Kinetics of Biotic and Abiotic CO Production during the Initial Phase of Biowaste Composting

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Abstract: Knowledge of kinetic parameters of CO production during biowaste composting is significantly important for the prediction of its course and estimation of total gas quantity. This allows increasing the control of the process, to minimize its negative impact on the environment and to protect the occupational safety of employees exposed to CO in the biowaste composting plant. For the first time, a full study of the influence of temperature and biowaste sterilization on the kinetics of CO production is presented. The lab-scale experiments used a mixture of green waste, dairy cattle manure, and sawdust in two variants: sterilized and non-sterilized samples. The process was carried out in controlled temperature reactors with measuring the concentrations of CO, O_2, and CO_2 every 12 h. CO production and k value increased with temperature. However, higher CO production was observed in biotic conditions between 10~50 °C, suggesting the biotic CO formation and 1st-order kinetics. The abiotic (thermochemical) process was more efficiently generating CO above 50 °C, described with a 0-order kinetic model. Additionally, the rate constant (k) value of CO production under biotic conditions was increasing up to a temperature of 60 °C, above which a slight decrease in CO production rate was observed at 70 °C. The presented results are the basis for further studies focused on the feasibility of (1) the mitigation and (2) valorization of CO production during the biowaste biostabilization are warranted.

Keywords: waste management; aerobic digestion; biowaste; carbon monoxide; green chemistry; biorenewables; kinetic modeling; GHGs mitigation; biotic; abiotic

1. Introduction

Carbon monoxide (CO) is an odorless gas, without color or taste, with a strong toxic effect on living organisms. It is also toxic to many microorganisms due to its ability to inhibit electron transport [1].

Composting and aerobic biostabilization processes of biowaste are sources of CO production, besides major ones such as natural forest fires and incomplete fuel combustion and also through biochar production [2]. Production of CO is a result of the biological decomposition of organic matter, which also generates CO_2, CH_4, H_2, nitrogen compounds, VOC, and H_2S [3]. CO emissions have been observed during the composting of green waste [4], green waste with manure [5], organic waste [2], and aerobic biostabilization of the municipal waste [6]. The detection of CO during the pioneering experiments was a surprise due to the widespread belief in its primary production by incomplete combustion [7].

In recent years, the interest in gas emissions during composting in a laboratory, semi-technical, and full-scale conditions has increased [8–11] and could be a part of the worldwide circular economy [12].

Initial work focused on gas sampling [13], gas concentrations in the compost pores or above the pile surface (CO_2, NH_3, N_2O, CH_4, and CO) [8], spatial and temporal variability of compost gases [14], the influence of the feedstock (source-separated organic household waste and food waste; [3]) (agricultural waste [15]), municipal waste [16], including the separated organic fraction [17], green waste [18], particle size on the gaseous products emission [19].

There is still a lack of systematic knowledge in the literature analyzing the kinetics of CO production during aerobic biostabilization of biowaste. To date, soil science provides the most important literature in the field of CO formation kinetics related to the decomposition of organic matter. Conrad and Seiler [20], studied the simultaneous production and utilization of CO in three types of soils and developed early models. CO consumption follows the 1st-order reaction kinetics:

$$\frac{dm}{dt} = k_{co}m \tag{1}$$

where:

k_{co}—CO consumption constant rate (s^{-1}),
m—the mass of CO (mg),
t—time (s),

while the CO production follows apparent zero-order reaction kinetics:

$$\frac{dm}{dt} = k_p \tag{2}$$

where:

k_p—CO production rate ($mg.s^{-1}$).

Since CO utilization and production occurs simultaneously, the mass balance is as follows:

$$\frac{dm}{dt} = k_p - k_{co}m \tag{3}$$

Integration with the boundary conditions of $t = 0$, $m = m_0$, and $t = \infty$, $m = m_e$, results in the CO utilization rate constant [18]:

$$k_{co} = \frac{1}{t} ln \frac{m_0 - m_e}{m - m_e} \tag{4}$$

where:

m_0, m_e—equilibrium values (mg).

CO production from biological processes occurs under both aerobic and anaerobic conditions, with the latter being greater and correlated with O_2 availability [3]. Researchers recognize the dual (biotic/abiotic) nature of CO production, but the question of the driving mechanisms remains open. Some argue that CO production is based on thermochemical processes and does not depend on the activity of microorganisms found in composted material [19]. These authors have shown that CO emissions are highly dependent on the temperature and amount of O_2 [5]. The temperature dependence and diurnal changes to CO emissions from soil were reported [21]. Other abiotic factors, such as UV radiation, type of material, humidity, and organic matter content, have an impact on CO generation from soil [22,23].

An interesting discovery was made by Rich and King [24], who found that CO is not only produced but also utilized by methanogenic, acetogenic, and sulfate-reducing bacteria in compost [25]. The biotic origin of CO is not fully understood; one hypothesis being that it is formed from abiotic substrates, including porphyrins, polyphenols, and aromatic acids [26].

Despite the agreement that CO production can occur via biotic and abiotic processes—its origins and mechanisms have not been explained exhaustively. It is also necessary to know the full impact of temperature as one of the key parameters of the waste composting process on CO production. Knowledge about the kinetic parameters of CO production during composting is significantly important for the estimation of its production rate and total quantity. This allows increasing the control of the process, minimizing its negative impact on the environment, increasing the sustainability, and, what is immediately more important, to protect the safety of employees exposed to CO in the biowaste composting plant. In the literature, there are many works dealing with the subject of workers' exposure related to the composting process exposure to volatile organic compounds (VOC), bacteria, fungi, organic dust, odors, leachates, molds, and other substances [27–31]. On the other hand, there are no sources providing information on the risk of CO poisoning; quantitative comparisons with the risk of other pollutants mentioned above do not exist either. However, this topic should not be ignored due to the toxicity of CO for humans and the nature of the work undertaken in composting plants. Most of the people employed perform manual labor, whose frequency and depth of breathing is increased due to their duties, which in turn increases their potential exposure to CO. Longterm exposure to CO may cause a number of diseases related to, among others, the respiratory and circulatory systems [32]. In the long-term, these results could also help save money in composting treatment plants. Designing the process to avoid CO emissions will reduce expenses related to the abstention & turnovers of employees at work or pay compensation for health impairment [33].

In recent work [4], we have indicated that CO production during biowaste aerobic digestion can be biotic and abiotic in nature. The main observation was that CO concentrations varied throughout the process, with the temperature being the main influencing factor. The temperature's effect was more significant in the case of non-sterilized samples. This observation highlights the fact that microorganisms are involved in net CO production. These results add to the small, yet growing evidence that biotic processes generate CO. What are the details of the kinetics of CO formation according to aerobic biowaste stabilization process parameters? Trying to answer this question requires reexamining the same dataset, i.e., CO, O_2, and CO_2 concentrations reported in [4], with extensive statistical analysis to reveal the kinetics of CO formation.

Therefore, this novel and significant research aim to explain the effect of compost process temperature and microorganisms activity on CO kinetics production. Additionally, this article, for the first time, comprehensively explains the effect of these parameters on the kinetics of CO production under biotic and abiotic conditions. The main research hypothesis and the driving force behind this work is the belief that understanding the kinetics of CO production during aerobic waste biostabilization and the factors influencing it will allow controlling of this production in the future. We believe that the ability to predict the course of CO formation during the process and the total amount of produced gas will enable the development of models to reduce the negative impact of CO not only on the environment but also on human health. The uniqueness of the presented research is also emphasized by the fact that there are no sources dealing with this topic in the literature, which makes us pioneers in this area. Due to the aspects of occupational health and safety, our results may be of interest to representatives of industry, mainly owners of composting plants.The presented data may also become a stimulus for the development of industrial and engineering solutions aimed at, on the one hand, ensuring the safety of human health and life, but also limiting the negative impact of the composting process on the environment. Additionally, due to environmental aspects, these results may be relevant to politicians, environmentalists, and other researchers dealing with the emission of harmful gases. The perspective of using the results presented in this paper is therefore important not only on a regional scale, concerning individual composting plants, but—what is important—on a global scale.

2. Materials and Methods

2.1. Waste Characteristics

Three fractions of waste were tested: dairy cattle manure, grass clippings, and pine sawdust. For the process of aerobic biostabilization of waste, it is recommended to keep the material moisture at 40–60% [31]. In order to obtain this parameter equal to ~59%, groups of waste used in the experiment were mixed in a mass ratio of 1:1:1. All samples were analyzed before and after the process for moisture and volatile solids (VS) content [32]. The respirometry activity (a.k.a. AT_4) of cattle manure, grass clippings, pine sawdust separately, and of the biowaste mixture were determined according to [33,34].

