. The assumed value is consistent with the organic waste bulk density ranging from ~200 to over 500 kg·m^{-3} [30]. A 50% dry matter content in the composted material was assumed based on [31], who reported 40~60% optimum humidity for the aerobic composting process.

The model used measured waste temperature during composting from Day 0 to Day 14. The values were calculated as the arithmetic means of the results obtained in the research on biowaste composting in a monitored compost bin [32] and dairy manure with sawdust [33] (Figure 2). The modeling was done only for the Day 0 to 14 period, because CO production consistently peaks during the first two weeks of the composting process [34].

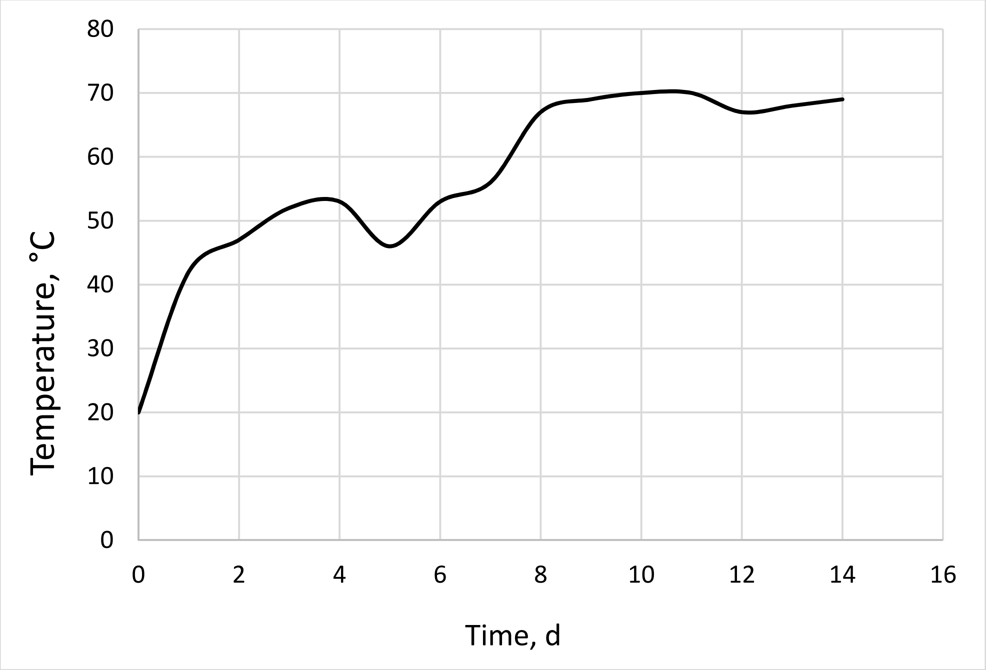

Figure 2. The typical temperature pattern during composting was used for CO accumulation modeling [32,33].

2.2.2. Composting Method

For the modeling purposed, it was assumed that organic waste was composted in relatively large, enclosed bioreactors used by waste management plants. The bioreactors were modeled as operating with and without ventilation and removal of process gases from the headspace; therefore, effectively modeling the accumulation of CO released from piles into the headspace. Headspace ventilation in the modeling is considered as the exchange of air collected in the bioreactor over the composted waste and is not synonymous with compost aeration, which is based on the forced introduction of air into the process, typically from the bottom of the pile. The process lasted 14 days and took place in the horizontal static reactor in a rectangular cross-section tunnel with forced aeration from the bottom. In practice, the "tunnel" ends serve as the feedstock inlet and processed compost outlet. The volume of this type of reactor can range from 10 to 500 m^3 [35]. A $5 \times 5 \times 20$ m (width × height × length), 500 m^3 total volume was assumed, resembling the [36,37].

The accumulation of CO in the headspace of the bioreactor was modeled in two scenarios for ventilation:

(a) no ventilation of bioreactor headspace through the entire process (with vigorous ventilation at the end of the process by opening the bioreactor before removal of stabilized product—the compost);
(b) with ventilation defined as a short, daily opening of the bioreactor to release accumulated CO and lower the concentration of CO in the headspace to the atmospheric level, followed by a bioreactor's closure.

For both scenarios (a) and (b):

(c) seven ratios of headspace-to-waste volume (H:W) in the reactor (4:1, 3:1, 2:1, 1:1, 1:2, 1:3, 1:4, and H:W, respectively) were considered for modeling of CO accumulation.

CO is considered a trace gas in the atmosphere; according to [38], global CO concentration background ranges between 0.05–0.12 ppm and estimated daily concentration ~8 ppm [39,40]. These levels were considered negligible, so the ambient CO levels were omitted in the modeling. The CO density of 1.145 $kg \cdot m^{-3}$ at 25 °C and 1 atm was used [41].

2.2.3. CO Inputs

In general, indoor CO thresholds are defined by numerous standards [42]. Most use the so-called 8-h time-weighted average concentration, ranging from 35 ppm [43], 50 ppm (in case of the chronic permissible exposure limit, PEL) [44], up to 10 $mg \cdot m^{-3}$ (~87 ppm) [41,45]. The immediately dangerous to life and health (IDLH) threshold is set at 1200 ppm (0.12%). For a longer period (10 h workdays), the ceiling threshold should never exceed 200 ppm [25].

Here, the 100 ppm of CO was adopted as a threshold value, defined as a maximum desired CO level inside the bioreactor headspace for potential short-term occupational exposure up to 10 min needed to perform short bioreactor maintenance works. The CO threshold value was estimated according to WHO guidelines, which are based on the maximum COHb level in the blood of people doing moderate physical work (90 ppm for 15 min) [38]. Moreover, CO concentration of 100 ppm is referred to as leading to some of the first symptoms of CO poisoning, like headaches [46,47]; higher values (~200 ppm) already lead to intoxication symptoms such as nausea and dizziness [48]. However, the Excel spreadsheet (Supplementary Materials) allows the modeling of all possible CO concentration scenarios and can be adjusted to adopted guidelines for the maximum gas threshold.

2.3. Analytical Procedures

Data for kinetic modeling of CO production during composting of organic waste were analyzed, excluding the lag-phase in microbial activity [49]. Nonlinear least-squares regression was used to determine the kinetic parameters of CO production, and the first-order reaction model was used [50]:

$$P_{CO} = P_{CO0} \cdot \left(1 - e^{-k \cdot t}\right) \tag{1}$$

where:

P_{CO}—cumulative CO production, $\mu g \cdot g^{-1}$d.m., at the given time, t
P_{CO0}—maximum CO production, $\mu g \cdot g^{-1}$d.m.
k—CO production constant rate, h^{-1}
t—time, h.

The CO kinetic parameters were determined based on the raw data published elsewhere [27,29].

Equations describing the influence of the composting temperature (T) on CO production constant rate (k) had a form of a polynomial to which regressions were fitted:

$$y = a1 + a2 \cdot x + a3 \cdot x^2 + a4 \cdot x^3 + a5 \cdot x^4 \tag{2}$$

where:

y—k
x—T
$a1$—intercept,
$a2$–$a5$—regression coefficients.

Equation (2) was used to estimate the constant rate (k) for different temperatures in the process (example shown in Figure 2). The influence of the temperature on (k) is described by Equation (3) and illustrated in Figure 3:

$$k = (-0.0043) + (0.0014) \cdot T + \left(9.73 \cdot 10^{-6}\right) \cdot T^2 + \left(-1.15 \cdot 10^{-6}\right) \cdot T^3 + \left(1.071 \cdot 10^{-8}\right) \cdot T^4 \tag{3}$$

where:

T—composting process temperature, °C

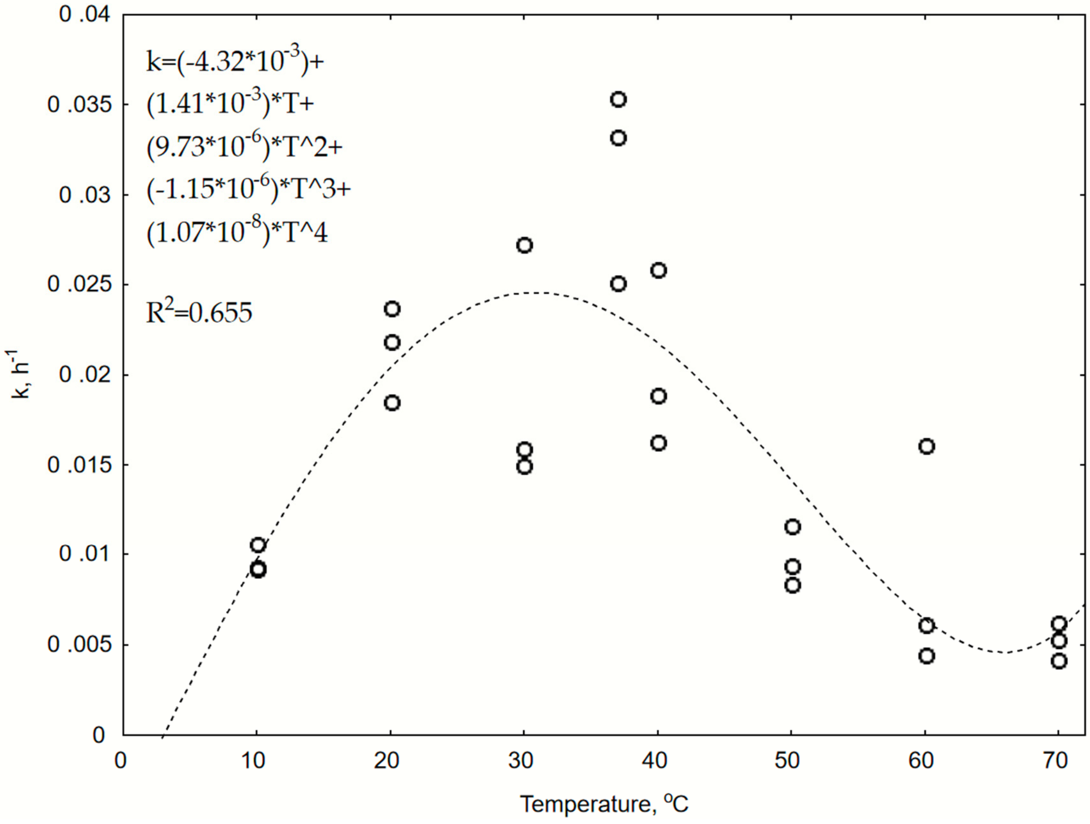

Figure 3. The influence of the composting temperature (T) on CO production constant rate (k).

The CO potential production (P_{CO}) as a function of (T) was described by Gompertz's model [51] (Equation (4)) and illustrated in Figure 4.

$$P_{CO} = 176.81 \cdot e(-e\,(-(0.1147) \cdot (T - (47.545)))) + (24.4963) \tag{4}$$

where:

T—composting process temperature on a particular day (from Day 1 to 14, °C).

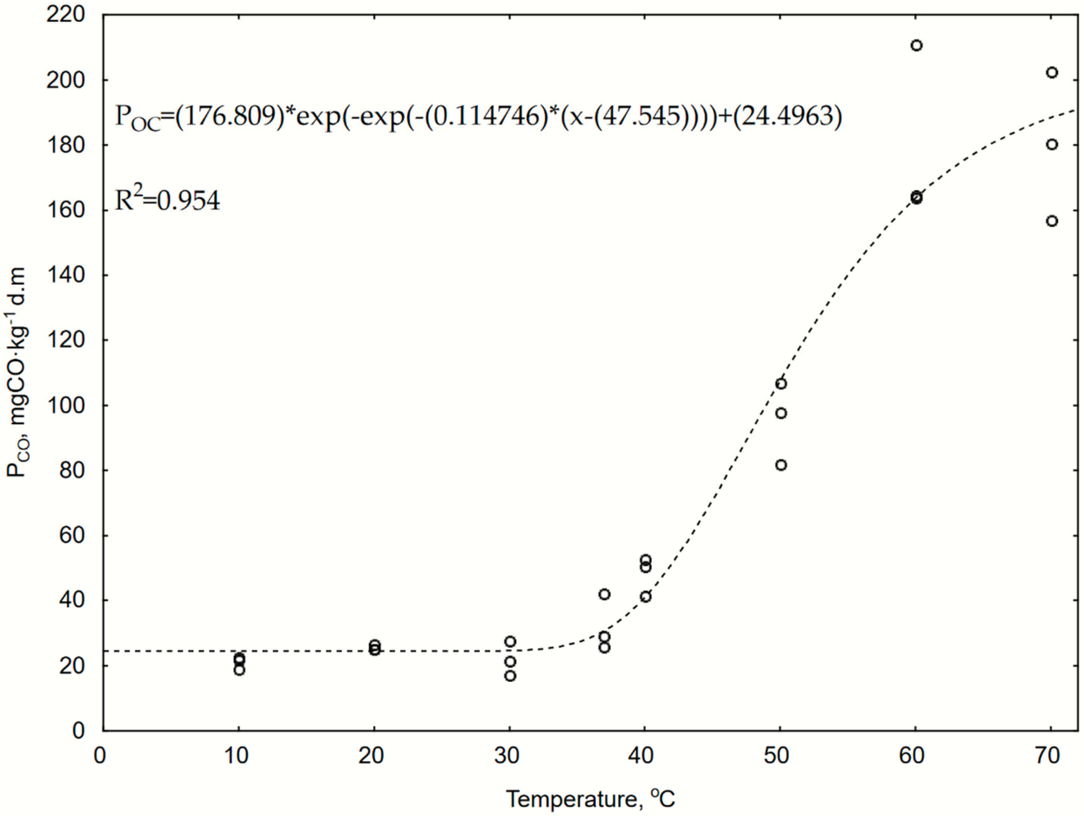

Figure 4. The influence of the composting temperature (T) on CO production potential (P_{CO}).

