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The effect of fermentation modes on the efficiency of organic waste treatment in batch bioreactors

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Introduction

The increase in the amount of solid organic waste is a global challenge requiring effective solutions (Chen, Zhang, and Yuan 2020; Alwaeli, Alshawaf, and Klasik 2022). Solid organic waste includes kitchen, food, agricultural, and garden waste (Chen, Zhang, and Yuan 2020; Mata-Alvarez, Macé, and Llabrés 2000). Since the global population is steadily growing, its demand for food production is also increasing (Chen, Zhang, and Yuan 2020). Consequently, industries and agriculture have to increase production to meet market needs. In this regard, the amount of organic solid waste daily produced amounts to over 400,000 tons in Europe (Mata-Alvarez, Macé, and Llabrés 2000).

The technologies for waste treatment require improvement since they are currently not efficient enough in degrading waste to utilize the existing resources effectively. Among the most common technologies are landfilling and incineration. However, the accumulation of organics in landfills causes their uncontrolled decay, resulting in the emission of greenhouse and toxic gases such as CO_2, CH_4, NH_3, H_2S, as well as toxic runoff containing organic acids and alcohols, along with the spread of pathogenic microorganisms. Incineration comes with its own costs, and also produces greenhouse gases and other toxic compounds (Chen, Zhang, and Yuan 2020; Pawnuk et al. 2022).

Therefore, there is an urgent need to develop new approaches for the effective degradation of solid organic waste. Biological processes are considered promising for providing effective degradation of organic waste (Chaijak and Sola 2023) while also yielding valuable by-products. For example, composting produces nutrient-rich compost that enhances soil fertility (Chen, Zhang, and Yuan 2020; Bernstad, Cánovas, and Valle 2017), while anaerobic fermentation can generate methane or hydrogen (Chen, Zhang, and Yuan 2020; Havryliuk et al. 2023).

Anaerobic fermentation of organic waste has been studied for over a century. Currently, the predominant process involves methane production and organic degradation. Various aspects of the process, including its stages, fermentation parameters, bioreactor design, etc. have been studied (Meegoda et al. 2018).

Methane fermentation has become a widespread technology. Methane can be obtained from various types of organic waste (Erdiwansyah et al. 2022). In Europe alone, over 17,000 bioreactors have been installed to meet the energy demands of agriculture and industry (Xue et al. 2020; Scarlat, Dallemand, and Fahl 2018). Presently, numerous small-scale bioreactors are being set up to supply methane to individual consumers and small enterprises (Katinas et al. 2019; Bakkaloglu et al. 2021).

Hydrogen production from anaerobic fermentation has gained popularity over the last several decades. Such type of fermentation not only degrades waste, meeting mankind's environmental protection needs, but also generates green energy carriers such as H. It contributes to minimizing greenhouse gas emissions and the greenhouse effect, since hydrogen combustion generates only water (Akhlaghi and Najafpour-Darzi 2020). Small-scale bioreactors can effectively treat solid organic waste directly at the sites of its accumulation, such as farms and cottages. In this case, not only does the amount of waste decrease, but also green energy is produced to meet energy needs and contribute to reducing the greenhouse effect (Khan et al. 2018).

Therefore, the goal of our work was to compare the efficiency of strictly anaerobic fermentation of multi-component solid organic waste with hydrogen synthesis, against waste treatment with pulsed air access in batch bioreactors. Both approaches are aimed to produce hydrogen and degrade waste, but their efficacy may differ based on the conditions created in bioreactors. Restricted air access favors increased hydrogen synthesis. However, this approach has limitations. Due to the lack of oxygen, the degradation of organics causes the accumulation of the intermediate products (organic acids and alcohols). The increased concentration of these compounds inhibits microbial growth followed by the