2.2. The Experiment Design and Procedure

The experiments were performed in laboratory conditions, in fixed temperature bioreactors, in triplicates, at the following temperatures: 10, 25, 30, 37, 40, 50, 60, and 70 °C, in sterile (abiotic) and non-sterile (biotic) conditions. For each bioreactor, 50~55 g of mixed biowaste was placed in the climatic chamber (POL-EKO, model ST-3, Wodzisław Śląski, Poland) under constant temperature conditions. The climatic chamber also shielded the bioreactors from natural light. The tests were carried out in 1 L bioreactors. The tyndalization method was used for biowaste sterilization—material in reactors was placed in the dryer for 1 h at 105 °C, 3 times with 24 h intervals. Figure 1 shows the experimental set-up.
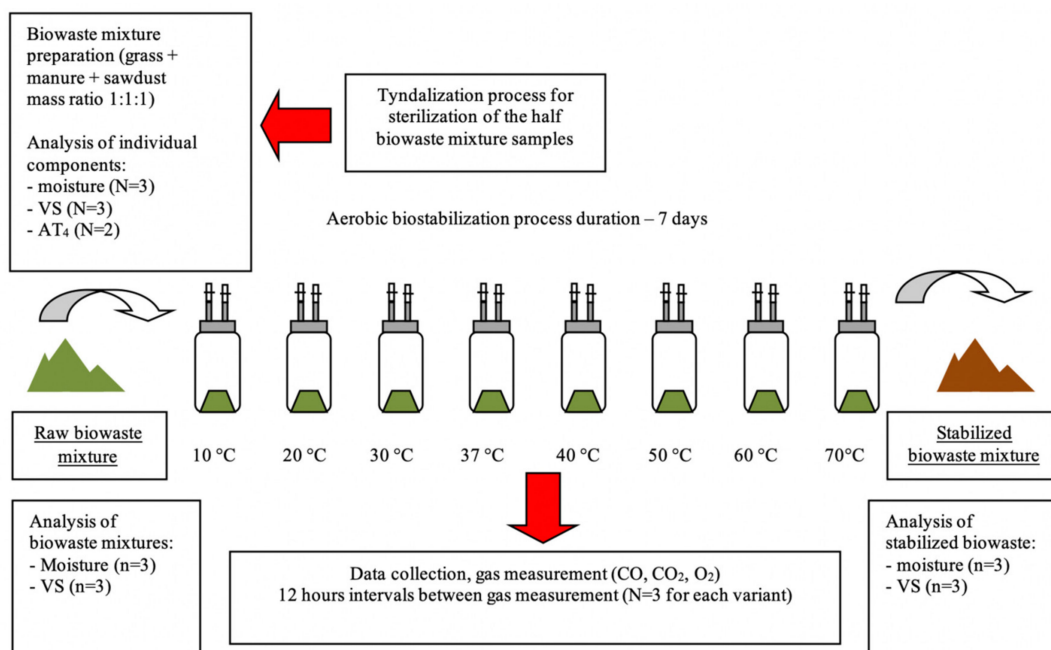

Figure 1. Scheme of the experimental set-up.

2.3. Gas Production Monitoring

The concentrations of CO, O_2, and CO_2 formed inside the reactors were monitored at a frequency of 12 h, starting at the twelfth hour from the set up of the experiment after incubating the reactors at a constant temperature. Measurements were carried out using a factory-calibrated Kimo KIGAZ 200 (Kimo Instruments, Chevry-Cossigny, France) exhaust gas analyzer. In each case, three replicates were performed, measuring the gas concentrations for ~50 s until the indicated values stabilized. The aerobic conditions in bioreactors' headspace were obtained by ventilation with the application of sterile filters (to keep sterile conditions in abiotic variants) during each gas measurement. Specific information about gas production measurement methods, air supplied to the process is described in our previous study [4].

2.4. CO Production Kinetics Determination

Data for kinetic analysis were analyzed by excluding the lag-phase [35]. Nonlinear least squares regression was used to determine the kinetic parameters of CO & CO_2 production and O_2 consumed. The cumulative curves of CO & CO_2 production and O_2 consumption concerning biowaste dry mass (d.m.) were determined [36]. The zero-order and 1st-order reaction models were compared. The zero-order equation for gas production is:

$$P = k \cdot t \tag{5}$$

where:

P—total production or consumption (CO, CO_2, or O_2), (mg·g^{-1}d.m.),
K—production or consumption rate (CO, CO_2, or O_2), (mg·g^{-1}d.m.·h^{-1}),
T—time, (h).

The 1st-order reaction equation for CO, CO_2, or O_2 production or utilization is:

$$P = P_0 \cdot \left(1 - e^{-k \cdot t}\right) \tag{6}$$

where:

P—total production or consumption (CO, CO_2, or O_2), (mg·g^{-1}d.m.),
P_0—maximum production or consumption (CO, CO_2, or O_2), (mg·g^{-1}d.m.),
k—production or consumption (CO, CO_2, or O_2) constant rate, (h^{-1}),
t—time, (h).

The k and P_0, calculated from nonlinear regression, were used to calculate the average production or consumption rate (r) of CO, CO_2, or O_2 according to:

$$r = k \cdot P_0 \tag{7}$$

where:

r—average production or consumption rate (r) of CO, CO_2, or O_2, (mg·g^{-1}d.m.·h^{-1}).

The same procedure (Equations (6) and (7)) was applied for the respiration activity (AT_4) of the biowaste sample's characterization.

2.5. Statistical Analysis

Statistical analysis for the results was performed using the Statistica software 13.3 (TIBCO Software Inc., Palo Alto, CA, USA). 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. Kinetic model parameters were statistically verified by estimating the value of the determination coefficient R^2 and Akaike information criterion (AIC) according to the following formula [37]:

$$AIC = n \, log\left(\frac{\sum \hat{e}_i^2}{n}\right) + 2K \tag{8}$$

where:

$\hat{e}_i$—the estimated residuals for a particular candidate model,
K—total number of estimated regression parameters.

3. Results

3.1. Material Properties before and after Composting

The initial moisture content in the biowaste mixture was ~59%. The final moisture content depended on the composting temperature. In general, for the 10~40 °C range, the final moisture content fluctuated near the initial value of 59%. For 25 °C, the moisture content decreased in the abiotic variant to 58%, while it increased to almost 62% for the biotic variant. For 30 °C, a decrease of moisture content in both variants was observed to 53% & 57% in abiotic & biotic variants, respectively. At 37 °C in the case of abiotic variant, the significant ($p < 0.05$) drop of the water content compared to the abiotic conditions at 25, 60, and 70 °C (Figure 2a), but less significant than at 30 °C (56.5% for abiotic and 58% for biotic conditions). At 40 °C, the differences between abiotic and biotic variants were significant ($p < 0.05$), and the water content amounted to 57% and 60%, respectively.
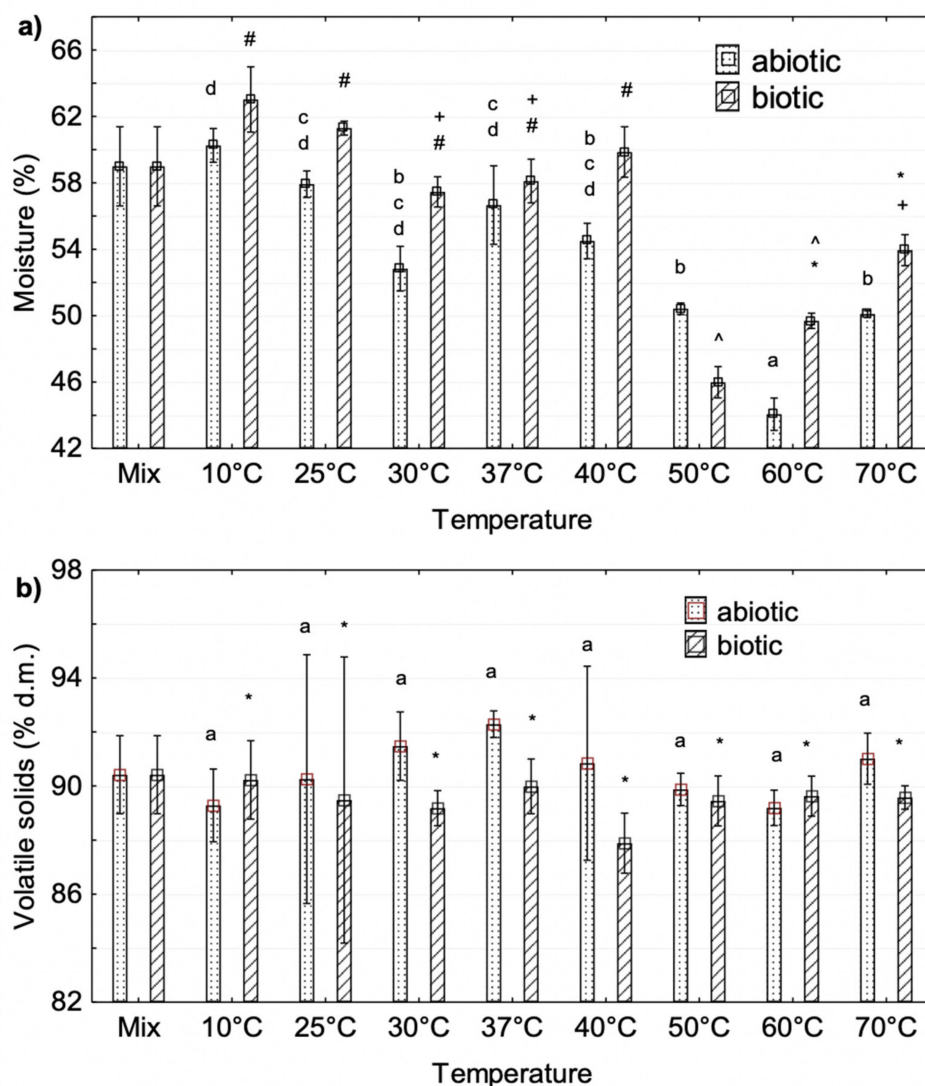