The prediction of CO concentration in a headspace was made with (k) estimation, according to Equation (3) and (P_{CO}) according to Equation (4). Mass of CO emitted from organic waste during a day (µg·d^{-1}) was calculated according to first-order kinetic:

$$M_{CO} = P_{CO} \cdot m_{dm} \cdot \left(1 - e^{-k}\right) \cdot 24 \tag{5}$$

where:

M_{CO}—the mass of CO emitted from organic waste during a day, mg·d^{-1},
m_{dm}—organic waste, dry mass, kg.

CO mass was then converted to normalized volume and changed to the CO concentration in the headspace in ppm according to [52]:

$$C_{gas} = \frac{M_{COc}}{V_h} \tag{6}$$

where:

C_{gas}—CO concentration, mg·m^3,
M_{COc}—the mass of CO accumulated, mg,
V_h—the volume of the headspace (above the organic waste), m^3,

$$C_{ppm} = C_{gas} \cdot \frac{R \cdot T_r}{MW \cdot P} \tag{7}$$

where:

C_{ppm}—gas concentration in parts per million, ppmv,
R—ideal gas law constant, R = 8.314 m^3·Pa·K^{-1}·mol^{-1},
P—atmospheric pressure, P = 101.32 kPa,
T_r—the temperature in the reactor, K,

MW—molecular weight of CO (g·mol^{-1}).

The rate of air exchange to maintain the CO concentration threshold value in the headspace (h^{-1}) was calculated according to:

$$r_{air} = \frac{C_{CO}}{C_{COmax} \cdot 24} \tag{8}$$

where:

C_{CO}—CO concentration in the headspace, ppm,

C_{COmax}—CO concentration threshold value, ppm.

The obtained rate of air exchange was also referred to as (i) the volume of air used to aerate the bioreactor per hour, m^3·h^{-1} (Equation (9)), and (ii) the volume of air to aerate the bioreactor per hour per one ton of wet mass of waste, m^3·(h·Mg w.m.)$^{-1}$ (Equation (10)), (Supplementary Materials—'4:1, 3:1, 2:1, 1:1, 1:2, 1:3, 1:4′ sheets).

$$r_V = r_{air} \cdot V_h \tag{9}$$

where:

r_V—air exchange rate referred to the volume of air per hour, m^3·h^{-1},

r_{air}—air exchange rate, h^{-1},

V_h—the volume of the headspace, m^3.

$$r_{Vm} = \frac{r_V}{m_{w.m.}} \tag{10}$$

where:

r_{Vm}—air exchange rate referred to the volume of air per hour per one ton of wet waste mass, m^3·(h·M gw.m.)$^{-1}$,

r_V—air exchange rate referred to the volume of air per hour, m^3·h^{-1},

$m_{w \cdot m \cdot}$– the mass of waste, Mg (wet basis).

Based on the assumed dimensions of the bioreactor and the input volume of waste in each of the considered variants, the height of the waste pile was determined (Supplementary Materials —"Height of waste pile" sheet).

All modeling of CO accumulation in the headspace of the bioreactor during organic waste composting was carried out using the Statistica software 13.3 (TIBCO Software Inc., Palo Alto, CA, USA) and Microsoft Excel spreadsheet (Supplementary Materials).

3. Results

3.1. CO Accumulation in the Headspace of the Bioreactor Without Ventilation

In each of the analyzed cases, the concentration of CO in the bioreactor headspace without ventilation significantly exceeded the accepted threshold value of 100 ppm. This concentration increased with the decrease of H:W, reaching on the first day from 1330 ppm for the lowest H:W (4:1) up to 21,200 ppm for the highest H:W (1:4) (Tables S4 and S16).

The considered scenario of CO accumulation in the headspace shows that in the event of a failure of the headspace ventilation system, the CO concentration reaches dangerous values for employees already within the first 24 h. The CO concentration can increase in the first week of the process from 12,750 ppm (H:W 4:1) to ~204,000 ppm (H:W 1:4) (Tables S4 and S16). Finally, on the 14th day (end of composting), the CO concentration will reach 22,500 ppm for the lowest H:W (4:1) to 361,000 ppm for the highest H:W (1:4) (Tables S4 and S16).

The CO concentration accumulated in the unventilated bioreactor headspace on the 14th day of the composting process reaches a value about 11 times higher than the highest concentration of this gas in the variant with ventilation, falling for each option on the third day of the process. Comparing the final values of both variants (without and with headspace ventilation), the CO concentration in the first case is about 17 times higher (Figures S3, S6, S9, S12, S15, S18, and S21).

3.2. CO Concentration in the Bioreactor Headspace with the Daily Release of Accumulated Gas

Modeling showed that even the daily release of gas accumulated in the bioreactor headspace is not sufficient to lower the CO concentration above the compost below 100 ppm (Figure 5). The daily CO concentration increased with the elevation in the proportion of composted waste, reaching the highest values for the variant with the lowest H:W (1:4).

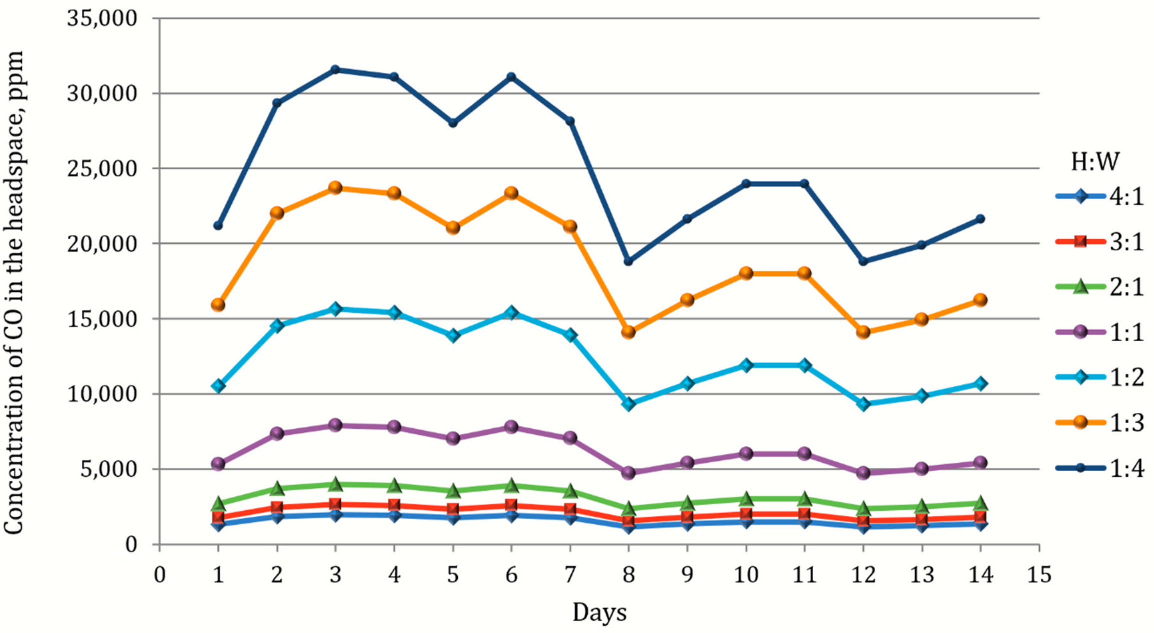

Figure 5. CO concentration in the daily release of gas accumulated in the bioreactor headspace.

In each of the considered variants, the headspace CO concentration was the highest in the first week of the process, reaching maximum on the third day ranging from 1970 ppm for H:W 4:1 to 31,600 ppm for an H:W ratio of 1:4 (Figure 5). The minimum production was recorded in all variants on the eighth day of composting. The CO concentration on the last day of the process for each case was close to the values on the first day of composting.

The CO concentration was characterized by the largest amplitude between the seventh and eighth day, with the difference between the values being greater for the lowest H:W of 1:4 (~9340 ppm). Daily fluctuations in CO concentration were generally greater for bioreactors with an increasing share of waste, especially for variants in which waste dominated (H:W of 1:2, 1:3, 1:4).

3.3. Air Exchange Rate to Mitigate CO Accumulation

The next stage of research was to model and find the continuous air exchange rate that could theoretically maintain CO concentrations in the bioreactor headspace below the assumed threshold of 100 ppm. The rate of air exchange correlated with the concentration of CO increased with the decrease of H:W (Figure 6). For the highest material load in the reactor (variant H:W 1:4), the air exchange rate was 16× greater than the analogous values achieved for the highest H:W (4:1). Among all the considered variants, for only H:W of 4:1, the rate of air exchange on each day of the process was <1.0 h^{-1} (Table S5). Similar lower values were also obtained in the second half of the process in the H:W 3:1 variant, when the CO concentration did not exceed 2000 ppm (Table S7). In the other variants, the air exchange rate varied in the min–max ranges 0.99–1.66, 1.96–3.29, 3.88–6.52, 5.88–9.87, and 7.8–13.2 h^{-1} for headspace to waste ratios 2:1, 1:1, 1:2, 1:3, and 1:4, respectively (Tables S9, S11, S13, S15, and S17).

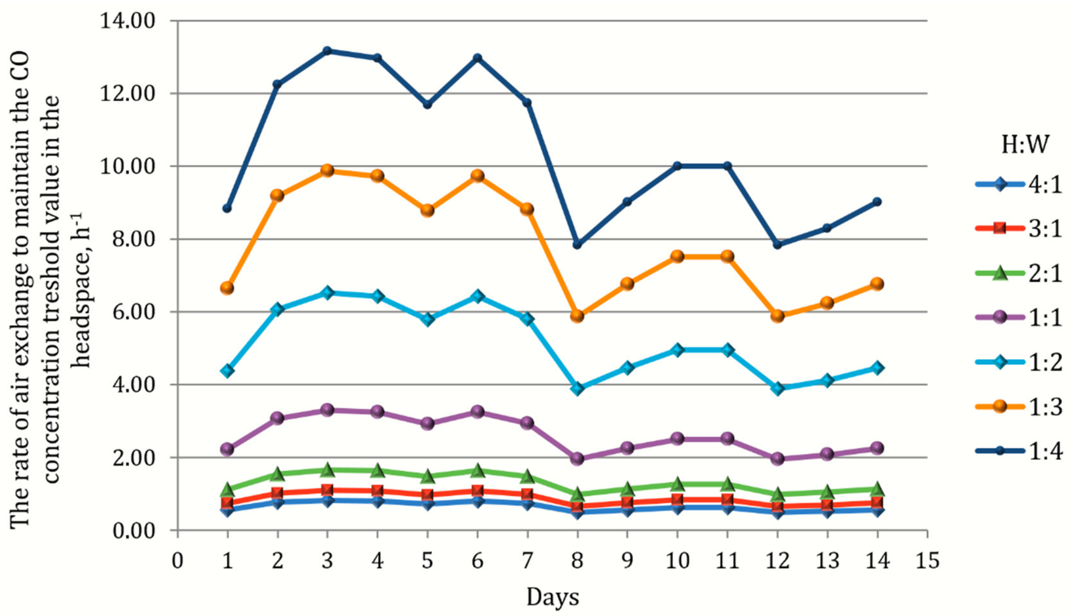

Figure 6. Rate of air exchange to mitigate CO accumulation and maintain the headspace CO concentration below the 100 ppm threshold.