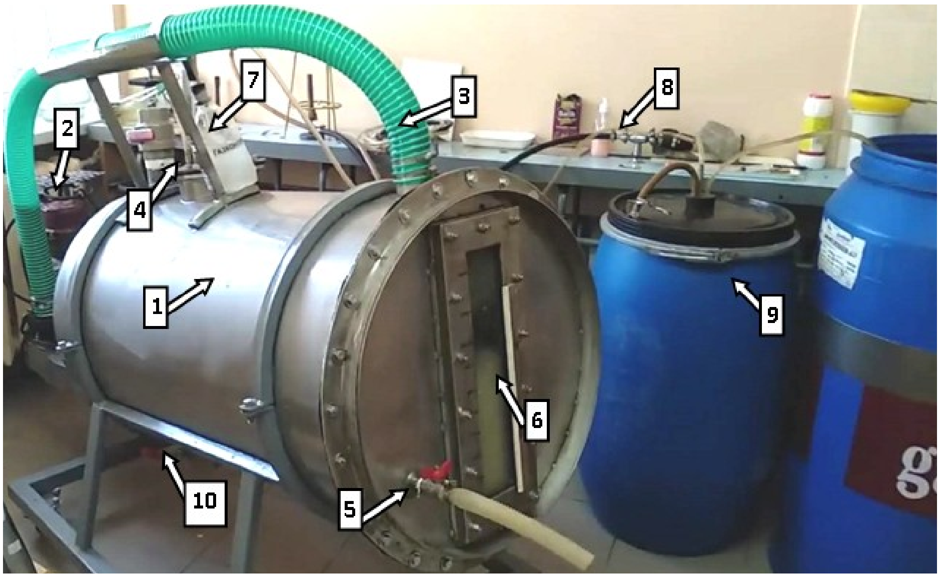

Figure. 1. Anaerobic batch bioreactor with the volume of working chamber 240 L: 1 – working chamber of the bioreactor; 2 – electric pump for waste mixing; 3 – hose for closed mass exchange of fermentation mixture; 4 – fittings to introduce microbial metabolism regulators and remove synthesized gas; 5 – fitting for culture liquid sampling; 6 – window for visual control; 7 – gas controller; 8 – tube to transport gas to the gas holder; 9 – water gas holder to store synthesized gas; 10 – fitting for draining culture liquid after the technological cycle completed.

Figure 2. Pulsed air access batch bioreactor with the volume of working chamber 300 L: 1 – working chamber of the bioreactor; 2 – fitting for culture liquid sampling; 3 – special lock for air access; 4 – electronic fermentation control unit; 5 – fittings to introduce regulators.

overall slowing down the process. On the other hand, air access can lead to more effective waste degradation through aerobic oxidation of organic acids and alcohols into the final product (CO_2), thus avoiding the inhibition of anaerobic microbial growth. However, the oxygen presence negatively affects hydrogen synthesis, as it requires strict anaerobic conditions.

The objectives of the research included the investigation of two types of organic waste treatment:

- fermentation of organics in strictly anaerobic conditions to maximize hydrogen yield, albeit potentially less efficient for waste degradation;
- treatment of waste using pulsed air access to enhacne waste degradation efficacy, albeit resulting in lower hydrogen yield.

Comparing these two approaches will aid in selecting waste treatment strategies based on customer needs during the future process scaling. The study will be of interest for the implementation of the small-scale bioreactors in households or small farms. However, large-scale implementation is currently limited by the need to investigate fermentation patterns in larger bioreactors, mixing modes, regulation of microbial metabolic activity, pH, redox potential of the fermentation mixture, etc.

Materials and methods

Anaerobic bioreactor

To study the dynamics of strictly anaerobic fermentation of multi-component solid organic waste with hydrogen synthesis, a batch bioreactor with a working chamber volume of 240 L was used (Fig. 1). The main components of the bioreactor are the working chamber where fermentation occurs, a pump for mixing the fermentation mixture, and a gas holder for collecting and storing the synthesized gas. The design of the bioreactor allows it to be hermetized by closing the fittings for waste loading and sampling. For fermentation, the bioreactor was hermetically sealed to provide anaerobic conditions and the accumulation of synthesized gas.