Figure 2. (**a**) Moisture content in biowaste (Mix—before the experiment) and residual biowaste after biowaste aerobic biostabilization under biotic and abiotic conditions under different thermal conditions,(**b**) volatile solids content in biowaste (Mix—before the experiment); letters and symbols (a, b, c, d, *, +, #) indicate homogeneity group according to Tukey'stest at significance level $p < 0.05$ (abiotic and biotic variant, respectively). The error bars are showing +/− standard deviation around the mean.

Composting above 50 °C significantly ($p > 0.05$) reduced the water content in all variants, below the optimum value of 59%. At 60 °C, the final moisture content was 40% and 49% for the abiotic and the biotic variant, respectively. It was the highest moisture loss, which may indicate the highest intensity of the process at this temperature. In the abiotic variant, a significant difference ($p > 0.05$) was observed between 60 & 10, 25 & 30 °C and between 70 & 10, 25, 30, 37, 40 and 50 °C (Figure 2a). Composting at 70 °C resulted in moisture content drop up to 50% in abiotic and 54% in the biotic variant. Significant differences ($p > 0.05$) were observed between the abiotic variant at 60 & 70 and 10, 25, 30, 37, 40, & 50 °C (Figure 3).
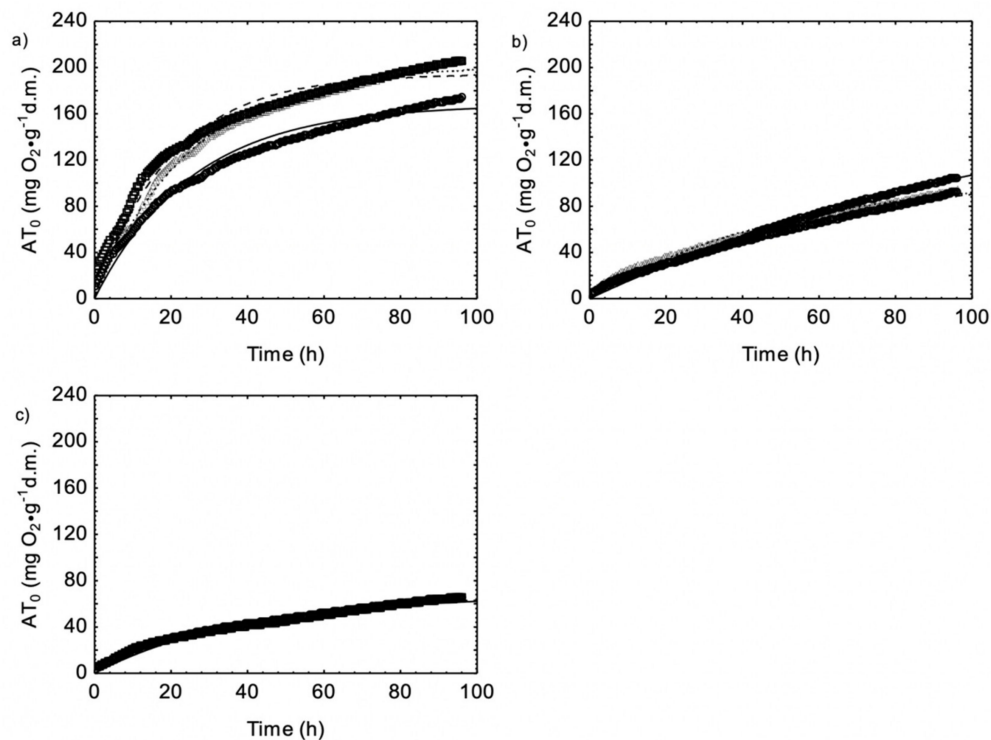

Figure 3. The respiration activity (AT0) of substrates (N = 3) and waste mix (N = 2); (**a**) grass, (**b**) manure, (**c**) mixture (different lines on figures mean repetitions).

The prepared biowaste mixture was characterized by a high content of volatile solids (~90% d.m., Figure 2b). However, no significant differences ($p > 0.05$) were observed during the 1-week aerobic biostabilization of the mixture (Figure 2b). In most variants, the content of organic matter was slightly numerically lower (not statistically significant) in non-sterilized biowaste in comparison to the sterilized under the same thermal conditions, which could be due to the activity of microorganisms under biotic conditions. The slight decrease (not statistically significant) in organic compounds is also due to the short process time (7 d). The highest ($p < 0.05$) differences between abiotic and biotic variants were observed at 30 & 40 °C (91.5% vs. 88.5%, & 91% vs. 88%, respectively) (Figure 2b).

Both two types of substrates (grass and manure) and the resulting biowaste mixture used in experiments were characterized by high respiration activity (Figure 3). The highest activity was obtained in the case of grass (188 mg $O_2 \cdot g^{-1}$d.m.), followed by manure (141 mg $O_2 \cdot g^{-1}$d.m.), and biowaste mixture (67.5 mg $O_2 \cdot g^{-1}$d.m.) (Table 1). The lowest AT_4 value for biowaste mixture was caused by the addition of inert sawdust. The kinetic rate constant for the O_2 demand of the obtained waste mixture was 0.026 h^{-1} (Table 1).

Table 1. The kinetic parameters of respiration activity for grass, manure, and biowaste mixture (shown in Figure 3).

Material	Repetition	AT$_4$		k		r		R^2
		(mg O$_2$·g d.m.$^{-1}$)	Mean ± SD	(h^{-1})	Mean ± SD	(mg O$_2$·g d.m.$^{-1}$·h^{-1})	Mean ± SD	-
Grass	1	167.92		0.0392		6.5751		0.9642
	2	194.59	188.11 ± 17.9	0.0503	0.0432 ± 0.006	9.7958	8.1500 ± 1.61	0.9675
	3	201.83		0.0400		8.0792		0.9898
Manure	1	190.74		0.0082		1.5707		0.9959
	2	124.44	140.63 ± 44.3	0.0130	0.0136 ± 0.006	1.6192	1.7558 ± 0.28	0.9897
	3	106.70		0.0195		2.0775		0.9787
Mixture	1	67.86	67.50 ± 0.5	0.0267	0.0263 ± 0.001	1.8138	1.7762 ± 0.05	0.9763
	2	67.15		0.0259		1.7385		0.9648

3.2. Dynamics of O$_2$ Consumption and CO$_2$ Production during Biowaste Aerobic Biostabilization

The O$_2$ consumption and CO$_2$ production were higher in all biotic variants compared with abiotic. This also indicated the successful sterilization process driven by biological & thermochemical mechanisms in biotic variants, while only thermochemical in abiotic. O$_2$ consumption in the biotic variant at 10 °C was 56.2 mg O$_2$·g^{-1}d.m. (Figure 4a), while the amount of CO$_2$ produced during the experiment reached 72.8 mg CO$_2$·g^{-1}d.m. (Figure 5a). At the same time, the amount of CO$_2$ produced in the abiotic variant was much lower and amounted to 4.04 mg CO$_2$·g^{-1}d.m (Figure 5a). Larger amounts of CO$_2$ generated and O$_2$ consumed were observed at 20 °C (Figures 4b and 5b). The total amount of O$_2$ consumed by microorganisms during 168 h was 6.03 mg O$_2$·g^{-1}d.m. under abiotic conditions and 112 mg O$_2$·g^{-1}d.m. in the biotic variant. At 30 °C, a further increase in O$_2$ consumed was observed (Figure 4c). The amount of CO$_2$ produced at this temperature was 6.75 mg CO$_2$·g^{-1}d.m. in abiotic and 174 mg CO$_2$·g^{-1}d.m. in biotic variant (Figure 5c). In the case of abiotic conditions, the effect of time was not statistically significant ($p > 0.05$) for the observed in CO$_2$ production and O$_2$ consumption at 10, 25, and 30 °C, indicating that these variants belonged to the same homogeneous group.