The minimum level of air exchange necessary to maintain the CO concentration below the limit value (100 ppm) increased with decreasing H:W (from 196 $m^3 \cdot h^{-1}$ for H:W = 4:1 up to 784 $m^3 \cdot h^{-1}$ for H:W = 1:4 on the 12th day, Tables S5 and S17). Generally, the required hourly air exchange exceeded 1000 m^3 for variants with the predominant share of waste over headspace, especially in the first week of the process (Tables S13, S15, and S17). Of all variants, the highest air flow required for effective removal of CO from the headspace was greater than 1320 $m^3 \cdot h^{-1}$ (third day of the process, H:W 1:4, Table S17).

Considering the wet mass of composted waste in every H:W ratio option, the minimum required airflow to remove CO reached the value of 4.26 $m^3 \cdot (h \cdot Mg\ w.m.)^{-1}$) on the eighth and 12th day of the process. Of all variants, the maximum required airflow per hour in terms of Mg of waste reached a value close to 7 $m^3 \cdot (h \cdot Mg\ w.m.)^{-1}$. These higher values were especially characteristic for the third, fourth, and sixth day of the process (Tables S5, S7, S9, S11, S13, S15, and S17).

3.4. The Height of the Waste Pile to Minimize the Risk of CO Accumulation

The height of the pile can then be determined based on the H:W for specific variants. The height of the waste pile in the bioreactor varied depending on the adopted H:W. The variant with the lowest fraction of waste was 1.0 m, gradually increasing through 1.25, 1.68, 2.5, 3.32, 3.75, up to 4.00 m (H:W ratio 3:1–1:4 respectively, the "Height of waste pile" sheet—Supplementary Materials).

4. Discussion

It is well known that compost aeration has a multidimensional impact on the biological aerobic waste treatment process. It affects the process temperature and activity of microorganisms and the degree of material decomposition [53]. The aeration of waste in the composting process has been recognized as one of the critical factors affecting both the process's course and the final quality of the resulting products. Thus, composting facilities use forced pile aeration technology for maintaining their efficiency. In addition, the designed systems try to provide appropriate conditions for biological processing using the lowest possible level of fan power for economic reasons [54]. Controlled aeration systems with feedback are also used to control the composted mass's oxygen concentration and humidity [55,56]. Compared to other areas of environmental technology, composting is overlooked in applying science-based models in practice [57].

In this manuscript, a different approach is taken, i.e., modeling the compost headspace air exchange rate depending on the kinetics of CO production inside the pile, which is different from other researchers' reports. This modeling driven by the CO production inside compost is essential, especially in closed aeration systems, which favor the accumulation of toxic gases in the headspace when the ventilation (of headspace) is not operating.

Temperature itself is also crucial in systems with controlled aeration. The airflow in the thermophilic phase can be even 2–3× higher than in the mesophilic phase (0.6 and 0.9 compared to 0.4 $L\cdot(min\cdot kg)^{-1}$ for the organic fraction municipal waste, respectively, [58], and 1.5–3.0 and 4.5–6.0 $m^3\cdot m^{-2}\cdot h^{-1}$ for organic waste [59]). These results are in contrast to the values obtained as a result of CO-driven modeling. The values indicated as characteristic for the mesophilic phase of composting are 3–5 times higher than those obtained in the modeling (compared to the first and third day of the process), while in the case of thermophilic conditions, they are 6–8× higher, analyzing only the lower threshold indicated by the investigators (0.6 $L\cdot(min\cdot kg)^{-1}$). On the other hand, taking into account the values indicated by [59], the value of the required air in the mesophilic phase of composting is consistent only with the data obtained for the 4:1 H:W variant (the calculated rate of air exchange didn't exceed 3.3 $m^3\cdot m^{-2}\cdot h^{-1}$ therein).

However, what is important in the modeling carried out is that an increasing trend of the required aeration along with the increase of the process temperature indicated by the authors was not observed. The highest airflow level was obtained for the second, third, fourth, and sixth day of the process when the assumed temperature was maintained at ~50 °C. From the ninth day of the process, when thermophilic conditions appeared (temperature close to 70 °C), the airflow required to remove CO dropped almost twice for each variant (1.7× lower values when comparing the aeration between the third and 12th day of the process). On the other hand, this information is in line with our previous article, which shows that the highest CO production is ~50 °C [29].

According to the modeling carried out, the minimum airflow to effectively remove CO to the limit value should not be lower than 0.49, 0.65, 0.99, 1.96, 3.88, 5.88, and 7.84 h^{-1} for the headspace to waste ratio equal to 4:1–1:4, respectively, which gives for each of the variants approx. 6–7 $m^3\cdot(h\cdot Mg\ w.m.)^{-1}$ in the first half of the process and approx. 4–5 $m^3\cdot(h\cdot Mg\ w.m.)^{-1}$ from the eighth day of composting. Among the values obtained in the modeling, mainly those relating to the first seven days of the process are consistent with the data indicated in the literature, and they concern a variety of processed substrates. A similar level of optimal aeration rates was obtained by [53], who used continuous and intermittent aeration during chicken manure with sawdust composting. Their indicated value of 0.5 $L\cdot min^{-1}\cdot kg\ OM^{-1}$ (organic matter) is consistent with the level of required aeration on the second–seventh day estimated here. These results are also confirmed by [60], who used *Penicillin mycelia dreg* as a substrate for the composting process, thus recommending an aeration rate of 0.5 $L\cdot min^{-1}\cdot kg\ OM^{-1}$. A similar level of aeration is also proposed by [61] for pig manure and corn stalks (0.48 $L\cdot min^{-1}\cdot kg\ OM^{-1}$), and by [62] for agricultural waste, while in the case of [62], it is the lower level of the range proposed by the study (0.5–1.16 $L\cdot min^{-1}\cdot kg\ OM^{-1}$). The aeration levels obtained as a result of modeling for the first days of the process are also consistent with the reports of [63] and [64], who successfully used aeration equal to 0.54 and 0.43 $L\cdot min^{-1}\cdot kg\ OM^{-1}$ for composting poultry manure and wheat straw, respectively.

As a result of the modeling, the level of the required aeration drops due to increasing of CO production constant rate k and connected CO concentration in the bioreactor's headspace from the eighth day of the process. It was influenced by the change of the process temperature from 56 to 67 °C between the seventh and eighth day; as it was observed during the own previous research, the k value of CO production increases at temperatures up to 60 °C, while it decreases at the process temperature close to 70 °C [29]. The recommendations of other researchers confirm the estimated aeration level during this period. Similar values (approx. 0.4 $L\cdot min^{-1}\cdot kg\ OM^{-1}$) are reported in the case of vegetable waste, such as maize stalks [65] and legume trimming residue [66], although for the latter substrate, it is the upper threshold indicated by the authors of the optimal aeration in process. The values of 0.3–0.9 $L \cdot min^{-1} \cdot kg\ OM^{-1}$ are also recommended by [67] as suitable for agricultural waste composting. Moreover, in a study by [68] using a mixture of grass trimmings and vegetable waste such as tomato, eggplant, and pepper in the process, the authors proved that due to the higher temperatures obtained and more effective decomposition of organic matter, the optimal level of aeration is 0.4 $L \cdot min^{-1} \cdot kg\ OM^{-1}$. Additionally, [58] have proposed the same aeration rate as appropriate for municipal solid waste treatment to reduce energy consumption in the composting process.

Our modeled values are, in comparison, many times lower than those in the literature considering the required level of aeration during composting in relation to its dry weight. The closest result was reported in [69], who recommended an aeration level of 0.2 $L \cdot h^{-1} \cdot kg\ DM^{-1}$ (dry matter) in their research on sludge composting. Similar values were maintained in the variants modeled here, especially from the eighth day of the process until its completion. However, the data indicated by other researchers for different types of substrates are many times higher: 3× for municipal waste (0.76 $m^3 \cdot day^{-1} \cdot kg\ DM^{-1}$ [70]), for a mixture of biosolids and woodchips 6× or 16× (1.41 and 3.0–3.8 $m^3 \cdot day^{-1} \cdot kg\ DM^{-1}$ [70] and [35], respectively), and twice or nearly 20× for animal manures (0.47–4.7 $m^3 \cdot day^{-1} \cdot kg\ DM^{-1}$ [71]). The values obtained in the modeling are also 10 or 15× lower than these indicated for by-products from sugar cane processing [72] and for waste-activated sludge [73]. Moreover, other authors indicated a decrease in aeration to a level 2× higher than that obtained here resulted in the formation of anaerobic conditions in the composted material. In addition, 15× higher aeration than estimated in modeling has also been reported for yard waste [70].

According to [74], it is recommended to maintain aeration at 10 $m^3 \cdot Mg^{-1}$ of waste during biological waste treatment. On the other hand, research by [75] indicated that with higher airflows equal to 23.3 kg [dry air]$\cdot h^{-1} \cdot m^3$ [compost], the activity of microorganisms present in the composted material increases. The same authors indicated that such higher aeration rates are preferable to lower flows (4.6 kg [dry air]$\cdot h^{-1} \cdot m^3$ [compost]), which is related to the influence of aeration on the process temperature. These recommended values significantly exceed the demand for air exchange obtained in the modeling carried out, indicating that the used and recommended air flows during composting are sufficient for effective removal of CO from the bioreactor headspace. The same trend can also be seen when comparing the results obtained with the model here with the values indicated by [76]. The optimal aeration proposed in [76] when composting chicken manure with straw and dry grasses (0.1 $m^3 \cdot min^{-1} \cdot m^{-3}$ [compost]) is 1.8–3× higher than the highest and lowest modeled value, respectively. However, the most similar results were obtained by [59]; according to the authors, the highest level of organic matter decomposition occurred with 2 and 4 $m^3 \cdot m^{-2} \cdot h^{-1}$ aeration. These values are similar to the variant H:W equal to 4:1 and 3:1. However, the authors' observations differ in terms of temperature at these levels of aeration—for the first one, it was 0 °C, while for the second one, it was 35 °C, i.e., the thermal conditions were much lower than in the modeled process.

With the assumed dimensions of the bioreactor, the height of the waste pile varied from 1.0 m in the case of H:W equal to 4:1, through 2.5 m with a ratio of headspace and waste of 1:1, up to 4.0 m for the largest share of waste (H:W 1:4). However, for each of the variants already, the initial CO concentration on the first day of the process exceeded the limit value of 100 ppm. The most effective removal of this gas was observed for the H:W 4:1 variant, where the concentration of CO did not exceed 2000 ppm throughout the process, and the height of the pile was 1 m. However, the observed 20× higher than the acceptable levels of CO returned note that this height is not optimal for the safety of composting plant workers handling of the material. This does not agree with the reports [77], who indicated that waste height of 1 m in the reactor leads to the most effective composting process. The same authors determined that the process runs effectively to pile of waste up to 1.6 m. However, it is worth emphasizing that the efficiency of the process as perceived by the authors was not considered in terms of minimizing gaseous emissions, including produced CO. The experiment was also conducted in pilot-scale reactors with different dimensions than presented here, strongly affecting the composting process. The modeling carried out shows that such a height (here present for the ratio H:W equal to 2:1—the height of waste pile 1.68 m) causes excessive CO accumulation, which on the first day of the process already reaches 2670 ppm. In the non-ventilated scenario, this gas's concentration may increase up to 45,500 ppm (14th day of composting). Accumulation of carbon monoxide in such a high concentration at the height of the waste pile in the bioreactor equal to and higher than 1 m indicates that the heap of material should be much lower, not exceeding several dozen centimeters, to ensure a reduction in the concentration of released CO. This is in accordance with the research by [73], where the effective pile operation can only be carried out to its height of 0.5 m.

Exceeding 2 m of waste height (H:W equal to 1:1) resulted in a gradual worsening of the conditions in the bioreactor, followed by daily CO concentrations exceeding over 300× the permissible value for the variant with the lowest headspace share (H:W 1:4, waste height = 4 m, third day of the process). Researchers [78] studying the composting process of sludge with woodchips determined a critical waste height of 2.0 m, above which the decomposition rate, the ability to control odors, and cost-effectiveness decrease. However, they pointed out that this critical value depends on the type of waste. It is influenced by, among others, humidity, porosity, and concentration of waste. Regarding the last of these parameters, if the height of composted mass is too high, the air spaces in the waste can be reduced, which can even cause inhibition of the process [77]. This can also lead to anaerobic conditions that may be beneficial for forming CO. It should be noted that the areas with reduced O_2 content in the composted biowaste have elevated CO concentrations [17].