Pulsed air access bioreactor

To investigate the patterns of solid organic waste degradation, a bioreactor with the volume of a working chamber of 300 L was used (Fig. 2). To provide pulsed air access during fermentation, it was periodically opened with simultaneous mixing of waste using a pump installed inside of the bioreactor. Under such a mode, periodic access to air provided aerobic oxidation of soluble microbial exometabolites that inhibited solid waste hydrolysis by aerobic microorganisms. When the bioreactor was hermetically sealed, anaerobic fermentation of solid waste took place.

Granular microbial preparation for fermentation of solid organic waste

Granular microbial preparation (GMP) was used to provide fermentation of solid organic waste and hydrogen synthesis (Fig. 3). GMP contains a diversified and concentrated microbial community originating from fermented sludge from a methane tank, regulators of microbial metabolism, as well as nutrients to initiate microbial growth. It was manufactured by mixing these components with tap water to obtain a dense, homogeneous dough-like mixture. The mixture was pressed through an extruder to obtain the form of granules and dried at +105 ºC to eliminate pathogenic microorganisms and promote the growth of spore-forming bacteria.

Fermentation conduction

To study the fermentation process, a mixture of solid organic waste (10 kg) was used. It contained 5 kg of potatoes and 5 kg of a mixture of bread, cooked pasta, meat, cucumbers, carrots, and apples in an equal weight ratio. The waste was cut into cubes with an edge length of about 1 cm. It was pasteurized for

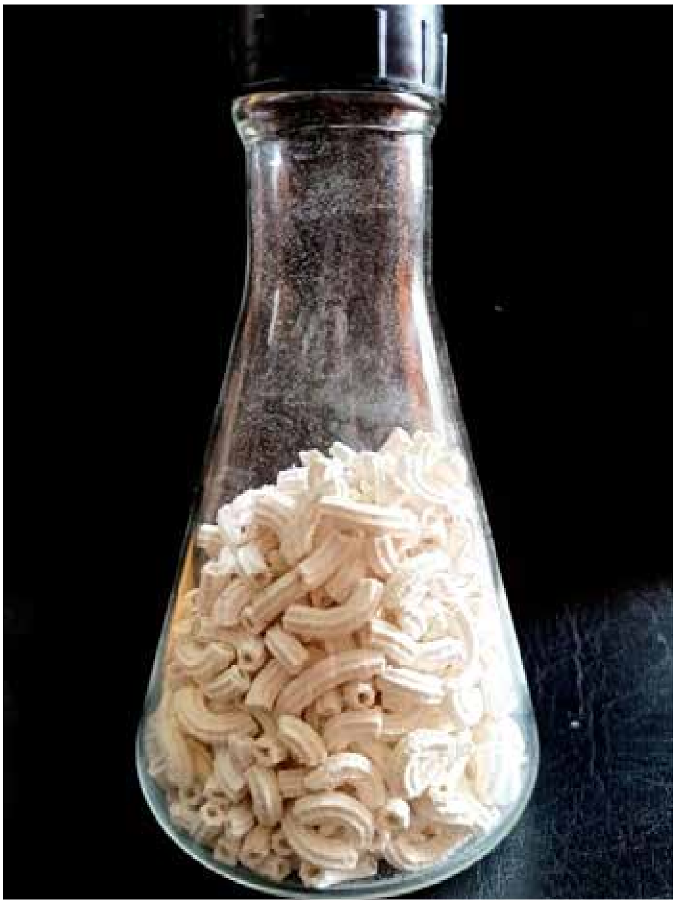

Figure. 3. The appearance of granular microbial preparation.