A significant statistical difference was observed only at the end of the process at 37 °C (132~168 h) (Table S3.12–S3.15, Supplementary Materials). A different outcome was noted in the case of abiotic conditions at 30 and 40 °C, i.e., much higher amounts of O$_2$ consumed and CO$_2$ produced in the case of 40 °C, a significant difference ($p < 0.05$) between variants, notably visible at the end of the process.

The peak of CO$_2$ produced (205 mg CO$_2$·g^{-1}d.m.) and O$_2$ consumed (160 mg O$_2$·g^{-1}d.m) was observed at 40 °C in the biotic variant (Figures 4e and 5e). The values obtained showed significant differences ($p < 0.05$) for most of the temperatures and belonged to a separate homogeneous group at most of the time of the experiment. At 50 °C, a decrease in the amount of CO$_2$ produced (153 mg CO$_2$·g^{-1}d.m) and O$_2$ consumed (130 mg O$_2$·g^{-1}d.m) were observed (Figures 4f and 5f) in biotic variants in comparison to 40 °C.

At 60°C, a decrease in the amount of CO$_2$ produced (116 mg CO$_2$·g^{-1}d.m) and O$_2$ consumed (101 mg O$_2$·g^{-1}d.m) under biotic conditions were observed (Figures 4g and 5g). The highest amount of CO$_2$ produced 46 mg CO$_2$·g^{-1}d.m in the entire experiment was observed at abiotic conditions under 60 °C (Figure 5g). The amount of O$_2$ consumed and CO$_2$ produced at 70 °C was very low (below 20 mg CO$_2$·g^{-1}d.m) and numerically similar regardless of the conditions (Figures 4h and 5h).

3.3. Kinetics of CO Production during Biowaste Aerobic Biostabilization

The accumulated amount of CO generated during the experiment at various temperatures is shown in Figure 6. Table S3.3 (Supplementary Materials) presents the best-fit reaction kinetics model (0- or 1st-order) selected based on the coefficient of determination (R^2) and Akaike information criterion (AIC). The amount of CO produced at 10 °C was 7.23 µg CO·g^{-1}d.m. under abiotic and 16.5 µg CO·g^{-1}d.m. biotic conditions (Figure 6a). At 20 °C, CO production was higher for both abiotic and biotic variants, and the final value was slightly higher for the abiotic (26.7 µg CO·g^{-1}d.m.) compared to 25.9 µg CO·g^{-1}d.m. for biotic (Figure 6b). At 30 °C, the CO production was 17.5 µg CO·g^{-1}d.m. (abiotic) and 21.5 µg CO·g^{-1}d.m. (biotic) (Figure 6c). Higher CO production

was observed at 37 °C (Figure 6d), especially for abiotic variant 43.4 μg $CO \cdot g^{-1}$d.m. compared to 31.4 g $CO \cdot g^{-1}$d.m. under biotic conditions. The opposite situation was observed during biostabilization at 40 °C (Figure 6e).
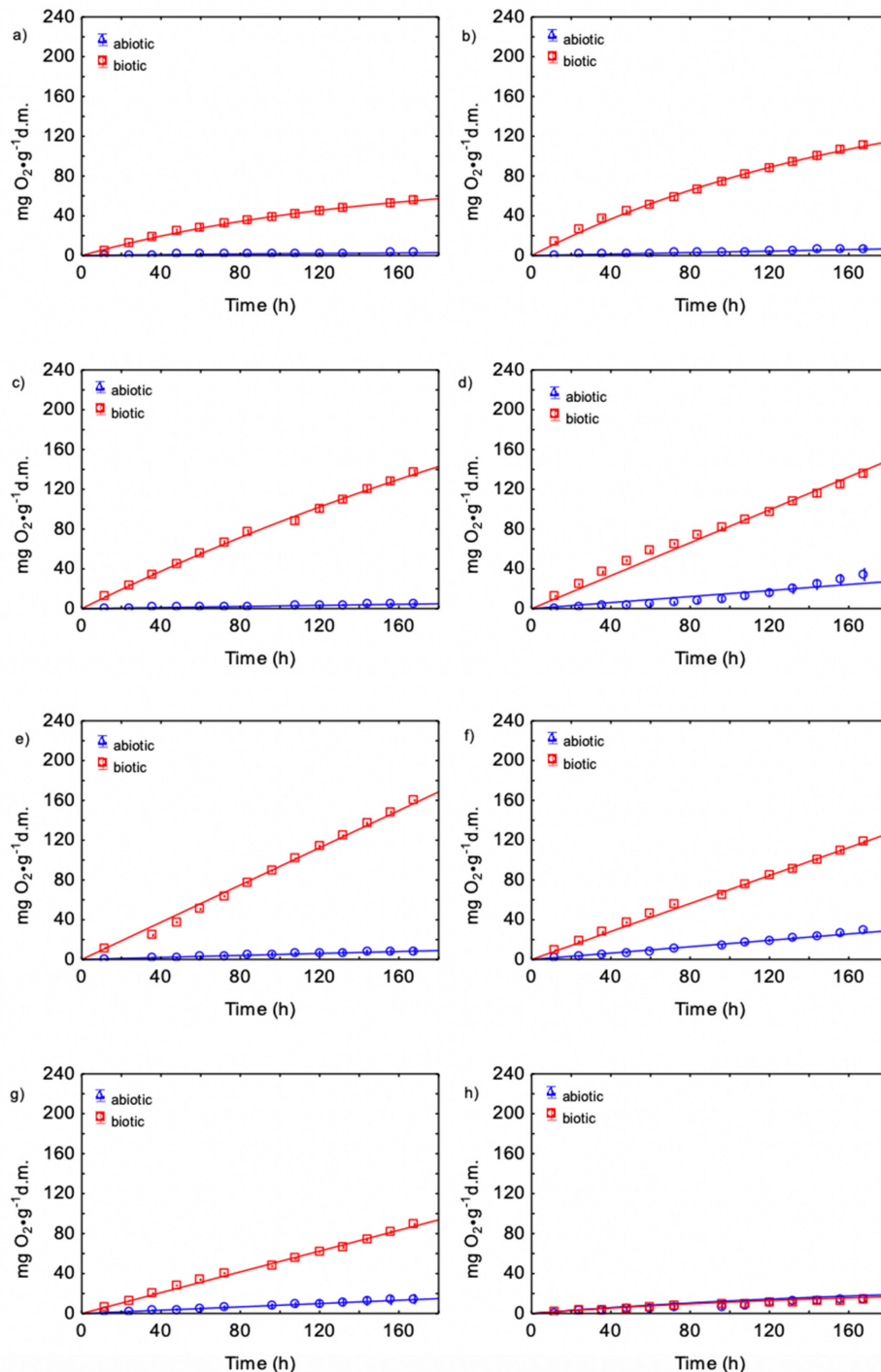