5. Conclusions and Process Recommendation

Due to the need to ensure the safety of plant employees who have direct contact with the composted material and inhale released gases, the limit value for the headspace CO concentration formed during the composting of organic waste was set at 100 ppm. The modeling of CO accumulation in the headspace of the bioreactor proved that the concentration of this gas exceeds the permissible value in each of the analyzed variants of the headspace-to-waste ratio (4:1, 3:1, 2:1, 1:1, 1:2, 1:3, 1:4 v/v). During the 14-day composting process, the CO concentration can reach a maximum value of 3.2% (31,600 ppm) and 36.1% (360,000 ppm) for reactors with the daily release of accumulated gas and without ventilation, respectively.

The values obtained from modeling prove that conducting compost aeration following the procedures recommended in the literature allows for effective removal of CO accumulating in the bioreactor headspace. For the adopted assumptions, the airflow necessary to remove CO to the permissible concentration should not be lower than 7.15 $m^3 \cdot (h \cdot Mg\ w.m.)^{-1}$. However, it should be emphasized that the values of headspace ventilation developed in the modeling are not equivalent to the airflow necessary for adequate compost aeration. As a result of the organic matter decomposition, other gases are also generated, such as CO_2 and water vapor, which should be taken into account in the case of forced aeration from the bottom of the compost pile. The results obtained here indicate the necessary air exchange rate for the reactor headspace should be sufficient in the case of reversed flow aeration, in which the ambient air enters the composted material towards the bottom of the pile where it is sucked in and treated. In addition, to prevent CO accumulation at a concentration exceeding 100 ppm, it is recommended to conduct a process for headspace-to-waste ratio higher than 4:1 with the height of waste pile < 1 m.

The optimal level of waste aeration for the removal of CO generated during the decomposition of organic matter contained in the substrate may depend on the type of material used in the process and other factors, such as its humidity or C/N ratio. Although the CO production modeling procedure proposed in this research is based on several assumptions, it is sufficient to facilitate effective management of the composting process. Excel spreadsheet in Supplementary Material in combination with basic information about the composting system and waste properties can provide composting plant operator practical information about possible CO accumulation in the headspace in the reversed flow aeration system in the case of failed ventilation, and alert when CO concentration exceeds the dangerous level for workers. Further development of the model by taking into account additional parameters, such as the generation rates of other gases during the composting, and the conventional system of forced aeration from the bottom of the pile is warranted.

Based on the simulations of CO accumulation during the composting process of organic waste in closed bioreactors, this method of biological waste treatment may pose a risk to composting plant workers' health or life. For this reason, it is recommended to implement the methodology of the risk assessment in waste composting plants on a technical scale following applicable regulations and guidelines. Particular attention should be paid to the ventilation of enclosed workplaces, ensuring sufficient amounts of fresh air, removing pollutants, as well as maintaining an efficient and failure-free air-conditioning or ventilation system [79]. It is also important to implement minimum requirements for personal protective equipment used by composting plant employees during bioreactors maintenance works, such as respiratory protection indicated by [80] in the case of working in containers or restricted areas with toxic gas or insufficient oxygen. The precautions mentioned above, together with the safety signs at work [81] and the mathematical model presented in this study, can increase the awareness of composting plant employees, and thus their caution and, consequently, safety.

Supplementary Materials: The following are available online at https://www.mdpi.com/1996-1073/14/5/1367/s1: Excel spreadsheet S1: 'Supplementary Materials.xlsx'.

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Appendix No. 3

Karolina Sobieraj
(Name and surname)

Wrocław, 22.06.2023
(Place and date)

Department of Applied Bioeconomy
Wrocław University of Environmental and Life Sciences
Wrocław, Poland
(Affiliation)

STATEMENT

I hereby declare that in the publication:

Karolina Sobieraj, Sylwia Stegenta-Dąbrowska, Jacek A. Koziel, Andrzej Białowiec, 2021. *Modeling of CO Accumulation in the Headspace of the Bioreactor during Organic Waste Composting*, Energies, 14(5), 1-17, doi: 10.3390/en14051367

my contribution included:
development of the concept and design of the study. I set the inputs for the model of CO accumulation. I performed the mathematical modeling of CO accumulation during waste composting in a bioreactor presented in the article. I prepared the text of the entire article, as well as a mathematical model, including tables and graphs describing it (supplementary materials). I have prepared a graphic abstract of the article. I obtained funds for the publication of the article.

Signed by /
Podpisano przez:

Karolina Natalia
Sobieraj

Date / Data:
2023-06-22 21:03

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(Date and signature)

Appendix No. 3

Sylwia Stegenta-Dąbrowska
(Name and surname)

Wrocław, 21.06.2023
(Place and date)

Department of Applied Bioeconomy
Wrocław University of Environmental and Life Sciences
Wrocław, Poland
(Affiliation)

STATEMENT

I hereby declare that in the publication:

Karolina Sobieraj, Sylwia Stegenta-Dąbrowska, Jacek A. Koziel, Andrzej Białowiec, 2021. *Modeling of CO Accumulation in the Headspace of the Bioreactor during Organic Waste Composting*, Energies, 14(5), 1-17, doi: 10.3390/en14051367

my contribution included:
the assistance in the description of the methodology used in the research, including the preparation of the text and equations from chapter *2.3 Analytical Procedures*. I prepared Figure 1, showing the model outline for the prediction of CO accumulation during composting of biowaste under different scenarios of compost headspace ventilation and temperature. During the review process, I made corrections to the manuscript and prepared responses for the reviewers. I obtained part of the funds for the research described in the article.

21.06.2023 Sylwia Stegenta-Dąbrowska
(Date and signature)

USDA
United States Department of Agriculture

Research, Education, and Economics
Agricultural Research Service

Appendix No. 3

Jacek A. Koziel
(Name and surname)

Bushland, Texas, June 22, 2023.
(Place and date)

U.S. Department of Agriculture, Agricultural Research Service
Conservation and Production Research Laboratory
Bushland, Texas
(Affiliation)

STATEMENT

I hereby declare that in the publication:

Karolina Sobieraj, Sylwia Stegenta-Dąbrowska, Jacek A. Koziel, Andrzej Białowiec, 2021. *Modeling of CO Accumulation in the Headspace of the Bioreactor during Organic Waste Composting*, Energies, 14(5), 1-17, doi: 10.3390/en14051367

my contribution included:

the assistance in the development of the mathematical model presented in the article and in the supplementary materials. While working on the manuscript, I participated in English language editing. I participated in the review process and the related modifications to the manuscript.

JACEK KOZIEL Digitally signed by JACEK KOZIEL
Date: 2023.06.22 17:49:16 -05'00'
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(Date and signature)

Jacek Koziel, Ph.D., Research Leader
Livestock Nutrient Management Research Unit
USDA-ARS Conservation & Production Research Lab
Bushland, TX 79102
Phone: 806-356-5744
Email: jacek.koziel@usda.gov
Google Scholar – ORCID – Web of Science

Appendix No. 3

Andrzej Białowiec
(Name and surname)

Wrocław, 21.06.2023
(Place and date)

Department of Applied Bioeconomy
Wrocław University of Environmental and Life Sciences
Wrocław, Poland
(Affiliation)

STATEMENT

I hereby declare that in the publication:

Karolina Sobieraj, Sylwia Stegenta-Dąbrowska, Jacek A. Koziel, Andrzej Białowiec, 2021. *Modeling of CO Accumulation in the Headspace of the Bioreactor during Organic Waste Composting*, Energies, 14(5), 1-17, doi: 10.3390/en14051367

my contribution included:
preparation of a mathematical model of CO accumulation in the bioreactor, in particular, the determination of the composting method and the kinetics of CO production. I have prepared Figures 2-4. While working on the manuscript, I participated in English language editing. I participated in the review process and the related modifications to the manuscript. I obtained part of the funds for the research described in the article. I was also the corresponding author.

Signed by /
Podpisano przez:

Andrzej Białowiec

Date / Data:
2023-06-21
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(Date and signature)

materials MDPI

Article

Assessment of emissions and potential occupational exposure to carbon monoxide during biowaste composting

Karolina Sobieraj [1]*, Karolina Giez [1], Jacek A. Koziel [2] and Andrzej Białowiec [1, 3]

[1] Department of Applied Bioeconomy, Wrocław University of Environmental and Life Sciences, 37a Chełmońskiego Str., 51-630 Wrocław, Poland, karolina.sobieraj@upwr.edu.pl, 113483@student.upwr.edu.pl, andrzej.bialowiec@upwr.edu.pl
[2] USDA-ARS Conservation and Production Research Laboratory, 2300 Experiment Station Rd, Bushland, TX 79012, USA, jacek.koziel@usda.gov
[3] Iowa State University, Department of Agricultural and Biosystems Engineering, 605 Bissell Road, Ames, IA 50011, USA
* Correspondence: karolina.sobieraj@upwr.edu.pl; Tel.: +48 601 530 343

Abstract: To date, only a few studies focused on carbon monoxide (CO) production during waste composting; all targeted on CO inside piles. Here, the CO net emissions from compost piles and the assessment of worker's occupational risk of exposure to CO at large-scale composting plants are shown for the first time. CO net emissions were measured at two plants processing green waste, sewage sludge, or undersize fraction of municipal solid waste. Effects of the location of piles (hermetised hall vs. open yard) and turning (before vs. after) were studied. Higher CO net emission rates were observed from piles located in a closed hall. The average CO flux before turning was 23.25 and 0.60 mg·m^{-2}·h^{-1} for hermetised and open piles, respectively, while after – 69.38 and 5.11 mg·m^{-2}·h^{-1}. The maximum CO net emissions occurred after the compost was turned (1.7x to 13.7x higher than before turning). The top sections of hermetised piles had greater CO emissions compared to sides. Additionally, 5% of measurement points of hermetised piles switched to 'CO sinks'. The 1-h concentration in hermetised composting hall can reach max. ~50 mg CO·m^{-3} before turning, and >115 mg CO·m^{-3} after, exceeding the WHO thresholds for a 1-h and 15-min exposures, respectively.

Keywords: solid waste management; waste treatment; environmental risk assessment; municipal waste; indoor air quality; gas emissions

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1. Introduction

Concern for the environment has led to initiatives and changes in regulatory frameworks worldwide and especially in Europe. The need to manage growing amounts of organic waste (biowaste) resulted in a renewed interest in the aerobic biological processing. The availability of biodegradable waste and its particular presorted types continues to grow, and includes, inter alia, food and kitchen waste, garden waste, agricultural waste and sewage sludge [1]. Moreover, industrial waste (e.g., from papermaking processes) is also treated at full-scale composting plants.

The first large-scale European composting plants in the 1970s and 1980s, treated mainly unsorted municipal solid waste (MSW). Since then, major process improvements have been implemented [2]. In 2019, the European countries used composting as the predominant waste treatment method, and 60% of the total biowaste weight was treated in ~3,400 facilities [3]. The new generation of composting plants has been managed with higher standards, including 'best available technologies' (BAT) [4]. One such standard requires hermitisation (i.e., enclosing compost piles indoors) to better control the process, improve the quality of the final product, and manage local emissions of odours and gaseous pollutants. However, hermitisation of composting raises concerns about the occupational health and safety for workers, due to emissions and accumulation of toxic gases, and inhalation exposure.

The composting process is a source of air pollutants, such as H_2S, SO_2, NH_3, dust, odours, volatile organic compounds (VOCs), endotoxins produced by bacteria, protozoan parasites and allergic fungi [5]. Toxic air pollutants are generated during various compost process stages, and in addition to the management operations including storage, sorting, grinding and turning [6].

One of the least known toxic gases emitted from composting is carbon monoxide (CO). CO is classified as a major ambient air pollutant which has immediate negative effects on human health and life. Emerging body of research has shown CO presence during composting of the undersize fraction of municipal waste, agricultural waste, green waste or fruit and vegetables [7–12]. However, it is worth emphasizing, that the research conducted to date on CO production during composting concerned its distribution *within* the composted material [7,8,13]; the literature does not provide information on net CO emissions *from* the pile surface into air above.