10 minutes and loaded into the bioreactor. 100 L) of tap water was added. The GMP (0.5 kg) was loaded to start fermentation. To adjust the initial pH of the mixture to 7, a saturated solution of Na_2CO_3 was added during fermentation if required. For both anaerobic fermentation and the process with pulsed air access, mixing was conducted only after 24 hours of cultivation to decrease the redox potential (-300…-200 mV) and to create deep anaerobic conditions. The mixing mode consisted of 5 minutes of mixing followed by 60 minute pause. In the fermentation process with pulsed air access, the bioreactor with a special lock was opened once a day, and simultaneous mixing was conducted for 1 hour to saturate the fermentation mixture with air. The fermentation was carried out at 25 ºC.

Determination of fermentation parameters

The values of pH and Eh were measured using the universal ionometer EZODO MP-103, equipped with combined electrodes featuring BNC connectors. Models PY41 and PO50 were used to measure pH and Eh, respectively.

The volume of synthesized gas was determined by observing the displacement of water from the gas holder into the water seal under the pressure exerted by the synthesized gas. After each measurement, the gas holder was replenished with water to its full capacity to avoid errors in calculating the composition of the gas phase.

The composition of the gas phase was determined using the standard gas chromatography method. The chromatograph was equipped with two steel columns: the first column (I) for the analysis of H_2, O_2, N_2, and CH_4, while the second column (II) for the analysis of CO_2. Column parameters: column I – length (l) = 3 m, diameter (d) = 3 mm, packed with molecular sieve 13X (NaX); column II – length (l) = 2 m, diameter (d) = 3 mm, filled with Porapak-Q carrier; column temperature: +60°C, evaporator temperature: +75°C, detector temperature (catharometer): +60°C, detector current: 50 mA, carrier gas: argon, gas flow rate: 30 cm^3/min. The gas concentration was calculated based on the peak square of the gas phase components (Berezkin 2000).

The concentration of dissolved organic compounds, measured in terms of total carbon content, was determined using the permanganate method (Suslova et al. 2014). In this method, a centrifuged sample (1 mL) was titrated with a 0.1% solution of $KMnO_4$ in the presence of 0.1 mL of concentrated H_2SO_4 until a light violet color was achieved. The amount of $KMnO_4$ required for complete oxidation of organic compounds was directly proportional to the carbon concentration in the sample.

Fermentation time (T, in days) refers to the time required for the degradation of waste material from its initial loading into the bioreactor until the end of the fermentation process (stabilization of fermentation parameters).

The coefficient of waste degradation (Kd) is the ratio of the initial and final weight of waste.

The yield of molecular hydrogen (VH_2, L) is the volume of H_2 synthesized by microorganisms from 1 kg of waste counted to absolutely dry weight.

Data analysis

The study was conducted in triplicate. Data analysis was carried out using the statistical platform of Microsoft Excel. Mean values and standard deviations (SDs) were calculated at a 95% confidence level.

Results and Discussion

Two technological modes of solid organic waste degradation were studied. The first mode provided strictly anaerobic fermentation in the bioreactor with a volume of 240 L. The second one was conducted in the bioreactor with a volume of 300 L. Periodical air access took place to provide not only anaerobic degradation of solid waste but also aerobic oxidation of soluble organics.

Anaerobic fermentation lasted for 4 days (Fig. 4). To facilitate the effective functioning of anaerobic microorganisms, no mixing was conducted during the first 24 hours of cultivation. During this period, the hydrolysis of solid organic waste accompanied by the accumulation of organic acids took place. This was evidenced by the decrease in pH from 7.54±0.1 to 5.3±0.2, as well as the increase in the concentration of dissolved organics from 127±5.4 to 225±16.9 mg/L, determined based on the content of total carbon (Fig. 4, a).