Figure 4. Cumulative O_2 consumption depending on the temperature under abiotic and biotic conditions under different fixed temperatures (**a**) 10 °C, (**b**) 20 °C, (**c**) 30 °C, (**d**) 37 °C, (**e**) 40 °C, (**f**) 50 °C, (**g**) 60 °C, (**h**) 70 °C. The error bars are showing +/− standard deviation around the mean. Raw data are shown in Tables S1.1 and S1.2 (Supplementary Materials, sheet 1). The kinetic values are shown in Table S2.1 (Supplementary Materials, sheet 4). Equations used in figures are shown in Table A1. The Tukey test $p < 0.05$ are shown in Tables S3.1–S3.28 (Supplementary Materials, sheet 6).
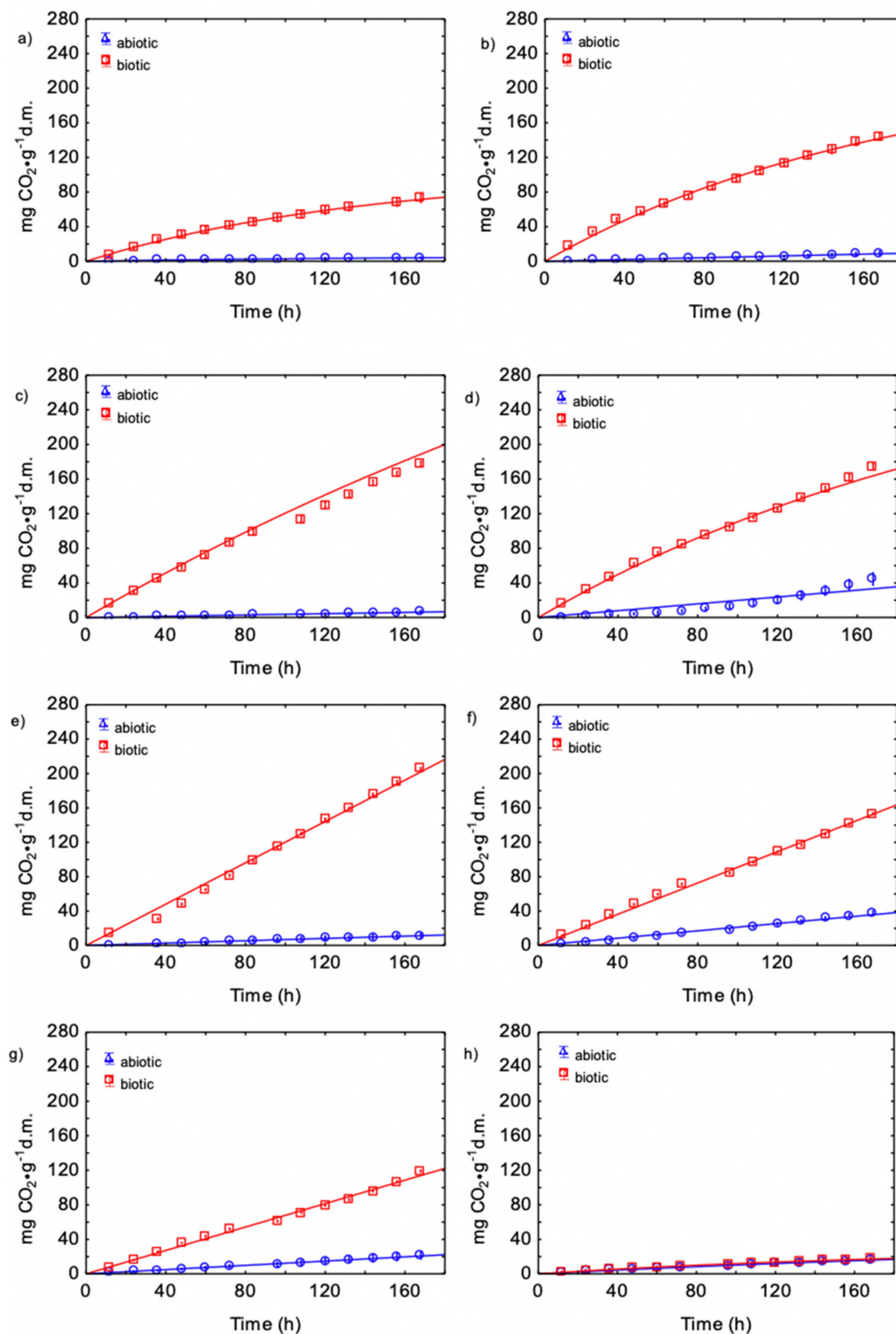
Figure 5. Cumulative CO_2 production depending on the temperature under abiotic and biotic conditions under different fixed temperatures (**a**) 10 °C, (**b**) 20 °C, (**c**) 30 °C, (**d**) 37 °C, (**e**) 40 °C, (**f**) 50 °C, (**g**) 60 °C, (**h**) 70 °C. The error bars are showing +/− standard deviation around the mean. Raw data are shown in Tables S1.3 and S1.4 (Supplementary Materials, sheet 2). The kinetic values are shown in Table S2.2 (Supplementary Materials, sheet 5). Equations used in figures are shown in Table A1. The Tukey test $p < 0.05$ are shown in Tables S3.29–S3.56 (Supplementary Materials, sheet 8).
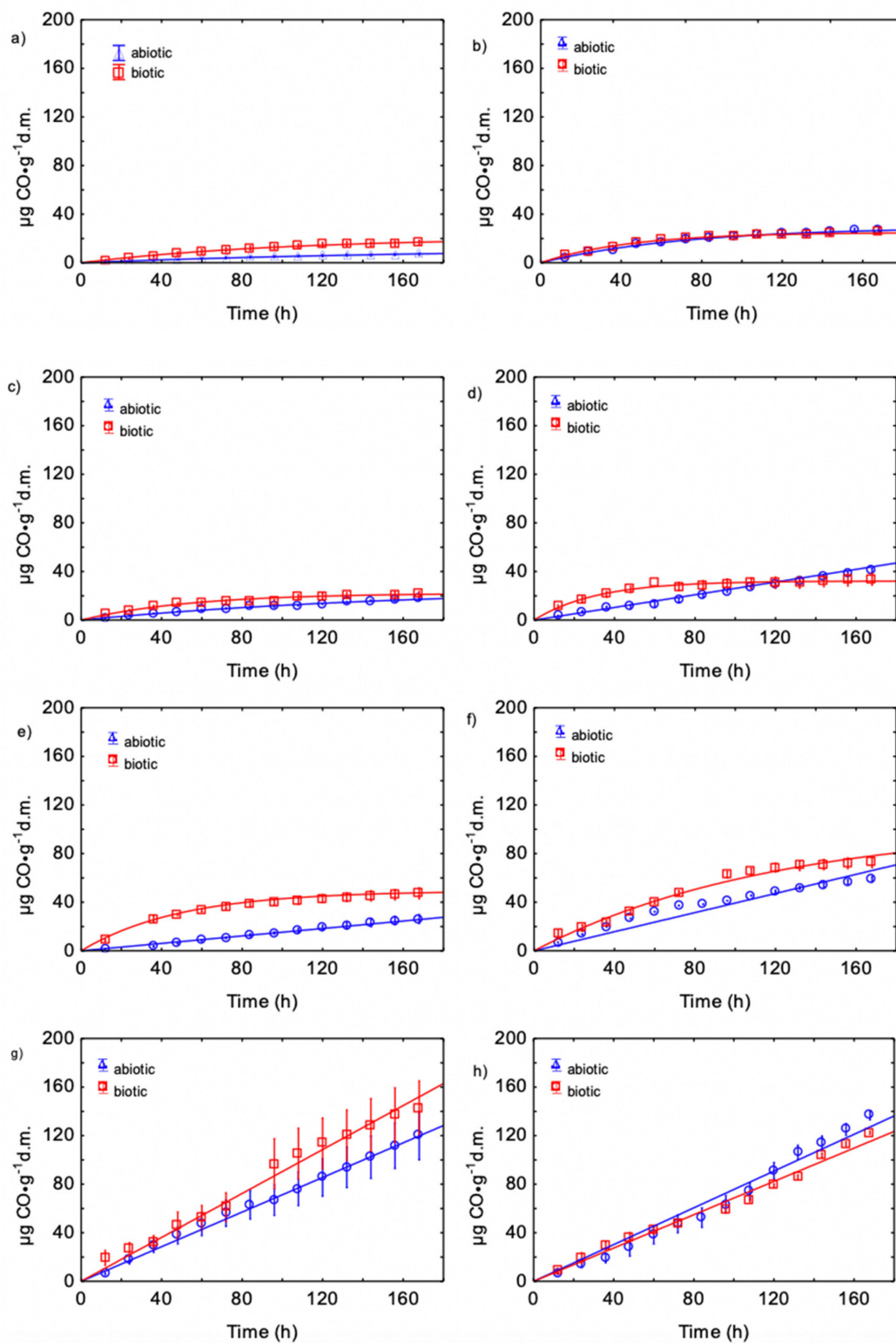
Figure 6. Cumulative CO production depending on the temperature under abiotic and biotic conditions under different fixed temperatures (**a**) 10 °C, (**b**) 20 °C, (**c**) 30 °C, (**d**) 37 °C, (**e**) 40 °C, (**f**) 50 °C, (**g**) 60 °C, (**h**) 70 °C. The error bars are showing +/− standard deviation around the mean. Raw data are shown in Tables S1.5 and S1.51 (Supplementary Materials, sheet 3). The kinetic values are shown in Table S2.3 (Supplementary Materials, sheet 6). Equations used in figures are shown in Table A1. The Tukey test $p < 0.05$ are shown in Tables S3.57–S3.84 (Supplementary Materials, sheet 9).

