Hydrogen Dark Fermentation for Degradation of Solid and Liquid Food Waste

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Abstract: The constant increase in the amount of food waste accumulating in landfills and discharged into the water reservoirs causes environment pollution and threatens human health. Solid and liquid food wastes include fruit, vegetable, and meat residues, alcohol bard, and sewage from various food enterprises. These products contain high concentrations of biodegradable organic compounds and represent an inexpensive and renewable substrate for the hydrogen fermentation. The goal of the work was to study the efficiency of hydrogen obtaining and decomposition of solid and liquid food waste via fermentation by granular microbial preparation (GMP). The application of GMP improved the efficiency of the dark fermentation of food waste. Hydrogen yields reached 102 L/kg of solid waste and 2.3 L/L of liquid waste. The fermentation resulted in the 91-fold reduction in the weight of the solid waste, while the concentration of organics in the liquid waste decreased 3-fold. Our results demonstrated the potential of granular microbial preparations in the production of hydrogen via dark fermentation. Further development of this technology may help to clean up the environment and reduce the reliance on fossil fuels by generating green hydrogen via recycling of household and industrial organic wastes.

Keywords: biohydrogen; green energy; fermentation; solid food waste; liquid food waste; environmental biotechnology

1. Introduction

The use of molecular hydrogen represents a promising alternative strategy for generating energy for industrial applications [1,2]. Hydrogen has a high specific energy density and is environmentally friendly since its combustion yields only water. The U.S. Department of Energy, the International Partnership for Hydrogen Economy, and the European Hydrogen Association have proposed a concept of replacing the fossil fuel-based economy with an alternative economy powered by hydrogen [3,4]. It has been suggested that by the year 2100 hydrogen will become a significant source of energy in the automotive industry, production of electricity, chemical manufacturing (ammonium derivatives, etc.), refineries, and production of electronics [3]. Hydrogen can be generated using various chemical, electrochemical, and biological approaches [3,5]. At present, most of it is produced using physical and chemical methods, which are expensive and require complex equipment and operational schemes [6]. The use of microbiological technologies can reduce the cost and increase the efficiency of hydrogen production, and, at the same time, help to protect the environment.

The microbial production of molecular hydrogen involves the direct and indirect biophotolysis of water, photofermentation, and dark fermentation [2,3,7]. Among these,
the dark fermentation emerged as a particularly promising approach due to the low cost and broad availability of renewable substrates [6,8]. This technology may also help to recycle large volumes of household and industrial organic wastes, the accumulation of which represents a growing global problem [9]. For example, the uncontrolled decay of solid food wastes (SFW) at landfills is accompanied by the emission of toxic filtrates and gases (mercaptans, hydrogen sulfide, ammonia), while the discharge of sewage containing concentrated liquid food wastes (LFW) leads to eutrophication [10,11]. Instead of simply wastes containing biodegradable organic compounds, these products can be fermented to generate the sustainable (green) hydrogen [12–14]. Under laboratory conditions, an efficient hydrogen synthesis was achieved by fermentation of distillery effluents, olive mill wastes, molasses production byproducts, and cassava processing wastewater [15,16]. The average rate of \( H_2 \) synthesis during the starch and sucrose fermentation by mesophilic microorganisms was sufficiently high and ranged between 64.5 mmol/L to 121 mmol/L of reactor/h (1.4–2.7 L \( H_2 / L \) of reactor/h) [17]. Such results make the dark fermentation a promising technology for the industrial-scale production of hydrogen [18]. However, the industrial implementation of such biotechnologies requires the detailed study to increase the productivity of hydrogen synthesizing microorganisms [19].

Anaerobic microorganisms capable of degrading organic compounds and synthesizing \( H_2 \) are widespread in natural and man-made ecosystems and can be applied to obtain hydrogen both in as pure cultures or microbial consortia [20]. Although pure cultures are extensively tested and have high metabolic activity, their use is limited by the risk of contamination and limited range of substrate utilization. As an alternative, a number of recent studies employed natural or artificial consortia consisting of different aerobic and anaerobic species that collectively ferment the substrate and produce hydrogen [21–23]. The advantages of applying microbial consortia include:

- the presence of microorganisms with diverse physiology that are capable of fermenting a wide range of substrates;
- the reduced contamination risk and ability to use non-sterile substrates;
- higher hydrogen yields due to the concerted action of a diversified community of \( H_2 \)-synthesizing microorganisms.