To date, modelling of CO production during composting in a lab-scale closed reactor has shown that the CO concentration can reach 36.1% without ventilation and 3.2% when accumulated process gas is released daily [14]. If scaled up, such CO concentrations would greatly exceed the acceptable inhalation exposure limits established by the World Health Organization (WHO), set at a peak CO concentration of 90 ppm for 15 min of physical work [15]. In general, CO concentration of 100 ppm causes a headache, while further symptoms (e.g., nausea, dizziness, general malaise) emerge at 200-300 ppm [16]. Monitoring the CO exposure is, therefore, important as health effects can be misdiagnosed for other ailments, such as influenza or food poisoning [17]. The chronic CO inhalation at a lower concentrations can adversely affect the respiratory, circulatory and nervous systems [18].

To date, the extent to which composting plant workers are at risk due to CO inhalation is not known and more research is needed. Measurement of CO emissions from large compost piles is challenging due to inherent spatial and temporal variability. The static flux chamber method is the commonly used for measuring gas emissions from large surfaces. Originally derived from soil gas emissions studies, flux chamber method was adapted for anthropogenic emissions sources. The method is based on the use of static (non-flow-through) chambers [19]. For static chamber method, the increasing gas concentration as a function of time is used to back-calculate flux from the enclosed surface [20], as demonstrated for the flux of greenhouse gases such as N_2O, CH_4 or CO_2 from soil [21]. In this research, the static flux chamber method was used for the operational simplicity needed for measurements at a large-scale plant.

Building on the research on CO production *inside* compost piles and aiming to bridge the knowledge gap in actual CO emissions *from* compost, we measured CO net emissions from surfaces of composted biowaste into air. To our knowledge, the CO net emissions assessment at large-scale composting plants was completed for the first time. This research was motivated by the need to assess the occupational risk of CO inhalation at composting plants and, if warranted, evaluate the need to implement the necessary safety measures. For this purpose, CO flux from compost piles was measured at two composting plants, one of which implemented current BAT guidelines for hermetisation. Effects of composting plant type (outdoors vs. enclosed indoors/hermetised) and compost pile turning were studied. Measured fluxes were used for modelling of potential occupational exposure to CO emissions.

2. Materials and Methods

2.1. Experiment matrix

Two composting plants representing differing technologies were selected. The first (Plant A) was in Rybnik, Poland, processing green waste (grass, leaves, branches) and sewage sludge from the "Boguszowice" wastewater treatment plant, 85 and 15% by fresh mass, respectively. Research focused on four compost piles located in the enclosed hall during September-October of 2021. The second (Plant B) was in Lubań, Poland, processing green waste (5 piles) and undersize fraction of municipal solid waste (1 pile), both in open yard.

Biowaste samples (approximately 10 kg each) were collected manually with a shovel from three random locations from every analysed pile. Each sample was then reduced to ~0.7 kg using the quartering method. The age of the composting piles ranged from 1 to 4 weeks (Plant A) and 4 to 8 weeks (Plant B). Experimental matrix is summarized in Table 1.

Table 1. Experiment matrix for CO emissions measurements from biowaste compost

Pile #	Age of the pile (weeks)	Compost Substrates	Emissions measurements series (per pile)	Season	Location (indoors/outdoors)
		Plant A			
1	2	Grass (80%), branches and wood (5%), sewage sludge (15%)	2	Autumn (Sep-Oct, 2021)	Enclosed hall (hermetised)
2	2		3		
3	3		3		
4	4		2		
		Plant B			
1	8	Green waste from backyards and parks	1	Winter (Feb, 2022)	Open yard
2	8		1		
3	6		1		
4	4		1		
5	3		1		
6	3	Undersize fraction of municipal waste (<80 mm)	1		

2.2. Biowaste characterization

Samples were analysed for the dry matter content in accordance with PN-EN 14346:2011 [22], at 105 °C with RadWag WPT/R C2 (Radom, Poland) with an accuracy of 0.01 g and thermal testing chamber KBC-65 (WAMED, Warsaw, Poland). The organic dry matter content was determined according to PN-EN 15169:2011 [23] at 550 °C using the muffle furnace Snol 8.1/1100 (Utena, Lithuania). The respiratory activity (AT_4) was measured as an indicator for compost stability using OxiTop Control system (WTW, Weilheim in Oberbayern, Germany) in accordance with [24].

2.3. Analysis of process gas emissions from compost piles

The measurement of CO emissions from compost piles was performed using the flux chamber method [25]. A plastic box with a volume of 0.071 m^3 was adapted to serve as a flux chamber. Two valves were installed onto the chamber, one for gas sampling and the other for pressure equalization. Gas sampling valve enabled connection with the Kimo KIGAZ 300 gas analyser (Sauermann-KIMO Instruments, France) via a silicone tube (Fig. 1) and CO concentration measurement (ppm). Ancillary measurements of CO_2

and O_2 was also conducted as they are considered co-dependent with CO [8,9]. Internal chamber temperature was measured (± 0.1°C) with a thermocouple.

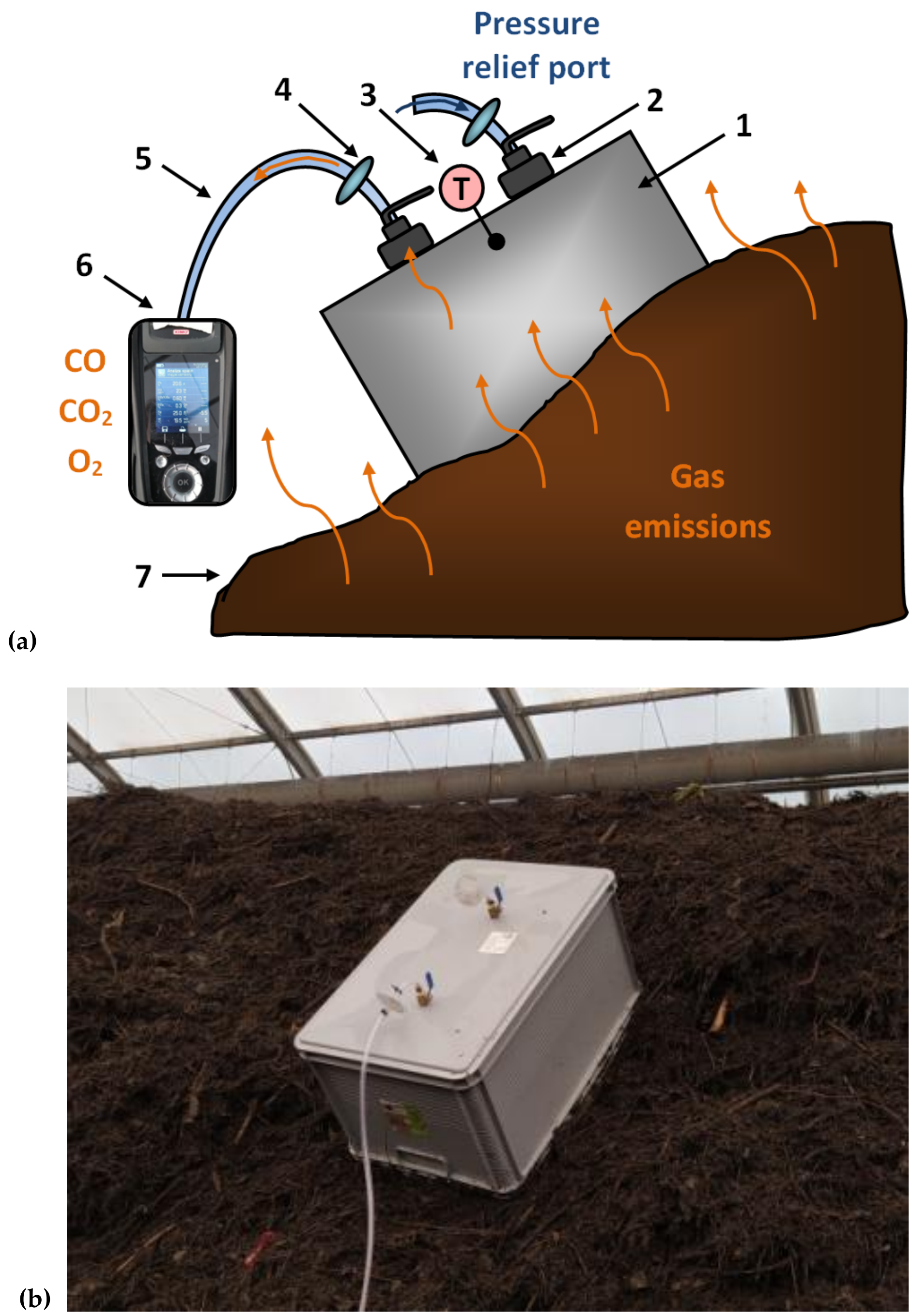


Figure 1. Flux chamber sampling of CO emissions: **(a)** cross-sectional schematic, 1 – flux chamber, 2 – valves, 3 – thermocouple, 4 – purification filter, 5 – silicon tube, 6 – gas analyser, 7 – composting pile; **(b)** flux chamber enclosing emitting surface of a green waste pile in hermetised composting Plant A

The flux chamber was placed on each pile in three locations along its length, on both sides and in its top (total of n = 9; D1-D9) according to the scheme (Fig. 2). Due to the difficult access to pile 6 in plant B, measurements were made only for D1-D3. To improve the enclosure of emitting surface during the measurement, the chamber was pounded into the pile or, in the case of a more homogenous material, gently pressed into the pile. The gas analyser was equipped with an internal pump (1 L·min^{-1}) which facilitated real-time concentrations measurement. After connecting and calibrating of the gas analyser, and placement on the pile surface, each measurement was carried out for 5 min, and its course (changes in CO concentration over time, ppm) was recorded with a camera (Xiaomi Redmi Note 8T, Beijing, China). Data was then processed manually, by entering real-time concentrations every 5 s into spreadsheet (summarized in Supplementary Material). After each measurement, the analyser was disconnected from the chamber and there was a short pause to flush remaining sampled gas and return to the ambient atmospheric levels (CO ~0 ppm, O_2 ~20.2%, CO_2 ~0%). Each measurement series (Tab. 1) was done once a day and included measurements of CO, CO_2 and O_2 concentrations *before* and *after* pile turning. Daily turning during initial phase of composting was facilitated by a self-propelled turner for windrows.

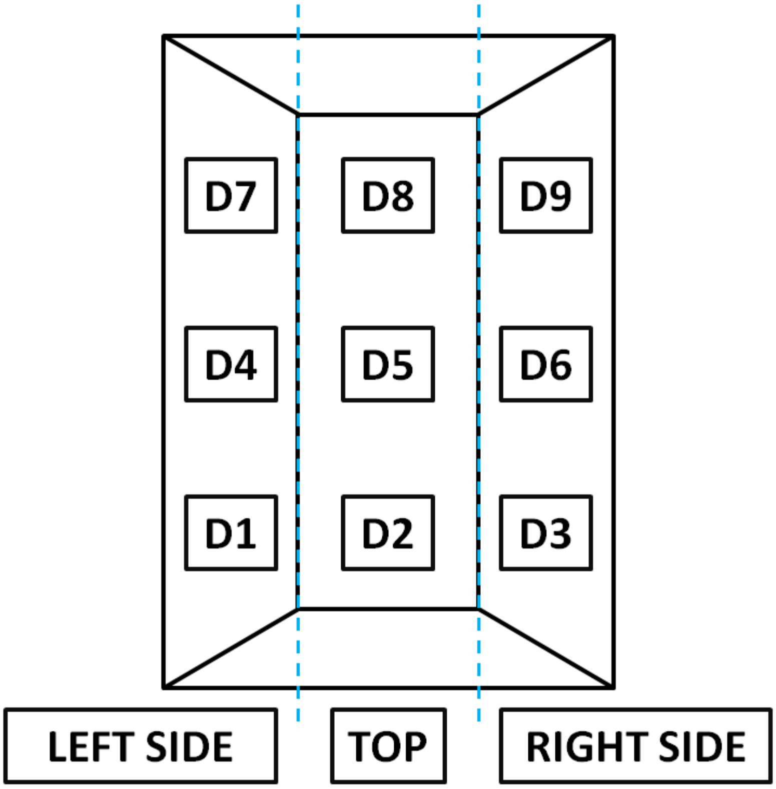


Figure 2. The top view of composting pile with the location of flux chamber placement for CO emissions measurements. Locations D1, D4, and D7 represent left side of the pile; D2, D5, and D8 – pile tops, while D3, D6, and D9 represent pile right side

2.4. Estimating CO emissions

The UK Environmental Agency's methodology (LFTGN07 Guidance on monitoring landfill gas surface emissions) [25] was adopted for estimating CO emissions. Measured CO concentrations (ppmv) were converted to mass/volume units (mg·m^{-3}) at standard temperature and pressure (273 K and 101.3 kPa) using:

$$c_m = \frac{c_V \cdot M_{CO}}{V_{CO}} \cdot \frac{273}{T} \quad (1)$$

where:

c_m – CO concentration, mg·m^{-3},

c_v – CO concentration, ppmv,

M_{CO} – molecular weight of CO, $M_{CO} = 28\times10^3$ mg·mol^{-1},

V_{CO} – molecular volume of CO at standard conditions, $V_{CO} = 0.0224$ m^3·mol^{-1},

T – analysed gas temperature during measurement.