It took 36 hours to create anaerobic conditions. During this time, the concentration of oxygen decreased from 21% to 0% (Fig. 4, b), and the redox potential of the liquid phase decreased from +326±24 to -251±21 mV (Fig. 4, a). After 24 hours of cultivation, pH values were maintained in the range of 7.2-6.5 through the addition of a saturated solution of Na_2CO_3 and regular mixing. This fermentation mode provided the

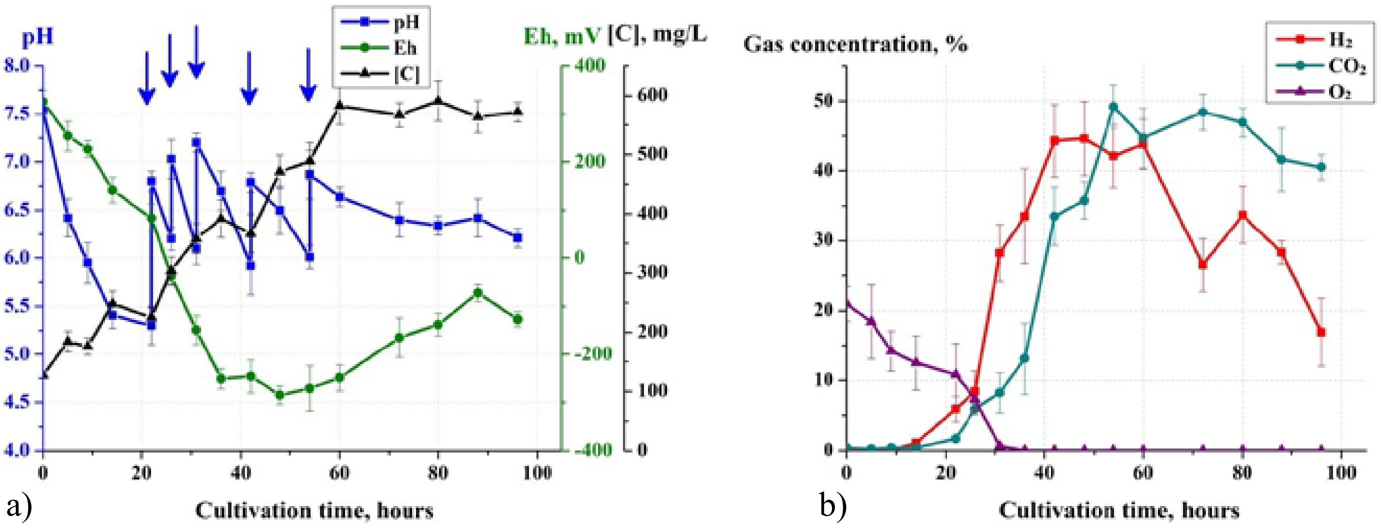

Figure. 4. Dynamics of strictly anaerobic fermentation of solid organic waste: a – pH, Eh, the concentration of soluble organic compounds; b – the concentration of H_2, CO_2, and O_2 in the gas phase (blue arrows indicate the points of Na_2CO_3 injection).

synthesis of H_2 starting from 14 hours of cultivation (Fig. 4, b). The maximum concentration of hydrogen was reached after 42 to 48 hours of fermentation.

The end of the process was indicated by the decrease in the concentration of H_2, an increase in the values of redox potential, as well as the stabilization of pH, concentration of dissolved organics, and CO_2. Strictly anaerobic fermentation resulted in a hydrogen yield of 54±4.1 L of H_2 per kilogram of waste, calculated based on the absolute dry weight of waste, and a Kd equal to 83±3.6. The concentration of dissolved organics was shown to increase from 127±5.4 to 572±16.5 mg/L. This could be explained by the accumulation of end products (alcohols and organic acids) from solid organic waste due to anaerobic hydrolysis.

For the fermentation with the pulsed air access, 7 days were required (Fig. 5). During the first 15 hours of cultivation, no mixing took place to initiate the hydrolysis of solid organic waste. During this period, pH decreased from 6.7±0.1 to 4.98±0.21, and the concentration of dissolved organics increased from 204±21.8 to 397±31.4 mg/L (Fig. 5, a). The redox potential continued to decrease from +260±37 to -42±9 mV until the aeration was conducted after 24 hours of cultivation.