Increasing temperature resulted in a further increase in CO production at 50 °C to 58.8 µg $CO \cdot g^{-1}$ d.m. (abiotic) and 73.0 µg $CO \cdot g^{-1}$ d.m. (biotic), at 60 °C to 120 µg $CO \cdot g^{-1}$ d.m. (abiotic) and 115 µg $CO \cdot g^{-1}$ d.m. (biotic), and at 70 °C to 137 µg $CO \cdot g^{-1}$ d.m. (abiotic) and 122 µg $CO \cdot g^{-1}$ d.m. (biotic) (Figure 6f). Significant differences ($p < 0.05$) in abiotic variants were observed between CO production under 10, 20, 30, 37 & 40 °C and temperatures of 50, 60 & 70 °C, which indicates that the temperature of 40 °C is a threshold value above which the CO production significantly ($p < 0.05$) increases. In biotic variants, significant differences were noted between the temperature of 10, 20, 30, & 37 °C and the temperature of 50, 60 & 70 °C for up to 60 h of experiment. At 70 °C, the differences were the same in both variants, due to the lethal effects of high temperatures on bacteria.

3.4. Kinetics of CO_2 and CO Production and O_2 Consumption during Biowaste Aerobic Biostabilization

Kinetics analyses of the production of CO & CO_2 and O_2 consumption were performed. The obtained results of O_2 consumption (Table S2.1, Supplementary Materials) and CO_2 production (Table S2.2, Supplementary Materials) during the biowaste aerobic biostabilization process differed significantly depending on the variant and temperature. Particularly high O_2 consumption and CO_2 production were observed in biotic variants.

The nature of O_2 consumption in the low-temperature range from 10 to 30 °C in both abiotic and biotic variants was nonlinear (Table S2.1, Supplementary Materials). At higher temperatures from 37 to 60 °C, the cumulative curves had linear character, while in the case of 70 °C, the 1st-order model better fitted to the results (Table A1). In the case of CO_2 production, a clear distinction into abiotic (mostly with a linear trend) and biotic (1st-order model) (Table S2.2, Supplementary Materials, Table A1) was observed. In the case of CO production, it was difficult to demonstrate one specific type of trend. At temperatures up to 30 °C, all analyzed samples followed the 1st-order model (Table S3.3, Supplementary Materials). At 37~40 °C, two different trends were observed, i.e., the linear for abiotic variants and the 1st-order for biotic conditions (Table A1). At 50 °C, all variants were characterized by the 1storder model, while at 60~70 °C, the CO production kinetics had an evident linear character (Table A1).

The k value had typical values for the composting process, 0.002–0.15 (d^{-1}) [38]. It was noted that O_2 consumption in the temperature range up to was significantly lower in abiotic variants. Above 40 °C, the k values were similar (Table S2.1, Supplementary Materials) both under biotic and abiotic conditions.

For CO_2 production at temperatures up to 37 °C, a slightly higher k value was observed in biotic variants. In the range of 40~70 °C, slightly higher k and r_{CO2} values were observed in the case of abiotic variants (Table S2.2, Supplementary Materials, Figure A1). Both in O_2 and CO production r_{O2} and r_{CO2} values were similar in abiotic conditions (Figure A1). In the case of CO production, the highest k values were observed in biotic variants ranging from 20 to 40 °C, i.e., from 0.018 to 0.031 h^{-1}. The k values were higher in all biotic variants than in abiotic (Table S3.3, Supplementary Materials; Figure A1).

4. Discussion

4.1. Biowaste Properties

The properties of biowaste and mixtures were shown and discussed in the previous article [4], Table S1 in Supplementary Materials. The experiment confirmed that the initial moisture content of dairy manure used to the experiment assumed typical values [38]. Organic matter content was similar to the previously observed by Fukumoto et al. [39] from 81.2% to 87.9% d.m. Similarly, the properties of grass did not differ significantly from the results available in the literature, i.e., moisture content of 70~80%, organic content 70~90% d.m. [40,41]. Due to the high moisture in both materials, it has been decided to add dried sawdust to obtain the optimal water content for the composting process (~60%, according to [42]). The sawdust used was characterized by a relatively high content of organic

substance compared to those used by Adhikari et al. [43], i.e., 75.0% compared to 53.7% d.m., which was the consequence of using fresh material.

Changes in moisture up to 5% in the material after the process observed during the present study are typical and consistent with data obtained earlier by other researchers. Sun et al. [44] explained this phenomenon by the process of water evaporation and its removal from headspace by the airflow. At lower temperatures (<30 °C), which do not allow for such intensive evaporation from the material, water is formed as a result of the decomposition of organic matter, both from chemical and microbiological sources [45]. This results in increased moisture of the waste [46,47].

The observed differences in the decomposition of the volatile solids between abiotic and biotic conditions were insignificant (especially at >50 °C), which may indicate that the thermochemical mechanism of organic matter decomposition dominated at higher temperatures. de Guardia et al. [48] noted that if the samples are similar to each other in organic matter content and moisture, their degradation is very similar, even despite different process conditions (temperature and moisture). Low organic matter decomposition could also indicate the influence of a large amount of hardly degradable lignocellulosic organic compounds from sawdust; even k value was similar to than proposed by [49] for the composting process. This sawdust constituted 33% of the sample, which is characterized by a high fraction of such substances [4].

Studies of various substrates made by Evangelou et al. [50] also demonstrated the high respiratory activity of microorganisms (AT_4) recorded in dairy manure and grass is the result of the high content of the easily-biodegradable organic substance (e.g., mono- and disaccharides, starch, proteins, and fats) in this biowaste. Sawdust was not tested for AT_4 activity due to the use of dry material. Low humidity creates an unfavorable environment for the development of microorganisms. For this reason, the addition of dried pine sawdust (used to reduce moisture to optimal values) caused a decrease in AT_4 to 67.5 mg $O_2 \cdot g^{-1}$d.m. in the biowaste mixture (Table 1).

All obtained results in the respiratory activity of microorganisms are typical for the type of material [51]. Dairy manure used for the tests showed a similar AT_4 value to chicken manure 94.7 and 109 mg $O_2 \cdot g^{-1}$d.m. [52] and to the organic fraction of municipal waste 90.9~119 mg $O_2 \cdot g^{-1}$ d.m. [48].

Similarly to previous research, if the respiratory activity was following the 1st-order model, we also used for the determination of the kinetic parameters. Nonlinear estimations for almost all samples were characterized by a very high coefficient of determination ($R^2 > 0.96$) (Table 1). The highest rate constant was obtained for grass ($k = 0.043$ h^{-1}); lower k value was noted for manure ($k = 0.0136$ h^{-1}) and biowaste mixture ($k = 0.026$ h^{-1}). The rate constants were comparable with the values obtained by other researchers for plant materials such as leaves ($k = 0.00129$ h^{-1}), branches ($k = 0.0025$ h^{-1}), grass ($k = 0.00125$ h^{-1}), and food waste ($k = 0.00044$ h^{-1}) [53]. In studies made by Fernandez et al. [54], k was from 0.0009 to 0.0018 d^{-1}. These values are in line with those proposed by Mason and Milke [49] for the composting process ($k = 0.0063$ h^{-1}) and were higher than for organic waste.

4.2. O_2 Consumption, CO_2 and CO Production during Biowaste Aerobic Biostabilization

CO_2 emissions are frequently used for estimating organic carbon decomposition [55]. In this research, it increased up to the 7th day of the process, but then a gradual decrease was observed. This phenomenon was also observed in laboratory conditions during composting garden waste [56,57] and in technical-scale analyzes when composting sewage sludge [44]. The amount of CO_2 produced during the 48 days of the process was about 50,000 g $CO_2 \cdot m^{-2}$, with 20,000 g $CO_2 \cdot m^{-2}$ produced during the next five days of the process [44].

This high initial CO_2 production is the result of the microbial activity that degrades easy-available volatile solids, such as simple sugars or organic acids [54]. This was confirmed by studies Nasini et al. [58], who observed a relationship between CO_2 emission and the level of microbial activity during static composting in piles of waste from olive oil pressing. The involvement of aerobic microorganisms in the production of CO_2 can also be seen in this research. For biotic variants, the amount of CO_2 produced was much higher than for abiotic. Furthermore, the cumulative

CO_2 production was the highest at 30, 37, and 40 °C, while the lowest in extreme thermal conditions, i.e., 10 and 70 °C. This may be due to the domination of mesophilic bacteria in the used biowaste mixture, for which the optimum temperature range is 30~40 °C [59]. Temperatures close to 10 and 70 °C caused inhibition of their metabolism, which affected the reduction of organic matter decompositions and the CO_2 production.

Analyzes conducted by Sommer and Moller [60] allowed the observation of the relationship between O_2 consumption and CO_2 emission. The presence of one gas inhibits the occurrence of another. This tendency can also be seen in the results presented here (cumulative O_2 consumption is inversely proportional to CO_2 production). Additional confirmation is also the rapid decrease in the amount of O_2 during the first 7 days observed earlier in the laboratory by Kulcu and Yaldiz [61] and in the technical-scale conditions [62]. Experiments carried out by [6] are also supported by the presented here findings. In the composting processes they analyzed, the initial decay in O_2 content was simultaneous with an increase in the CO_2 concentration in the material due to the decomposition of simple organic compounds. The correlation of CO_2 production with O_2 consumption in the present study and their dependence on the activity of microorganisms concerning temperature was also consistent with reports Puyuelo et al. [63]. They emphasized that temperature and O_2 consumption are identified as key parameters for assessment of the microbial activity.