CO flux for each measurement location (D1-D9 on compost pile, Fig 2) was calculated using:

$$Q = \frac{V}{A} \cdot \frac{dc}{dt} \tag{2}$$

where:

Q – CO flux, mg·m^{-2}·s^{-1},

V – volume of the flux chamber, V = 0.071 m^3,

A – emitting surface area of compost pile enclosed by the flux chamber (flux chamber footprint), A = 0.23 m^2,

dc/dt – rate of change of measured CO concentration in the flux chamber with time, determined by plotting CO concentrations on chart with the x-axis representing time (s) and the y-axis representing the mass concentrations (mg·m^{-3}), mg·m^{-3}·s^{-1}.

2.5. Modelling of CO emissions in the composting plant

The modelling of CO emissions during 1 h of operation of the enclosed (hermetised; airtight) composting hall with a 1,000 m^3 of headspace, with a total area of piles of ~1200 m^2 was performed. The 1 h period was chosen for modelling due to the average worker time for turning one pile, and therefore 60 min of exposure to CO emissions per pile. The 'worst-case-scenario' was assumed, i.e., no ventilation in the composting hall and CO emissions allowed to accumulate. The mass of emitted CO during t = 1 h for both 'before' and 'after' turning of the compost material was:

$$m_{CO} = Q_a \cdot A_p \cdot t \cdot n \tag{3}$$

where:

m_{CO} – mass of the emitted CO during t = 1 h for both before and after compost turning, mg,

Q_a – averaged flux of CO from measurement locations D1-D9 on compost pile, mg·m^{-2}·s^{-1},

A_p – surface of n=1 compost pile, $A_p = 300$ m^2,

t – time, t = 3600 s,

n – number of piles inside hermetised composting hall, n = 4.

CO concentration in the headspace of the composting hall after t = 1 h accumulation in both 'before' and 'after' compost turning scenarios was:

$$C_{CO} = \frac{m_{CO}}{V_{hall}} \tag{4}$$

where:

C_{CO} – CO concentration in the headspace of the composting hall after accumulation for t = 1 h for both before & after compost turning, mg·m^{-3},

V_{hall} – volume of the headspace of the airtight composting hall, $V_{hall} = 1{,}000$ m^3.

CO concentration in the headspace of the composting hall after accumulation (C_{CO}) was then converted to the ppm values:

$$C_{CO\,V} = C_{CO} \cdot \frac{M_{CO}}{V_{CO}} \cdot \frac{273}{T} \tag{5}$$

where:

$C_{CO\ v}$ – CO concentration in the headspace of the composting hall after accumulation for t = 1 h for both before/after compost turning, ppm.

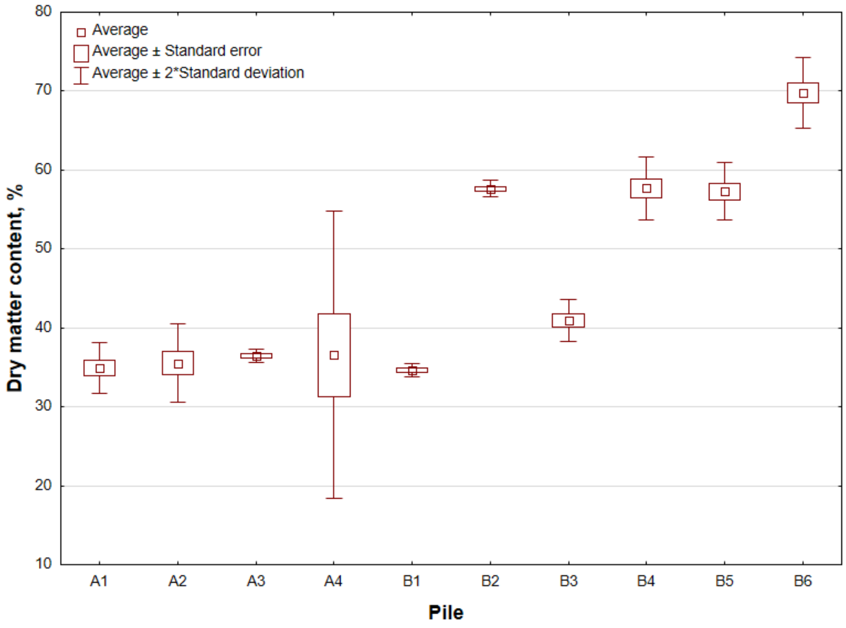
2.6. Statistical Analyses

All data were analysed using Statistica StatSoft Inc., TIBCO Software Inc, i.e., estimating the measurements mean, standard deviation, conducting the correlation analyses between CO emissions and CO_2, O_2 concentration and temperature.

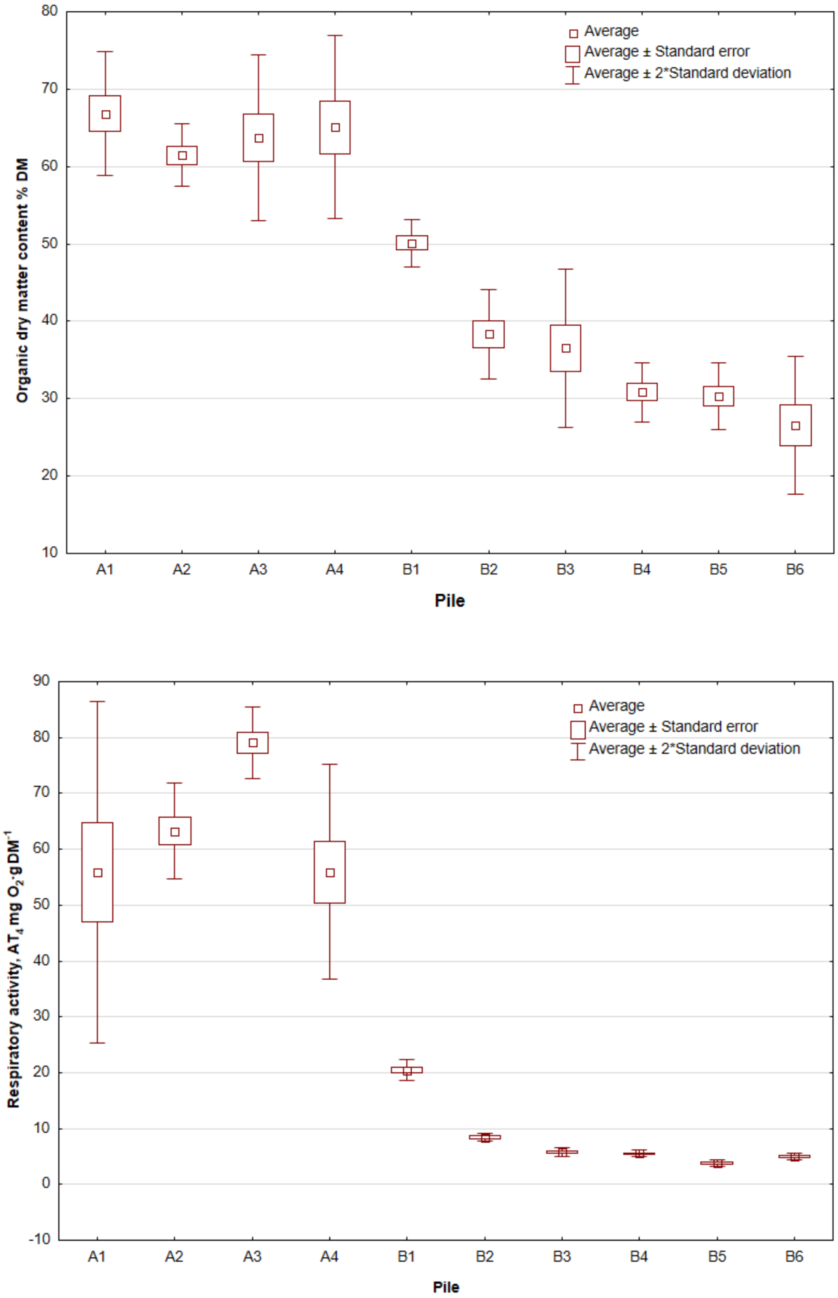
3. Results

3.1. Compost biowaste characterization

Compost piles in Plant A (hermetised) were characterized by similar dry matter content (DM) and dry organic matter (OM) content (DMO) (Fig. 3). The DM values were ~35% and ranged from 34.9% (pile 1) to 36.6% in pile 4. For DMO, the highest mean value was noted for pile 1 (66.8% DM), and the lowest (61.4% DM), was obtained for pile 2. Different biowaste properties were observed at Plant B (open yard), where DM varied from 69.8% in case of pile 6 to 34.6% for pile 1. DMO levels ranged from 26.6% DM to over 50% DM. Clearly, the process parameters were more difficult to control in an open yard operation.



(a)



(b)

(c)

Figure 3. Compost properties for piles 1-4 in Plant A (hermitised, A1-A4) and Plant B (open yard, B1-B6): **(a)** dry matter content, %; **(b)** organic dry matter content, % D.M.; **(c)** respiratory activity AT_4, mg O_2·g DM^{-1}

The respiratory activity was different for piles in Plants A and B. In general, the compost in Plant B (open yard) can be classified as stabilized material (AT_4 <10 mg O_2·g DM^{-1}) [26]. The exception was pile 1, for which the AT_4 > 20 mg O_2·g DM^{-1}. In turn, Plant A (hermetised) piles were characterized by high respiratory activity where the limit value for stabilized compost was exceeded, and AT_4 ranged from 52.3 to as high as 80.3 mg O_2·g DM^{-1}.

3.2. CO fluxes from composting piles

The assessment of CO net emissions at large-scale composting plants was completed. Detailed measured CO concentrations and CO flux estimations are summarized in Excel spreadsheets in Supplementary Materials. Tables 2 and 3 summarize the spatial distribution of CO flux from piles before and after turning, in a hermetised and open yard plants, respectively.

Table 2. Spatial distribution of CO flux (Q) from compost piles in hermetised plant (Plant A) before and after turning

Pile #	**Measurement series**	**Turning**	**CO flux from measurement locations D1-D9 on compost pile (Q), mg·m^{-2}·h^{-1}**									**Avg. CO flux, mg·m^{-2}·h^{-1}**
			LEFT SIDE			**TOP**			**RIGHT SIDE**			
			D1	**D4**	**D7**	**D2**	**D5**	**D8**	**D3**	**D6**	**D9**	
1	1	Before	13.22	-5.22	-0.78	12.34	7.67	9.22	10.89	11.78	3.22	6.93 ± 6.47
		After	135.13	2.33	-5.33	194.92	-2.11	-4.89	136.80	1.78	1.78	51.16 ± 80.22
	2	Before	33.34	20.67	19.23	12.34	26.23	16.00	28.56	15.34	19.00	21.19 ± 6.85
		After	75.35	68.12	37.45	59.12	33.34	38.12	59.12	67.57	31.45	52.18 ± 17.04
2	1	Before	9.89	9.56	0.33	20.67	37.12	6.33	11.00	6.78	0.11	11.31 ± 11.46
		After	142.47	-1.78	-3.78	350.28	86.46	1.00	188.37	3.00	-2.00	84.89 ± 123.05
	2	Before	10.45	10.56	9.67	53.56	27.56	15.11	13.89	16.67	17.67	19.46 ± 13.90
		After	17.89	44.01	12.67	90.57	15.67	28.78	26.89	41.34	20.56	33.15 ± 24.13
	3	Before	50.45	37.56	22.11	59.68	77.35	15.11	54.23	38.90	17.56	41.44 ± 20.99
		After	87.57	100.46	37.12	79.24	72.68	48.56	75.79	78.24	38.90	68.73 ± 22.15
3	1	Before	27.89	43.12	74.24	33.23	37.01	39.01	41.90	31.67	49.90	42.00 ± 13.78
		After	110.35	99.69	120.13	160.81	47.34	55.79	108.35	86.24	79.79	96.50 ± 34.45
	2	Before	11.34	19.67	28.00	18.00	33.01	22.78	-1.00	11.34	25.34	18.72 ± 10.31
		After	43.79	61.57	83.90	36.78	84.79	91.13	44.79	66.90	69.01	64.74 ± 19.74
	3	Before	5.56	18.56	15.89	8.45	0.04	24.56	15.89	13.67	15.00	13.07 ± 7.33
		After	15.34	48.79	78.12	13.22	72.68	158.81	16.34	54.68	76.68	59.41 ± 45.79
4	1	Before	35.67	20.67	26.67	35.12	20.61	25.23	46.34	14.56	23.67	27.62 ± 9.76
		After	59.57	127.80	92.46	61.68	129.47	81.57	55.90	110.69	108.13	91.92 ± 28.89
	2	Before	1.78	24.00	61.23	77.68	19.03	11.34	5.89	20.89	55.23	30.79 ± 27.03
		After	68.01	76.12	93.79	109.02	102.13	106.80	78.24	84.35	101.91	91.15 ± 14.92
Avg. CO flux, mg·m^{-2}·h^{-1}	Before		21.05 ± 18.16			26.71 ± 19.37			21.20 ± 15.58			
	After		64.30 ± 42.95			80.13 ± 70.60			63.72 ± 43.28			