The combination of aeration every 24 hours with the period of anaerobic fermentation required more time for the degradation of solid organic compounds. However, it facilitated more efficient oxidation of dissolved organics in the liquid phase. Active hydrolysis resulted in the accumulation of solid organic compounds, while access to oxygen promoted the oxidation of dissolved organics, especially during the final stage of fermentation when anaerobic activity diminished. The final concentration of dissolved organics was 187±26.8 mg/L. The hydrogen yield was 19±2.8 L from 1 kg per solid waste, with Kd values averaging 86±5.2.

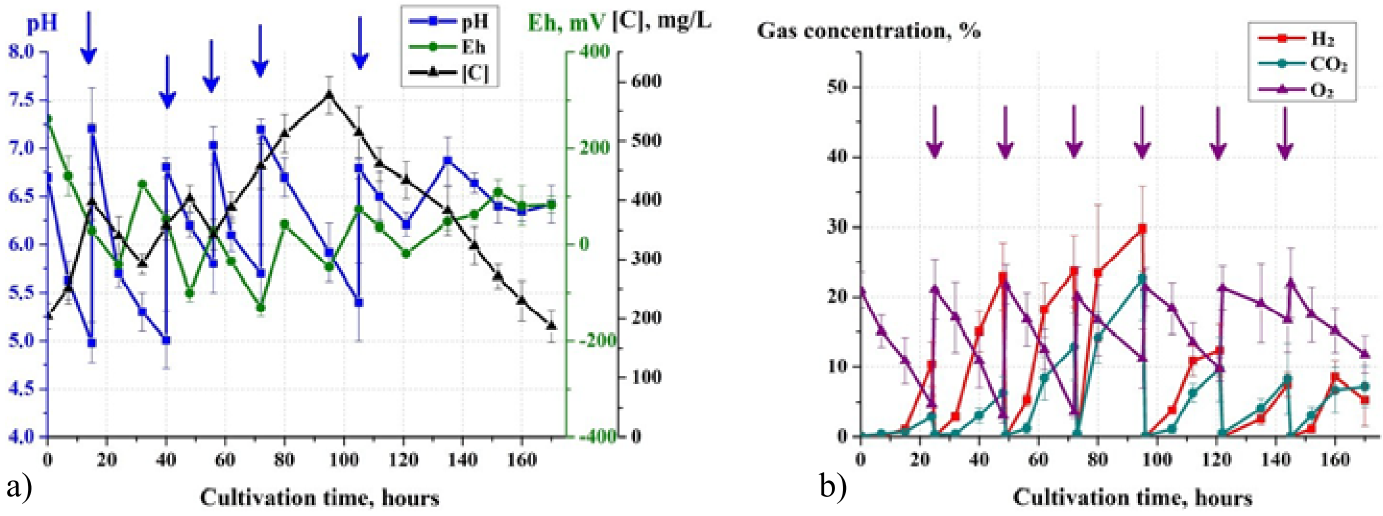

Figure. 5. Dynamics of fermentation of solid organic waste with the pulsed air access: a – pH, Eh, the concentration of soluble organic compounds; b – the concentration of H_2, CO_2, and O_2 in the gas phase (blue arrows indicate the points of Na_2CO_3 injection; violet arrows show the points of aeration).

Thus, the efficiency of hydrogen synthesis was 2.8-fold higher during strictly anaerobic fermentation. This is natural, as anaerobic hydrogen synthesis was inhibited with pulsed air access. The duration of anaerobic fermentation was 1.75-fold shorter. Solid waste degradation efficiency was similar for both methods. However, the efficacy of removing dissolved organics from the liquid phase was three times higher with pulsed air access during fermentation.