The application of microbial communities also reduces the cost of preparation of raw materials, which significantly simplifies the fermentation process and makes it more economical [2,24,25]. The ability of pure cultures and consortia to grow and consume certain substrates is determined by the presence and activity of complementary metabolic pathways and resistance to the physical and chemical environmental factors [26,27]. Thus, the efficacy of substrate fermentation and hydrogen synthesis can be significantly improved by optimizing the growth conditions and carefully controlling and regulating the microbial metabolism [28,29]. To improve the substrate conversion and \( H_2 \) yield, we developed the granular microbial preparation containing an optimized community of highly active hydrogen synthesizing microorganisms capable of fermenting mixed organic substrates. The goal of the work was to study the efficiency of hydrogen obtaining and decomposition of solid and liquid food waste via fermentation by granular microbial preparation.

2. Materials and Methods

2.1. The Production of Granular Microbial Preparation

The granular microbial preparation used in this study was manufactured as follows. A 0.5 kg batch of pasteurized crushed organic substrate (equal parts \((w/w)\) of potatoes, cabbage, carrots, and pasta) was combined with 10 mL of pasteurized soil extract (10 g of soil in 50 mL of water) and 2 L of sterile tap water. The mixture was loaded into a fermenter, hermetically sealed with rubber stoppers containing fittings to drain the synthesized gas, and allowed to ferment for 7 days at 30°C. The resultant culture fluid containing hydrogen synthesizing microorganisms was combined with proprietary starter substrates and regulators of microbial metabolism to manufacture GMP. The GMP granules
were produced using an extruder, dried in a ventilated laboratory furnace, and stored until needed at ambient temperature in sealed glass jars (Figure 1).

![Figure 1](image_url)

**Figure 1.** The appearance of the granular microbial preparation used in the study.

### 2.2. Model Substrates for Assessing the Dark Fermentation Efficiency

Two types of model substrates were used to study the dark fermentation efficiency. The model solid food waste (SFW) contained equal parts (w/w) of raw and cooked potatoes (mixed at 1:1 ratio), tomatoes, zucchini, cucumbers, carrots, cabbage, apples, parsley, boiled chicken fillet, cooked macaroni, and bread. The components were ground, mixed, and formed into 1–2 cm cubes. Apple juice purchased from a local grocery store was used as a model liquid food waste (LFW) substrate. Since the juice was used from hermetically closed sterilized bottles it was not additionally treated before loading into the bioreactor.

### 2.3. The Fermentation Process

The fermentation of solid and liquid model food waste was carried out with and without GMP and regulators of microbial metabolism. To study the dynamics of SFW fermentation, 2 kg of model substrate was combined with 140 g of CaCO₃, 300 mL of 0.01% bromothymol blue (BTB), 20 g of GMP, 6 L of boiled tap water, and loaded into a 20 L horizontal bioreactor (Figure 2). Control treatments were fermented in the absence of GMP to model the untreated waste in landfill site. In the other case, the waste mixture was treated with the 85–90 °C tap water for 10 min. Then water was removed, the waste was loaded to the bioreactor following the described above procedure, and 20 g of the GMP was added. The fermentations were also performed with and without pH adjustment, which was attained by adding 5% solution of NaOH to the bioreactor to maintain pH in the optimum (6.0 pH to 7.0 pH) range. The reactor was closed with rubber stoppers modified to allow sampling of the culture fluid, addition of reagents, and gas removal. The synthesized gas flowed through the gas controller to the gas holder. The liquid inside the bioreactor was periodically mixed (15 min. stirring/30 min. pause frequency) with paddles rotating at 24 rpm. The fermentation was carried out for 7 days at 30 °C.