Table 3. Spatial distribution of CO flux (Q) from compost piles in open-yard plant (Plant B) before and after turning

Pile #	**Turning**	**CO flux from measurement locations D1-D9 on compost pile (Q), mg·m^{-2}·h^{-1}**									**Avg. CO flux, mg·m^{-2}·h^{-1}**
		LEFT SIDE			**TOP**			**RIGHT SIDE**			
		D1	**D4**	**D7**	**D2**	**D5**	**D8**	**D3**	**D6**	**D9**	
1	Before	1.56	0.44	2.22	0.78	0.44	1.56	0.44	0.33	0.78	0.95 ± 0.67
	After	3.78	1.56	2.22	6.89	3.11	1.56	8.00	1.89	0.78	3.31 ± 2.52
2	Before	0.44	0.44	0.33	0.44	0.33	0.44	0.78	0.44	0.44	0.45 ± 0.13
	After	4.56	4.56	1.89	5.78	1.89	3.45	4.11	1.22	3.11	3.40 ± 1.51

3	Before	0.33	0.44	0.44	0.33	1.22	1.22	0.33	0.44	0.44	0.58 ± 0.37
	After	12.78	6.67	8.89	4.22	11.0	6.33	11.22	7.22	2.89	7.91 ± 3.33
4	Before	0.44	0.44	0.44	0.44	0.22	0.33	0.33	0.44	0.44	0.39 ± 0.08
	After	4.67	7.45	4.22	2.89	4.00	3.45	3.78	2.22	6.78	4.38 ± 1.72
5	Before	0.44	0.33	0.44	0.44	1.00	0.33	0.33	0.44	0.44	0.47 ± 0.21
	After	4.56	6.56	3.45	3.78	4.67	7.00	8.11	3.45	3.22	4.98 ± 1.80
6	Before	1.56	-	-	0.44	-	-	1.22	-	-	1.07 ± 0.57
	After	10.00	-	-	6.45	-	-	12.89	-	-	9.78 ± 3.23
Avg. CO flux, mg·m^{-2}·h^{-1}	Before	0.67 ± 0.57			0.62 ± 0.41			0.50 ± 0.23			
	After	5.49 ± 3.08			4.78 ± 2.38			5.06 ± 3.59			

At Plant A, a higher average CO flux was measured at the top of the piles, compared with CO flux from the sides. The CO flux was 26.71 mg CO·m^{-2}·h^{-1} vs. 21.05 and 21.20 mg CO·m^{-2}·h^{-1}, and 80.13 mg CO·m^{-2}·h^{-1} vs. 64.30 and 63.72 mg CO·m^{-2}·h^{-1} for top, left and right side of the piles before and after turning, respectively (Tab. 2). At Plant B, the left side of the pile was emitting more CO compared with the top (Tab. 3). The highest CO fluxes here were measured on the left side of the piles (0.67 and 5.49 mg CO·m^{-2}·h^{-1}, before and after turning respectively).

Piles in hermetised hall generated more CO emissions than those outdoors, both before and after compost turning. The average CO flux in all cases was higher after the material was turned; the increase varied from 1.7x to 7.4x for plant A (hermetised, Tab. 2) and from 3.5x to 13.7x for plant B (open yard, Tab. 3). The lowest recorded average CO flux was 6.93 and 0.39 mg CO·m^{-2}·h^{-1}, while the highest reached ~100 and ~10 mg CO·m^{-2}·h^{-1} (with max. values equal to 350 and 12. 9 mg CO·m^{-2}·h^{-1}, values for plant A and B, respectively).

Importantly, a negative CO flux was recorded at 9 measurement points in hermetised plant A (5% of total measurement locations, Tab. 2). In most cases, negative CO fluxes were observed after material turning (points D7, D5, and D8 for pile 1, measurement series 1; D4, D7, and D9 for pile 2, series 2). The CO sinks were not distributed evenly, i.e., most of them were located at the sides of the piles (>50% 'CO sinks' occurred on the left side and two of them on the right). The strongest 'CO sink' achieved -5.22 mg CO·m^{-2}·h^{-1} (point D4 in pile 1 before turning, series 1), while the weakest – -0.78 mg CO·m^{-2}·h^{-1} (point D7, the same pile).

Based on the data presented in Tables 2 and 3, overall net CO emission factors for hermetised and open composting piles were developed (Tab. 4) for the before and after turning for both hermetised and open plants. The average CO flux was lower before the compost is turned. In the 'before turning' scenario it reached 23.25 and 0.60 mg CO·m-2·h-1 for Plants A and B, respectively, and 69.4 and 5.11 mg CO·m^{-2}·h^{-1} after the turning. The before/after turning ratio was higher for hermetised piles (0.34 vs. 0.12 for piles located outdoors). However, the range of before/after ratios was broad. For hermetised plant it ranged from negative (-5.37) up to >6, while for open yard piles it ranged from 0.03 to 1.00.

Table 4. Summary of averaged CO fluxes for hermetised (Plant A) and open (Plant B) piles

	Average CO flux (Q), mg·m^{-2}·h^{-1}	
	Plant A (hermetised)	Plant B (open)
Before	23.25 ± 17.75	0.60 ± 0.42
After	69.38 ± 53.79	5.11 ± 3.01
Before/after ratio	0.34	0.12

Before/after ratio range (min. – max)	-5.37 – 6.62	0.03 – 1.00

3.3. CO concentration accumulation in the hermetised composting plant

The modelling of CO emissions during 1 h of operation of the enclosed (hermetised) composting hall with a cubature of headspace 1,000 m^3, processing green waste with an annual capacity of 60,000 Mg (one-time area of piles in the hall ~1,200 m^2) was performed. Modelling has shown that the concentration of accumulated CO in the hall headspace during 1 h in 'before turning' scenario can reach from 8.3 to even 50.4 $mg \cdot m^{-3}$ (Tab. 5). In each of the analyzed cases, this concentration increased after turning the material, reaching values from 1.7x to over 7x higher, i.e., raising concerns about the potential occupational risk during a typical 1 h-long pile turning. In the 'after turning' scenario, CO levels in the hall headspace after 1 h reached >60 $mg \cdot m^{-3}$, exceeding 100 $mg \cdot m^{-3}$ in 4 analyzed cases. The maximum modelled CO concentration was 110.3 $mg \cdot m^{-3}$.

Table 5. Concentration of accumulated CO in the hall headspace during 1 h modelled for hermetised plant

Pile #	Measurements series	Concentration of accumulated CO in the hall headspace during 1 h			
		Before turning		After turning	
		$mg \cdot m^{-3}$	ppm	$mg \cdot m^{-3}$	ppm
1	1	8.31	9.68	61.39	71.50
	2	25.43	29.61	62.62	72.93
2	1	13.57	15.81	101.87	118.65
	2	23.35	27.20	39.78	46.34
	3	49.73	57.92	82.47	96.06
3	1	50.39	58.69	115.80	134.87
	2	22.46	26.16	77.69	90.48
	3	15.68	18.27	71.29	83.03
4	1	33.14	38.60	110.30	128.46
	2	36.94	43.03	109.38	127.39
Average ± standard deviation		27.90 ± 14.48	32.50 ± 16.86	83.26 ± 25.36	96.97 ± 29.53

3.4. Relationship between CO and other process gases and temperature

Correlation analysis showed that in Plant A (hermetised) CO emissions followed measured CO_2 concentrations (Pearson correlation coefficient r ranged from 0.55 to 0.91) and negative correlation with measured O_2 concentration (r ranging from -0.78 to -0.91, Tab. 6), both before and after turning. This is in contrast to the observations of other researchers, reporting that the increased availability of O_2 stimulates the production of CO related to thermal degradation of OM [7,9]. No statistically significant correlations between those gases were obtained for Plant B (open yard, Tab. 7). More research is needed to evaluate the kinetics of CO, CO_2 and O_2 as the effect of turning and its frequency.

Table 6. Correlation between CO and other process gases and temperature in Plant A (hermetised) for a probability level of α=0,05; statistically significant correlation coefficients are marked in red, r – Pearson correlation coefficient

			CO			
			Pile 1	Pile 2	Pile 3	Pile 4
Before turning	CO_2, %	r	0.18	0.55	0.64	0.86
		p value	0.463	0.003	0.000	0.000
	O_2, %	r	-0.071	0.15	-0.78	-0.87
		p value	0.778	0.468	0.000	0.000
	Temperature, °C	r	-0.03	-0.4	0.85	-0.17
		p value	0.282	0.031	0.000	0.510
After turning	CO_2, %	r	0.91	0.64	0.85	0.37
		p value	0.000	0.000	0.000	0.136
	O_2, %	r	-0.91	-0.82	-0.78	-0.35
		p value	0.000	0.000	0.000	0.159
	Temperature, °C	r	0.09	0.35	0.56	0.41
		p value	0.720	0.075	0.002	0.091

Table 7. Correlation between CO and other process gases and temperature in Plant B (open) for a probability level of α=0,05; statistically significant correlation coefficients are marked in red, r – Pearson correlation coefficient, nd – no data

			CO					
			Pile 1	Pile 2	Pile 3	Pile 4	Pile 5	Pile 6
Before turning	CO_2, %		nd					
	O_2, %							
	Temperature, °C							
After turning	CO_2, %	r	-0.05	-0.10	-0.12	-0.01	0.03	nd
		p value	0.905	0.804	0.751	0.989	0.946	
	O_2, %	r	0.43	nd	-0.25	-0.44	-0.56	
		p value	0.244		0.520	0.233	0.113	
	Temperature, °C	r	-0.73	-0.08	0.53	-0.10	-0.20	0.71
		p value	0.027	0.838	0.143	0.808	0.610	0.499

There was no statistically significant correlation between CO emissions and temperature, as observed by other researchers [9,10,13,27]; the only exceptions were pile 2 (before turning) and pile 3 (before and after turning) in Plant A and pile 1 in plant B (after turning). However, the data obtained was inconsistent; for pile 2 and pile 1 (Plant A and B, respectively) the correlation was negative, in the first case the r was low (-0.42, Tab. 6), while in the case of pile 3 (plant A) the correlation was strongly positive (r equal to 0.85 and 0.56 before and after turning the material, respectively).

4. Discussion

To date, only a few studies focused on the CO production during waste composting; all were targeted on CO *inside* piles. Here, data of CO net emission *from* compost piles is shown for the first time. The comparison of process-based CO emissions for 'before' and 'after' compost turning is important both in terms of occupational safety, and for improved inventory of CO sources in local and regional air quality.

Regarding the occupational safety, the topic of CO emissions accumulation in enclosed spaces is rarely discussed in the context of waste management. Related studies were conducted mainly in relation to the storage of wheat, rape, wood pellets or during the processing of such materials, e.g. wood drying, in rooms similar in nature to closed composting halls [28,29]. For the first two, emission factors reached up to 200 mg CO·ton^{-1} (rape) and 9 mg CO·ton^{-1} (wheat grain) per day. Moreover, the recorded CO levels in the storage and processing of wood materials exceeded the permissible values for warehouses [30].