Given the annual increase in the amount of solid organic waste produced by cities, industrial, and agricultural facilities, there is an urgent need for effective treatment approaches. Incineration of waste or landfill accumulation are not suitable due to the emission of toxic and greenhouse gases, as well as other environmentally hazardous compounds. Therefore, fermentation of solid organic waste is considered the most promising method for cost-effective waste treatment as well as to obtain molecular hydrogen (Zhang et al. 2018; Parthiba Karthikeyan et al. 2018).

The proposed work aimed to contribute to the study of approaches for degrading solid organic waste through strictly anaerobic fermentation and the process involving pulsed air access.

Though anaerobic digestion has traditionally been used to produce methane (with energy content 56 kJ/g), its application for hydrogen, as an energy carrier with a higher energy content (143 kJ/g), is now considered a promising direction. Strictly anaerobic fermentation, as used in our study, is estimated to be an effective process due to its simplicity, independence from oxygen and light, ability to utilize a wide range of substrates, and high hydrogen yield (54±4.1 L/kg of waste). Literature data show a wide range of results in hydrogen production depending on substrate, pre-treatment, fermentation conditions, and other factors. For example, Marone et al. (2014)was taken in consideration. Batch experiments were carried out, under two mesophilic anaerobic conditions (28 and 37 °C reported obtaining 0.99 L H_2/kg of biomass from leaf-shaped vegetable waste at 28°C and 1.98 L H_2/kg of leaf-shaped vegetable waste with potato peels at 37°C. Another study showed a hydrogen yield ranging from 1.33 to 5 L/kg of food waste biomass at 35°C (Marone et al. 2014; Shimizu et al. 2008)was taken in consideration. Batch experiments were carried out, under two mesophilic anaerobic conditions (28 and 37 °C, as well as 65 L H_2/kg of organic waste (Wang and Zhao 2009).

A much higher yield of hydrogen was shown to be achievable from glucose-containing substrates, such as pork manure with glucose amendments (147.1-202.7 L H_2 per 1 kg) (Wu et al. 2009). Therefore, when comparing literature data with the results obtained in our study, it is noteworthy that the hydrogen yield aligns with the typical range for such multi-component organic waste. Substrates with low biodegradable organics content (leaves and vegetable pills), yielded less hydrogen, whereas glucose amendments significantly enhanced yields. Drawbacks of the process, such as the accumulation of volatile fatty acids and competition from microorganisms, can be solved through optimization and regulation of the process to achieve higher hydrogen yield (El Bari et al. 2022). This approach, based on the application of GMP and pH optimization, was studied and confirmed in our recent research (Tashyrev et al. 2022; V. M. Hovorukha et al. 2019; V. Hovorukha et al. 2020).

In this study, fermentation inhibition was controlled by regulating and maintaining the pH within the range 6.5-7.2, along with regular mixing. The accumulation of organic acids, known intermediate products of anaerobic process (Lim, Zhou, and Vadivelu 2020)the amount of acidogenic and methanogenic microorganisms will affect the output VFA concentration in a wastewater treatment process. In this study, sequencing batch reactor (SBR, can pose environmental hazards (Xiao and Wu 2014). To reduce the concentration of dissolved organic compounds, including volatile fatty acids, we studied an alternative fermentation approach. This method involved initiating waste fermentation through a strictly anaerobic process, followed by regular aeration and mixing of the fermentation mixture. This approach facilitated the hydrolysis of solid organic waste, leading to hydrogen synthesis and the oxidation of dissolved organics to purify the liquid phase. Our method was shown to be effective in degrading soluble organic compounds, reducing their concentration from 572±16.5 to 187±26.8 mg/L within 3 days. Despite inhibiting hydrogen synthesis, yielding only 19±2.8 L from 1 kg of solid waste, the efficiency of solid waste decomposition was close to that of the strictly anaerobic process.