The fermentation of LFW was carried out in 2 L vessels containing 1.5 L of apple juice and 3 mL of the BTB indicator. To stimulate the process of fermentation, juice packages were opened prior to experiment and left at 25 °C for 12 h. As with SFW, the control treatments were fermented without GMP. The LFW was also fermented with and without pH adjustment, which involved the addition of 5% NaOH to achieve the starting pH of 7.0. The containers were hermetically sealed and the fermentation of LFW was carried out for two weeks at 30 °C.
Figure 2. A 20-L bioreactor used for the dark fermentation of food wastes.

The following parameters were monitored: pH, redox potential (Eh), gas volume, the concentration of H$_2$ in the gas phase, as well as total organic carbon (TOC) concentration in the culture fluid. The completion of the process was judged by the stabilization of pH and increase in the redox potential of the medium, termination of gas synthesis and visual degradation of solid waste particles. Bromothymol blue was used as a pH indicator with the following color range: pH > 7—blue, pH = 7—green, 5 < pH < 6—yellow, and pH <5—bright yellow. This compound was also used as a visual redox indicator, as bromothymol blue turns colorless at Eh ≤ −200 mV (pH = 7.0–7.8).

2.4. Assessment of the Fermentation Parameters

The pH and redox potential of the medium were measured by the potentiometric method with a pH–150 MA pH/millivoltmeter and measuring electrodes (Antech, Gomel, Belarus). A porous glass electrode ESC-10603/4 was used for pH measurements, while an EPV-1 platinum electrode and a chlorine silver EVL-1M3 reference electrode were used to measure the redox potential. The volume of gas was determined by the amount of water displaced from the gas holder into the intake manifold under the pressure of the synthesized gas. The H$_2$ concentration was determined by gas chromatography [30]. The analysis of H$_2$, O$_2$, N$_2$ and CH$_4$ was performed with a 3 m × 3 mm i.d. molecular sieve 13X (NaX) (RealSorb, Yaroslavl, Russia) steel column, and CO$_2$ was detected with a 2 m × 3 mm i.d. Porapak Q (Merck KGaA, Darmstadt, Germany) steel column. The following parameters were used in the analysis: column temperature 60 °C, evaporator temperature 75 °C, detector temperature 60 °C, and detector current 50 mA. Argon with flow rate of 30 cm$^3$/min was used as a carrier gas. The concentration of H$_2$ was calculated by the squares of peaks of its components [30].

The concentration of soluble total organic compounds (TOC) in the fermentation medium was measured using the permanganate method [31]. In this assay, 1 mL aliquots of the culture fluid were mixed with 0.1 mL of concentrated sulfuric acid and heated in a boiling water bath. Next, 0.1 mL of 0.1% potassium permanganate solution was added resulting in the development of light pink color due to the oxidation of organic compounds by MnO$_4^{2-}$. The absorbance of samples was measured at 540 nm, and the amount of total carbon was calculated based on a standard calibration curve [31].

2.5. The Calculation of the Efficiency of Model Food Waste Digestion

The efficiency of the fermentation of the model food waste was determined using the following parameters:

- Waste degradation time ($T$, days)—defined as the amount of time passed between the start and the end of the fermentation (i.e., the termination of the gas synthesis);
• Hydrogen yield—calculated as the amount of H$_2$ (L) synthesized per 1 kg (measured in total solids (TS)) or 1 L (measured in TOC) of waste;

• Waste degradation coefficient ($K_d$)—the degree of waste digestion. For SFW, this parameter was calculated as the ratio of initial and final weight of the waste based on TS ($K_d = m_1 : m_2$, where $m_1$ is the initial weight of dry waste; $m_2$ is the weight of dry detritus). To determine the initial weight, the waste was dried to completion at 105 °C and weighed. To determine the weight of detritus, the non-fermented residues after fermentation were washed in distilled water, in the solution of weak organic acid, and then again in distilled water. The washed residues were dried at 105 °C and weighed. For LFW, the $K_d$ was calculated as the ratio of the initial and final concentrations of TOC in the culture medium.