According to the study conducted here, the CO accumulation in hermetised compost halls should also be of concern. Based on emissions modelling, averaged CO level before turning reached nearly 30 mg CO·m^{-3}, and after – more than 80 mg CO·m^{-3}, with single values exceeding 50 and 100 mg CO·m^{-3}, respectively. According to WHO guidelines, the 30 mg CO·m^{-3} should not be exceeded during 1-h work and 100 mg CO·m^{-3} during 15 min of moderate physical activity [31]. This is important because of the toxic CO impact on human health. Prolonged exposure to CO causes the formation of carbohydrate hemoglobin (COHb) due to the higher affinity of CO for hemoglobin compared to O_2 [31].

The *duration* of the high CO concentrations in hermitised plans is also important in the context of the exposure of composting plant workers. Composting facilities often work continuously with three 8-h shifts. A typical worker repeats scheduled turning of piles over entire shift, and thus, may be exposed to increased CO emissions throughout the entire 8 h of work. The initial phase of exposure to CO starts with the first pile turning. COHb concentration increases rapidly at the beginning of exposure to a constant CO concentration [31]. Stabilization takes place after 3 h, and the steady state, when the CO concentration in alveolar breath and ambient air is ~equal, is achieved after 6-8 h, i.e., practically during one work shift in a closed composting hall [32]. Moreover, high CO levels may be present in closed halls for a longer period, even several months during cool season when the ventilation is low. During the research on emissions from wood pellets, the CO concentration was equal to 21 mg·m^{-3} even after 3 months from the beginning of storage of this raw material [30]. This is particularly important due to the fact that long-term exposure to lower CO levels results in much greater health impact than short-term exposure to high concentrations of this gas. The health consequences of chronic CO exposure include, inter alia, heart failure, asthma, stroke, tuberculosis, pneumonia, cognitive memory deficits or sensorimotor changes [15]. Human activity level during exposure to CO is also important. Considering that compost plan workers of the composting plants sometimes handle waste manually, it should be taken into account that in combination with long shifts in hermetised environment with high levels of CO and potentially other highly toxic gases such as H_2S, and moderate-to-high activity (and therefore inhalation rate) pose synergistically elevated risks.

Moreover, the CO levels may increase again during composting with increasing ambient temperature [30]. The peaks of higher CO concentration were observed after 100 days from the start of the process, when the temperature reached 80 °C [27]. This means that in the context of exposure of workers to the negative effects of CO, monitoring should be carried out throughout the process, not only in its initial stage. In addition, it is possible that piles originally considered as 'safe' (with lower CO net emissions), such as those processed outdoor in Plant B, when moved to a composting hall with more favourable thermal conditions, may again exhibit higher CO emissions.

Taking into account the spatial variability of gaseous emissions from compost piles, the CO gradient distribution indicates that its level is higher in top of the piles [27]. This is confirmed by the observations made for hermetised Plant A, where ~1.2x higher CO fluxes, both before and after turning, were measured at the top of piles. A similar situation was also noted during the storage of wood pellets [30]; the highest values, significantly exceeding the permissible levels of CO emissions, were recorded at the top of the pile. It was also noted in case of other pollutants emission, such as VOCs and N_2O [27,33]. This tendency is related to the so-called 'chimney effect' in the pile, which is caused by the temperature profile within the material and occurs as a result of convection [33,34]. In this way, the warmer gas migrates from the core of the pile due to buoyancy leaves it through the top, while the cooler air enters the sides of the pile, close to the ground [35]. The chimney effect was observed in this research for CO emissions from the pile. This is important from occupational safety of plant employees who work with pile levelling. Additionally, CO, being slightly lighter than air, rises in the enclosed hall and accumulates in its upper part [15]. Thus, high-off-the-ground cabin location of common machinery (excavators, turners, or shredders) may result in greater risk to operators exposure to CO emitted from the top of the piles. On the other hand, the chimney effect was not noted in the case of open piles in Plant B, where the higher average CO flux occurred on the left side of the pile. This may be related to the influence of external conditions, such as wind direction. This is confirmed by research conducted by [27], who explain the asymmetric distribution of process gases in the pile with higher pressure and pore gas dilution in the area of the pile not sheltered from the wind.

It should be emphasized that compost can not only be a ‘source’ but also a ‘sink’ of CO, which in hermetised plant occurred in 5% of flux measurement locations. Emerging evidence have shown that CO production during composting has a twofold character and is based on (1) the activity of microorganisms (biotic CO production), and on (2) thermochemical processes dependent on temperature and O_2 concentration (abiotic CO production) [9]. Furthermore, when the CO production is biotic, net CO emission is the result of the CO formation by bacteria and its metabolism (microbial oxidation); the enzyme carbon monoxide dehydrogenase (CODH) plays a key role controlling both processes [36]. The same situation was observed with soils [36]; early research dating back to the 1970s identified soils not only as a CO producers, but also as the main sinks of atmospheric CO [37]. The nature of CO uptake is mainly based on microbial activity, as confirmed by studies of autoclaved soil and the use of antibiotics [37–39]. For this reason, CO consumption is also limited by the concentration – an increased level of CO can inhibit the metabolism of bacteria. An important element of the biotic CO uptake studied for soils is also the fact that these processes occurred under both aerobic and anaerobic conditions [38]. This issue becomes important in the context of studies on aerobic and anaerobic bacteria functioning in an environment with >1% CO concentration, which use the enzyme carbon monoxide dehydrogenase (CODH) to metabolize CO [40]. Due to the bidirectional activity of this enzyme, enabling the reversible process of CO oxidation to CO_2, it can be hypothesized that, apart from bacteria that only produce/consume CO, there are also strains that carry out both of these processes. The responsibility of microorganisms for ‘CO sinks’ in composting piles in this research may also affect the spatial distribution of spots with negative CO fluxes. About 78% of them occurred on both sides of the piles, creating chimney effect of CO uptake on the pile sides and emission of CO from the top of the pile. Since CO and O_2 concentration were positive correlated, this effect could be caused by the transfer of aerobic CO-metabolizing microorganisms from sites with less nutrient availability to areas with higher O_2 concentration and decomposable OM content.

The second aspect of this study, i.e., the determination of CO net emission factors from open and hermetised piles before and after turning (Tab. 5) is needed for atmospheric air quality modelling and CO source inventories. Open yard Plant B had a much lower CO emission potential compared with hermetised Plant A. However, according to the research conducted by [30], the outdoor composted material emits most of the gases in warm season. The authors associated this with the close correlation of CO concentration and temperature, which is especially visible in thermophilic conditions [30]. During present study, CO fluxes from open piles were estimated in winter, when the ambient temperatures were low. It is also worth noting that no statistically significant correlation between CO concentration and temperature was observed. However, it should be remembered that the dependence of CO production on temperature refers to the thermal conditions *inside* the composted material [8]. The temperature measured in these studies prevailed in flux chamber headspace, i.e., directly above the pile. Considering the ambient conditions (low temperatures in winter), it can be assumed that the temperature in the flux chamber correspond to the conditions under which CO was *net emitted*.

5. Conclusions and recommendations

Research on CO net emissions from biowaste composting on industrial scale has shown its dependence on turning and plant type (open yard vs. hermetised). Higher CO net emission rates were observed for piles located in an enclosed composting hall, separated from ambient conditions (23.25 and 69.38 mg CO·m^{-2}·h^{-1} before and after turning, respectively). In each of the analyzed cases, maximum CO emissions occurred after compost turning. The areas with increased CO emissions for hermetised piles were the tops with 'CO sinks' spots on the sides, showing the 'chimney effect' of CO distribution. Modelling of CO emissions during 1-h of work in a closed hall has shown that it can reach max. ~50 mg CO·m^{-3} (59 ppm) before turning, and >115 mg CO·m^{-3} (135 ppm) after, exceeding the WHO thresholds for an 1-h and 15-min exposures, respectively.

The results show that due to the nature of work in composting plants (operating machine with cabins high above ground, occasional manual labour, 8-h shifts), personal protective equipment should be implemented for workers exposed to CO emissions (e.g., personal CO detectors, appropriate breathing masks with filters). This is especially important for people working with biowaste turning or manual levelling on top of piles. Additionally, it is recommended that the time spent in the closed composting hall be shortened to a minimum and limiting activities to moderate physical effort. Access to composting halls should be limited only to authorized persons, equipped with appropriate safety equipment, and following protocols. Automating turning and eliminating workers exposure could be developed and implemented to the composting practice. Due to the CO tendency to accumulate in the upper part of halls, it is also recommended to install alarms, especially above compost piles. Since CO emissions are variable and may increase with the temperature, reaching several peaks throughout the process, it is recommended to monitor it continuously throughout the composting process, not only in its initial stage. Engineering design should consider adequate ventilation for operations involving human operators.

Since this study has shown that compost can be considered not only as a 'producer', but also as a 'sink' of CO, based on studies on CO consumption conducted for soils, it can be hypothesized that during bio-waste composting aerobic and anaerobic bacteria are responsible for the CO uptake, possibly using the CODH enzyme to metabolize CO. Further research identifying the mechanisms of biotic CO uptake should be conducted as a future strategy for CO emission mitigation.

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Supplementary Materials: All measured CO concentrations, CO fluxes estimation according to the UK Environmental Agency's methodology and modelling of CO concentration in closed composting hall can be found in the Supplementary Material.

Author Contributions: Conceptualization, K.S. and A.B.; methodology, K.S..; validation, A.B.; formal analysis, K.S. and J.A.K..; investigation, K.G..; resources, K.S. and K.G.; data curation, K.S. and J.A.K.; writing—original draft preparation, K.S.; writing—review and editing, A.B. and J.A.K.; visualization, K.S.; supervision, A.B. and J.A.K.; project administration, K.S. and K.G..; funding acquisition, K.S. and K.G. All authors have read and agreed to the published version of the manuscript.

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Data Availability Statement: The data presented in this study is contained within the article and its supplementary material.

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(Name and surname) (Place and date)

Department of Applied Bioeconomy
Wrocław University of Environmental and Life Sciences
Wrocław, Poland
(Affiliation)

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Karolina Sobieraj, Karolina Giez, Jacek A. Koziel, Andrzej Białowiec, 2023. *Assessment of emissions and potential occupational exposure to carbon monoxide during biowaste composting*, Materials (under review)

my contribution included:

I prepared the text of the entire article, including the introduction part, materials and methods, description of the results, discussion, conclusions and recommendations. I characterized the compost properties, that were presented in Table 2. I prepared Tables 1-8 and Figures 1-2. I calculated CO fluxes from composting piles, which are indicated in Tables 3 and 4. I performed modeling of the concentration of accumulated CO in the hall headspace shown in Table 6. I conducted statistical analyzes, the results of which are presented in Tables 7 and 8.

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Karolina Natalia
Sobieraj

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Appendix No. 3

Karolina Giez
(Name and surname)

WROCŁAW, 21.06.23r.
(Place and date)

Department of Applied Bioeconomy
Wrocław University of Environmental and Life Sciences
Wrocław, Poland
(Affiliation)

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my contribution included:
the revision of the final version of the manuscript. I participated in the calculation of CO fluxes from composting piles, which were indicated in Tables 3 and 4, and in the modeling of concentration of accumulated CO in the hall headspace showed in the Table 6.

21.06.23r. Giez Karolina
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Appendix No. 3

Jacek A. Koziel
(Name and surname)

Bushland, Texas, June 22, 2023.
(Place and date)

U.S. Department of Agriculture, Agricultural Research Service
Conservation and Production Research Laboratory
Bushland, Texas
(Affiliation)

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my contribution included:

I proposed the use of the UK Environmental Agency's methodology for estimating CO emissions. I assisted in the evaluation of CO emission from biowaste composting and modelling of CO emissions in the composting plant described in the sections *2.4.Estimating CO emissions* and *2.5. Modelling of CO emissions in the composting plant*. I participated in the preparation of Figure 1. I participated in the revision of the manuscript and English language editing.

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Jacek Koziel, Ph.D., Research Leader
Livestock Nutrient Management Research Unit
USDA-ARS Conservation & Production Research Lab
Bushland, TX 79102
Phone: 806-356-5744
Email: jacek.koziel@usda.gov
Google Scholar – ORCID – Web of Science

Appendix No. 3

Andrzej Białowiec
(Name and surname)

Wrocław, 21.06.2023
(Place and date)

Department of Applied Bioeconomy
Wrocław University of Environmental and Life Sciences
Wrocław, Poland
(Affiliation)

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my contribution included:
developing the concept and design of the study, in particular the use of flux chamber method for measurements of CO emission from biowaste pile during composting on a technical scale and calculations used for the estimation of CO emission presented in the supplementary material of the manuscript. I participated in the revision of the article and English language editing.

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Andrzej Białowiec

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