No information was found in the literature regarding the investigation of fermentation with pulsed air access similar to our approach. However, research data confirm our findings on the potential to reduce organic content in the liquid phase through aerobic treatment. For instance, aerobic treatment of poultry manure for 14 days resulted in a 2.01% reduction in total organic carbon (Rubežius et al. 2020). Additionally, cyanobacteria have been reported to reduce the weight of kitchen waste by 40 % (Gill et al. 2014). In comparison, our approach demonstrated 33 % reduction in dissolved organics concentration. Further support for our studies comes from comparative study on the efficiency of aerobic and anaerobic degradation of organic waste at bioreactor landfills. This study revealed that aerobic treatment required approximately 6 times longer to achieve a reduction in organic concentration compared to anaerobic treatment (Erses et al. 2008). Our study corroborates these findings, demonstrating higher efficacy of waste degradation via pulsed air access compared to anaerobic methods, consistent with existing literature data.

Thus, strictly anaerobic fermentation was shown to be promising for fast degradation of solid organic waste with hydrogen production, while fermentation with pulsed air access was useful for the treatment of both solid and liquid organic waste.

Conclusions

A comparison of the efficiency of two approaches for fermenting solid organic waste was conducted. Strictly anaerobic fermentation yielded a high hydrogen output (54±4.1 L/kg of waste) and reduced the weight of solid waste (83±3.6). However, it did not effectively removed dissolved organics, which remained at 572±16.5 mg/L. Fermentation with pulsed air access achieved a similar level of solid organic waste degradation (86±5.2) but with a lower hydrogen yield (19±2.8 L). Notably, it succeeded in reducing the concentration of dissolved organics to 187±26.8 mg/L. Both fermentation approaches demonstrated efficiency in degrading solid organic waste. Strictly anaerobic fermentation is preferable for hydrogen production, whereas fermentation with pulsed air access is better suited for obtaining a purified liquid phase. These approaches are promising for future biotechnological applications after their optimization.

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Conflict of Interest

The authors declare no conflict of interest.

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Wpływ trybów fermentacji na efektywność oczyszczania odpadów organicznych w bioreaktorach okresowych

Streszczenie. Ilość stałych odpadów organicznych stale rośnie. Jest to spowodowane wzrostem potencjału przemysłowego i rolniczego, a także nieefektywnością istniejących technologii przetwarzania odpadów. Biotechnologie mogą zapewnić skuteczne, przyjazne dla środowiska rozwiązania w zakresie przetwarzania odpadów. Dlatego celem naszej pracy było porównanie efektywności ściśle beztlenowej fermentacji wieloskładnikowych stałych odpadów organicznych z syntezą wodoru i przetwarzania odpadów z pulsacyjnym dostępem powietrza w bioreaktorach okresowych. Podczas fermentacji kontrolowano następujące parametry: pH, potencjał redoks (Eh), stężenie rozpuszczonych substancji organicznych oraz zawartość H_2, O_2 i CO_2 w fazie gazowej. Efektywność oceniano poprzez czas trwania procesu, obliczenie stosunku początkowej i końcowej masy odpadów (Kd) oraz uzysk wodoru cząsteczkowego. Uzyskane wyniki wykazały wysoką skuteczność degradacji odpadów organicznych w obu wariantach. Masa odpadów zmniejszyła się odpowiednio 83-krotnie i 86-krotnie. Czas fermentacji w warunkach ściśle beztlenowych wynosił 4 dni, natomiast w trybie z pulsacyjnym dostępem powietrza 7 dni. W pierwszym wariancie uzyskano 2,8-krotnie większy uzysk wodoru (54±4,1 L/kg odpadów), w drugim zmniejszono stężenie rozpuszczonych związków organicznych w płynie pofermentacyjnym. Fermentacja jest skuteczną metodą przyspieszonej degradacji stałych odpadów organicznych. Fermentacja ściśle beztlenowa okazała się przydatna w potrzebie przyspieszenia procesu. Tryb z pulsacyjnym dostępem powietrza pozwala nie tylko na degradację odpadów stałych, ale także na oczyszczenie płynu pofermentacyjnego.