2.6. Theory/Calculation

The potential thermodynamic efficiency of the fermentation of organic substrates and hydrogen production can be estimated based on the consumption of carbohydrate polymers (starch, cellulose, etc.) [32]:

$$\text{Starch} \rightarrow C_6H_{12}O_6 + 2H_2O = 2\text{CH}_3\text{COOH} + 2\text{CO}_2 + 4\text{H}_2, \Delta G_o = -184 \text{kJ/M.} \tag{1}$$

The efficiency of the hydrogen synthesis and waste degradation can be optimized by controlling several key parameters, including the pH and redox potential of the culture medium, the concentration of organic compounds or the ratio of solid waste and liquid phase in the bioreactor, the waste mixing mode, the process temperature, and the type of microbial community used in the fermentation [29,33,34]. The microbial growth during fermentation results in the acidification of the medium, which decreases to pH of 4.0–5.0 [35,36]. The adjustment and maintenance of pH during the fermentation within neutral range (pH = 6.0–7.0) accompanied by waste mixing were theoretically and experimentally prove to speed up the degradation of waste and increase the hydrogen yield [33,37]. In addition, the pH adjustment at the start of the process can significantly shorten the lag phase of the fermentation [38].

3. Results

Two different sets of experiments were used to investigate the fermentation of SFW. The control experimental setup simulated the spontaneous decay of SFW in landfills and involved fermentation without the addition of granular microbial preparation, regulation of microbial metabolism, and mixing. Alternatively, the fermentation was performed with granular microbial preparation in the presence of metabolism regulators and substrate mixing. In the control fermentation, a sharp drop of pH from 6.8 pH to 5.7 pH was observed within the first 10 h of the process (Figure 3).

![Figure 3](image.png)  
**Figure 3.** The inhibition of solid food wastes (SFW) fermentation in the absence of the granular microbial preparation and pH regulation.
Concomitantly, the redox potential of the medium decreased from +295 mV to −352 mV and then remained at −300 . . . −200 mV. However, there the synthesis of hydrogen remained low and started rising slowly after 20 h, peaking at 23% after 28 h of cultivation. In the absence of pH adjustment and substrate mixing, the rapid consumption of easily accessible organic compounds resulted in the sharp acidification of the medium. These processes slowed the microbial growth, which is in agreement with studies reporting the inhibition of microorganisms by organic acids [39,40]. The hydrogen production was low and yielded only 9 L H₂/kg of TS. The waste particles in the fermenter remained visually intact, and the coefficient of waste degradation Kd (i.e., the ratio of the initial and final weight of waste) equaled only 3.

Collectively, the fermentation of the model solid food waste with autochthonous microorganisms closely resembles the unregulated digestion of SFW taking place in landfills. There, the microorganisms start to proliferate due to the large amount of available organic compounds, but later their growth is inhibited by the rapid accumulation of toxic acidic exometabolites. This slows the waste decay for months or even years and leads to environmental pollution [41]. The lack of specialized microbial groups accounts for inefficient hydrogen production.

The efficiency of SFW degradation and the synthesis of molecular hydrogen markedly improved when the fermenter was seeded with granular microbial preparation and the fermentation process was actively controlled (Figure 4).

Figure 4. Effective fermentation of SFW using the granular microbial preparation (the arrows indicate the loading of pH regulators).

Within the first 8 h of cultivation, the pH of the medium decreased from 6.9 to 5.9, and Eh dropped from +312 mV to −326 mV, indicating the rapid microbial growth and active consumption of the substrate. The periodic addition of pH regulators and mixing of the substrate allowed to maintain pH within the range (6.0–7.0) optimal for the microbial growth. The addition of CaCO₃ to the culture medium also helped to neutralize the accumulation of toxic organic acids. As the result, the pH changed more gradually. The mixing of SFW aided in the uniform distribution of microorganisms and regulators throughout the growth substrate. Under these conditions, the redox potential remained within the range optimal for the hydrogen synthesis (−357 mV to −348 mV). As a result, the hydrogen concentration reached 39% after 21 h of cultivation. The efficiency of the waste degradation in the presence of GMP increased by 30-fold and the organic waste was completely degraded in 5 days resulting in Kd of 91. The hydrogen yield was 102 L H₂/kg of TS, which was an 11-fold improvement compared to control conditions.