A characteristic feature of CO production during composting is its increased production in the early stages of the process. The researchers reported high concentrations of CO during composting of green waste with grass immediately after a few hours starting the experiment [19]. In the experiments of all of the cited authors [4,7,19,64], the CO content decreased after a period of 1 to 2 weeks. In our previous research [6], CO emissions from the biostabilization process of organic fraction of municipal solid waste CO emission significantly decreased in the 3rd week of the full-scale experiment. This tendency is also visible in the present results. For process variants carried out at 10, 20, 30, and 37 °C after the initial increase in the amount of CO produced, the CO production stagnated (with 40 °C only in the case of biotic variant).

CO production rate decreased in the later stages of biostabilization can be explained by two factors. The first is the consumption of CO by bacteria as a result of oxidation CO to CO_2 [5]. The second is based on the dependence between the content of CO and O_2 in the biowaste matrix. [3,7,19,64] reported the highest CO production under aerobic conditions. The O_2 depletion causes a decrease in the level of CO, and thus affects the increase in CO_2 concentration, which could affect the results obtained.

In this study, the highest CO production was characterized by high-temperature biostabilization variants (60, 70, and 50 °C, respectively). The lowest CO production was obtained at 10 and 30 °C. These results are consistent with those obtained by [4]. In their experiment, the highest CO production was observed at 65 and 50 °C, while the lowest amounts of CO were generated during composting of green waste at 5 °C.

4.3. Kinetics of O_2 Consumption, CO_2 and CO Production during Biowaste Aerobic Biostabilization

Many authors recognize the benefits of predicting gaseous products from composting, such as CO_2 production and O_2 consumption and other GHG, but still less data regarding CO kinetics production [65,66]. Knowledge of the kinetics of the composting process allows its modeling, which in turn could improve the optimization [67]. To date, researchers have proposed several models for process kinetics, mainly based on the physical and biochemical properties of composts [5]. Mathematical models most often refer to 0, 1st, or higher-order kinetics [50,68]. These models can effectively describe the evolution of changes in process parameters, such as mass balance, heat balance, organic matter degradation, C mineralization, or changes in the C, N, P, K content [53,69], O_2 demand, or CO_2 production [67,70]. The proportion of nutrients have an extreme influence on compost properties [71]. No studies are presenting a full picture of the kinetics of COproduction as a function of the composting process temperature. Due to a gap in the literature, the comparison in this paper was made mainly based on other process parameters obtained by other researchers. The CO_2 production stems from

the oxidation of readily degradable carbon compounds, and consequently, from the nature of the substrates, O_2, moisture, and microbial activity [72]. The CO_2 emission rate is also an indicator of the rapid degradation of total organic matter and strong microbial activity [73]. The carbon mineralization of organic wastes follows a combined two-step kinetic: initial rapid phase 1st-order followed by 0-order, slow phase [74], thus, the rationale to use of these two models to find kinetic parameters of composting. Results from this study confirmed that zero and 1st-order kinetic suits both for the production CO_2 and O_2 consumption (first stage of composting), which is confirmed with high R^2 and low AIC results (Tables S2.1 and S2.2, Supplementary Materials). CO_2 production and O_2 consumption rate during biotic conditions were typical for the first stage of composting, as were observed by other researchers [46,70,75]. In the abiotic environment, k value was lower due to a lack of microorganism (sterilization process). O_2 consumption rate was only a reason for thermochemical reactions inside easily biodegradable fraction. Hosseini and Aziz [76] confirmed that the increase in temperature in the composting pile results in an increase in the consumption rate at which chemical (hydrogen) bonds in substrate compounds like polysaccharides cellulose and lignocellulose are broken. As suggested by Ma et al. 2020 [77] this biodegradation, is made by complex enzymes and requires the synergistic action of various microorganisms. The same conditions influenced the CO production rate. We confirmed that the threshold value of CO production kinetics, below 40 °C is driven by biotic processes, while above 40 °C by thermochemical. However, the net CO production above 40 °C was limited by the biodegradation of thermochemically produced CO. This is because the optimal temperature for most microorganisms is 38.5 °C, with a maximum from 63 °C to ~67 °C, which, as the authors note, is not in line with the process temperature that we observed during composting in real condition, i.e., 60~70 °C [78]. It can be explained that the change in curvature observed is due to a change in the control regime of the oxidation reaction. Initially, it is governed by the biotic process (with a pseudo-first-order kinetics) until a temperature is reached in which a thermochemical regime governs (with zero-order kinetics). In the presented experiment, the confirmation of this trend was also noticed. In thermophilic conditions >50 °C, the CO_2 production was significantly reduced. However, the CO production increased with temperature, which confirms thermochemical nature, independent of microorganisms, at least at high temperatures. This observation is also confirmed with the kinetic model, i.e., <50 °C the best-fit model is 1st-order kinetic, but >50 °C, the best-fit model was a 0-order in both abiotic and biotic conditions (Table A1). This trend can be clearly observed in r_{O2} consumption and r_{CO2} production rate present in Figure A1. This information is true for 0- and I-order kinetics.

Although a higher CO production was observed at temperatures <50 °C, the k and CO production rate was always lower in abiotic conditions, even in thermophilic conditions, when it was similar for both variants. We also observed that in biotic conditions, where the maximum CO production was at 60 °C, while the maximum k was at 37 °C. Some explanation can be elucidated from the results of Tang et al. [79] and Yu et al. [80], who noted that the k value of organic matter decomposition under thermophilic conditions was ~20% higher than under mesophilic conditions, and the maximum thermophilic composting activity at temperatures in the range of 50~60 °C. This was a result of thermochemical decomposition at high temperatures, as well as the microbial activity. It has been experimentally shown that the rate of microbial activity, as well as the process of hydrolysis, are closely related, and the relationship is as follows—the reaction rate increases in the range of 20 to 40~50 °C, then after exceeding 50 °C begins to decrease rapidly (Figure A1) [74]. This effect is also clearly visible in this experiment and noticed in our previous research carried out on a technical scale [8,71] when the k value was the highest between the 2nd and 4th week of tests at a temperature in a pile is >60 °C.

Chen et al. [81] identified that key factors affecting the emissions of GHGs the most are: organic nitrogen (Org N, 33.0%), DOC (20.5%), TN (14.6%), and TOC (10.2%). They believed that this characteristic of waste properties could also affect CO production. The results of this research create possibilities of predicting CO production during composting of organic waste, which can be useful, for example, to the modeling of CO concentrations and emissions in waste treatment plants [71].

Such modeling will allow the minimization of CO emissions from the process and the simultaneous use of engineering and technological solutions to optimize the decomposition of organic matter. Additionally, it will enable the development of recommendations and implementation of solutions limiting the negative impact of CO on the health of composting plant employees who have direct contact with the process. In the long term, the use of the predictive model to reduce CO emissions from the waste composting process will contribute to reducing air pollution and the associated adverse effects on public health, including the economy. Exposure to harmful compounds in the air is one of the main causes of pulmonary and cardiovascular diseases, resulting in the development of chronic diseases and premature mortality of the population. The reduction of these effects as a result of reducing pollutant emissions is defined as 75–85% of all estimated economic benefits of improving air quality [82]. So, it can be said that functioning in a healthier and cleaner environment will increase the standard of living.

Due to the limited knowledge in composting process CO production, we recommend more studies in this area. There are several pathways to explore, including composting trials with different feedstock, scaling up to larger composting volumes, exploring the influence of environmental stressors, and a more comprehensive assessment of the toxicity of emitted gases. This new data can improve mathematical models and help with the mitigation of CO production during composting and storage of organic waste. Another possibility is the production of CO and its use for the production of biorenewables.

5. Conclusions

For the first time, a full study of the influence of temperature and biowaste sterilization on the kinetics CO production is presented. The experiment showed an increase in CO production and k value with temperature. Depending on the conditions (biotic or abiotic) and the temperature, different CO production processes were observed. In both variants, the CO production increased. However, in the temperature range from 10~50 °C, higher CO production was observed in biotic conditions, suggesting that the biological transformation is responsible for CO formation. Above 50 °C, the CO production was higher under abiotic conditions, suggesting that the thermochemical process was more efficient. Additionally, the constant rate (k) value of CO production under biotic conditions was increasing up to a temperature of 60 °C, above which a slight decrease in CO production rate was observed at 70 °C. The 1st-order model described the CO production in <50 °C range; the 0-order model was better for >50 °C.