The effectiveness of GMP was further tested during the fermentation of model liquid food waste exemplified by apple juice with the TOC content of 6.5 g/L. In the control treatment, the fermentation was performed using undefined microorganisms involved in the juice souring. Under these conditions, the pH remained almost constant (pH = 3.5–3.6) throughout the entire fermentation even in the absence of adjustment (Figure 5). The redox potential of the medium decreased slightly from +368 mV to +262 mV. The substrate
degradation efficiency was low, and the TOC decreased by only 1.25-fold reaching 5.2 g/L after 14 days of fermentation. Although the oxygen concentration in the gas phase decreased from 21% to 2.5% within two days of cultivation, the synthesis of hydrogen did not commence. The inhibition of microbial growth was observed after 14 days of fermentation. Under these conditions, the coefficient of LFW degradation ($K_d$) defined as the ratio of the initial and final concentration of the TOC, was only 1.25.

**Figure 5.** The inhibition of fermentation of liquid food wastes (LFW) in the absence of the granular microbial preparation and pH regulation.

In contrast to the spontaneous fermentation, the efficiency of the degradation of LFW and hydrogen production increased markedly in the presence of the granular microbial preparation and pH adjustment (Figure 6). Under these experimental conditions, the pH remained within the range optimal for microbial growth (pH = 6.0–6.9) during 14 days of cultivation due to the periodic addition of NaOH aliquotes.

**Figure 6.** An effective fermentation of LFW in the presence the granular microbial preparation. Vertical arrows indicate the addition of pH regulators.

The growth of microorganisms lowered the $E_h$ of the medium from +358 mV to −283 mV within 48 h and decreased the oxygen concentration from 21% to 1.4% in 27 h. These changes were followed by the synthesis of hydrogen, the concentration of which reached 34% after 72 h of fermentation. The efficiency of the substrate degradation increased in the presence of GMP by 2.4-fold, and the concentration of organic compounds in the culture fluid decreased from 6.5 g/L to 2.2 g/L. After two weeks, the hydrogen synthesis ended and the residual levels of TOC in the substrate remained constant. The final yield of hydrogen was 2.3 L H$_2$/L of juice (or 0.5 L H$_2$/g of TOC). No methane was observed during the fermentation of both solid and liquid waste.
4. Discussion

The efficiency of hydrogen production by microorganisms depends on their species, growth substrate, and culture conditions. The published studies identify *Clostridium* sp. as the best performing hydrogen synthesizing bacteria capable of H$_2$ yields close to 250 L H$_2$/kg glucose [42]. Similarly, pure cultures of *Bacillus* spp. have been shown to produce up to 60 L H$_2$/kg hexose using different simple substrates [43]. However, the use of pure cultures in the fermentation of multicomponent substrates is restricted by the metabolic capacity of bacteria [15]. Here, the use of microbial consortia proved to be beneficial, and mixed microbial cultures enriched for *Clostridium* and *Bacillus* increased the H$_2$ yield three-fold [43]. We expanded this line of research by developing a granular microbial preparation (GMP) that contain a stable community of hydrogen–producing bacteria and tested this product in the hydrogen dark fermentation of organic waste. The use of GMP in the fermentation of SFW increased the yield of biohydrogen 11-fold from 9 L to 102 L H$_2$/kg of TS compared to the control treatment. The application of GMP to the fermentation of liquid food waste provided the yield of 2.3 L H$_2$/L of substrate.

The molecular hydrogen can be produced using a wide range of organic substrates [15]. Ideally, these substrates should be readily digestible by microorganisms and high in carbohydrates [44]. Simple carbohydrates have been used as model substrates for studying the process of hydrogen production, and the fermentation of starch and sucrose yields of up to 2 L of H$_2$/L of reactor/h [17,45]. However, the use of simple carbohydrates in the large-scale hydrogen production leads to a significant increase in the cost of such technologies [46,47]. The use of inexpensive food waste represents a cost-effective alternative way of generating H$_2$. Food waste has a great biotechnological potential due to the high moisture content (72–85%), high substrate concentration (COD: 19–346 g/L), carbohydrates: 25–143 g/L, and high carbon to nitrogen (C/N) ratio (9–21) [14]. Plant substrates rich in carbohydrates are the best raw material for hydrogen production [48]. Hydrogen yields from the fermentation of rice or potatoes are 20 times higher compared to substrates rich in fat (fatty meat) or protein (eggs and lean meat) [49]. Therefore, cheap and renewable substrates, such as byproducts of food processing and culinary waste should be used in industrial implementation of the biohydrogen technologies [48,50–52]. Although such substrates yield less hydrogen, their low cost and large volumes make the process cost-effective.

It is known that hydrogen yield increases under pH of 6.0–7.0 [53,54], redox-potential of −300 . . . −250 mV, temperatures of 20–45 °C, which are ideal for the growth of mesophilic microorganisms [2,14,55], and the optimal substrate concentrations and fermentation mode [29,34,56–58]. Using these parameters, two-step systems for the production of H$_2$ and CH$_4$ were developed and used for the treatment of solid food waste, cheese whey, olive mill sewage, mixtures of crushed garbage and shredded paper waste, and sewage sludge. The use of a 200–L (working volume of 110 L) H$_2$ reactor and a 500 L (working volume of 340 L) CH$_4$ reactor resulted in the hydrogen output of 5.4 m$^3$/m$^3$ of reactor/day, and methane output of 6.1 m$^3$/m$^3$ of reactor/day [59–61]. However, according to the published data, the overall efficiency of the hydrogen fermentation of food waste remains low and results in 40%–80% decrease in the substrate weight ($K_d$ = 2.5–5.0) [62–64]. In addition, the time of the waste digestion is too long (up to 1 month) making such technologies unprofitable.

We propose to improve the efficiency of food waste fermentation by using granular microbial preparations containing highly active hydrogen synthesizing microorganisms and by regulating the main fermentation parameters. Such approach shortened the duration of the fermentation by 2–5-fold (from 1 month up to 6–14 days) [65,66]. The application of GMP also improved the efficiency of hydrogen fermentation of solid and liquid food waste by 30-fold and 2.4-fold, respectively. The coefficients of waste degradation ($K_d$) were 91.0 and 3.0, respectively, for SFW and LFW.

The opportunity to reduce the volume of solid waste is extremely urgent taking into account the rate of its accumulation [2,8]. In this regard, our study was focused on the efficiency of solid waste particles removal and calculation of the ratio of waste
weight (\(K_d\)) indicating the overall reduction of waste weight and volume. However, the fermentation of solid organic waste is accompanied by the accumulation of the products (organic acids, alcohols) [64]. Further research should focus on the characterization of metabolic products to get deeper insight into details of the fermentation process. The preliminary characterization of the fermentation of vegetable waste resulted in 2–3 g/L of total organic acids (unpublished data). The following improvement of the approach of solid food waste fermentation should be supplemented with the quantification and analysis of fermentation products. The study of the efficiency of soluble organics removal is the important stage for further development of the biotechnologies for decomposition of solid waste with \(H_2\) synthesis and the leachate treatment avoiding the pollution of the environment with the fermentation products.

According to the literature, the price of 1 m\(^3\) of \(H_2\) is $2.7 and the average cost of treating 1 t of food waste is about $16 [19]. Based on these parameters and our results, the fermentation of 1 t of food waste should produce 102 m\(^3\) of \(H_2\) worth approximately $275. Taking into account the cost of waste treatment, brings the revenue from the processing a ton of waste to about $260. This makes the approach of recycling organic waste for the production of green hydrogen potentially very attractive. Further studies should focus on the improvement of the efficiency of the process and prospects of its scaling.

5. Conclusions

The results of this study demonstrated the potential of granular microbial preparations in the production of hydrogen via dark fermentation. The use of GMP in the regulated fermentation of organic wastes resulted in the efficient degradation of the substrate and markedly increased production of biohydrogen. Further development of this technology may help to clean up the environment and reduce the reliance on fossil fuels by generating green hydrogen via recycling of household and industrial organic wastes.

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