These observations indicate that the kinetic process of CO production is both abiotic and biotic. Observation of CO emissions under abiotic conditions indicates the thermochemical nature of CO production. Own previous studies have shown a significant impact of O_2 consumption on CO production under both abiotic and biotic conditions. Higher CO emissions under abiotic conditions at >50 °C indicated that microorganisms were metabolizing thermochemically produced CO in the biotic variant. However, in the psychrophilic and mesophilic temperature range (10~40 °C), higher CO production under biotic conditions indicated the presence of CO producing microorganisms. The presented results are the basis for further studies on the possibility of controlling CO production during the biowaste aerobic biostabilization process.

Conflicts of Interest: The authors declare no conflict of interest.
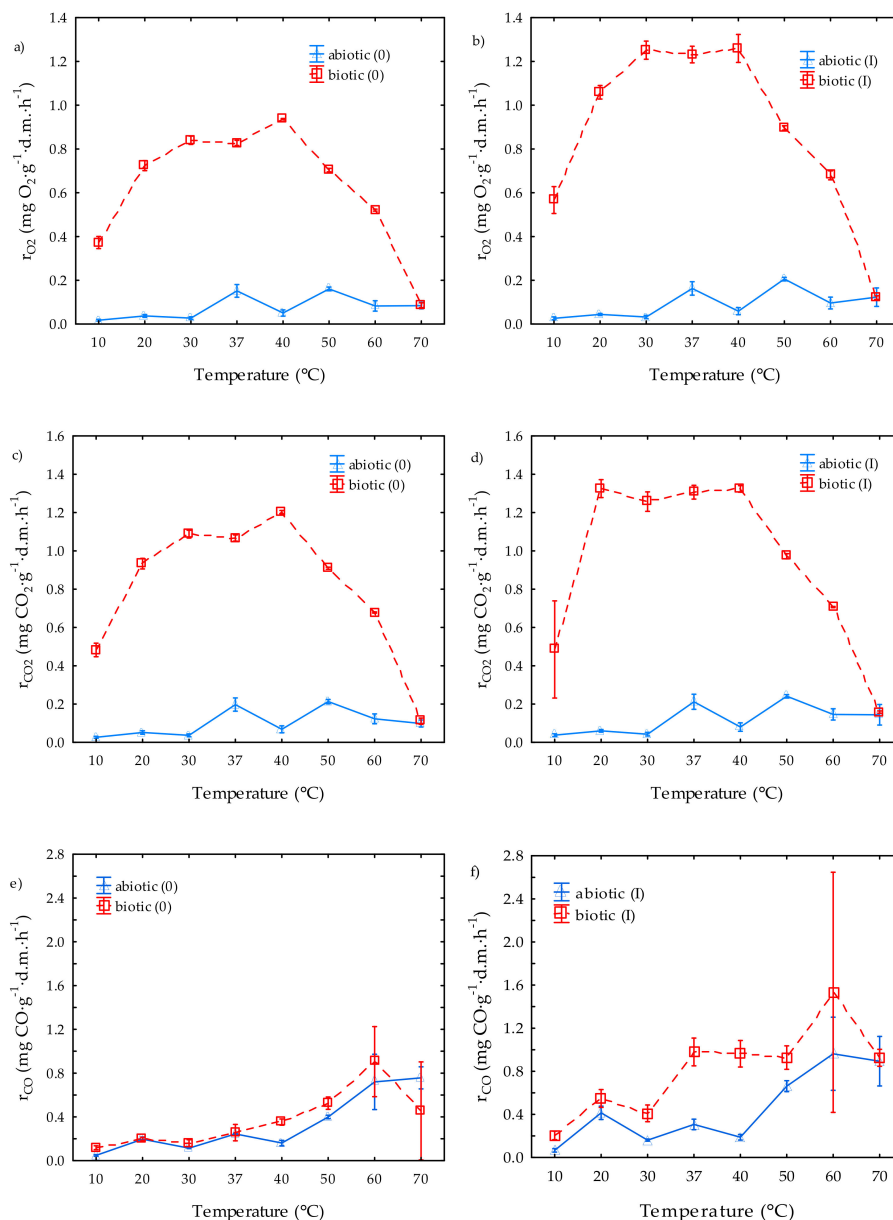
Appendix A

Figure A1. Average production and consumption rate (r_{O2}) (**a**) O_2 consumption 0-order kinetic, (**b**) O_2 consumption I-order kinetic, (**c**) CO_2 production 0-order kinetic, (**d**) CO_2 production I-order kinetic, (**e**) CO production 0-order kinetic, (**f**) CO production 0-order kinetic. The error bars are showing +/− standard deviation around the mean. The kinetic values are shown in Tables S2.1–S2.3 (Supplementary Materials, sheets 4–6).

Table A1. Kinetic model of O_2 consumption and CO_2 and CO used for process estimation (based on average results and R^2). The kinetic values are shown in Tables S2.1–S2.3 (Supplementary Materials, sheets 4–6).

Production or Consumption	Temperature °C	Abiotic	Biotic
O_2	10	$O_2 = 4.16\cdot\left(1 - e^{-0.0064\cdot t}\right)$	$O_2 = 77.92\cdot\left(1 - e^{-0.0072\cdot t}\right)$
	20	$O_2 = 19.46\cdot\left(1 - e^{-0.0032\cdot t}\right)$	$O_2 = 159.23\cdot\left(1 - e^{-0.0067\cdot t}\right)$
	30	$O_2 = 15.93\cdot\left(1 - e^{-0.0028\cdot t}\right)$	$O_2 = 174.37\cdot\left(1 - e^{-0.0072\cdot t}\right)$
	37	$O_2 = 0.1516\cdot t$	$O_2 = 0.8277\cdot t$
	40	$O_2 = 0.0505\cdot t$	$O_2 = 0.9362\cdot t$
	50	$O_2 = 0.1606\cdot t$	$O_2 = 0.7034\cdot t$
	60	$O_2 = 0.0827\cdot t$	$O_2 = 0.5207\cdot t$
	70	$O_2 = 30.61\cdot\left(1 - e^{-0.0053\cdot t}\right)$	$O_2 = 25.29\cdot\left(1 - e^{-0.0060\cdot t}\right)$
CO_2	10	$CO_2 = 6.39\cdot\left(1 - e^{-0.0060\cdot t}\right)$	$CO_2 = 68.05\cdot\left(1 - e^{-0.0071\cdot t}\right)$
	20	$CO_2 = 0.0516\cdot t$	$CO_2 = 228.41\cdot\left(1 - e^{-0.0058\cdot t}\right)$
	30	$CO_2 = 0.0369\cdot t$	$CO_2 = 610.86\cdot\left(1 - e^{-0.0022\cdot t}\right)$
	37	$CO_2 = 0.1975\cdot t$	$CO_2 = 421.37\cdot\left(1 - e^{-0.0032\cdot t}\right)$
	40	$CO_2 = 0.0681\cdot t$	$CO_2 = 1.2022\cdot t$
	50	$CO_2 = 0.2410\cdot t$	$CO_2 = 1049.7\cdot\left(1 - e^{-0.0010\cdot t}\right)$
	60	$CO_2 = 0.1232\cdot t$	$CO_2 = 0.6782\cdot t$
	70	$CO_2 = 35.51\cdot\left(1 - e^{-0.0050\cdot t}\right)$	$CO_2 = 42.58\cdot\left(1 - e^{-0.0064\cdot t}\right)$
CO	10	$CO = 13.04\cdot\left(1 - e^{-0.0053\cdot t}\right)$	$CO = 21.09\cdot\left(1 - e^{-0.0097\cdot t}\right)$
	20	$CO = 30.08\cdot\left(1 - e^{-0.0138\cdot t}\right)$	$CO = 25.62\cdot\left(1 - e^{-0.0214\cdot t}\right)$
	30	$CO = 28.36\cdot\left(1 - e^{-0.0057\cdot t}\right)$	$CO = 22.14\cdot\left(1 - e^{-0.194\cdot t}\right)$
	37	$CO = 95.57\cdot\left(1 - e^{-0.0038\cdot t}\right)$	$CO = 32.32\cdot\left(1 - e^{-0.0312\cdot t}\right)$
	40	$CO = 0.1608\cdot t$	$CO = 48.28\cdot\left(1 - e^{-0.0204\cdot t}\right)$
	50	$CO = 77.48\cdot\left(1 - e^{-0.0088\cdot t}\right)$	$CO = 95.64\cdot\left(1 - e^{-0.0098\cdot t}\right)$
	60	$CO = 0.7194\cdot t$	$CO = 0.9047\cdot t$
	70	$CO = 0.7563\cdot t$	$CO = 0.4543\cdot t$

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