EREM 74/4
Journal of Environmental Research, Engineering and Management
Vol. 74 / No. 4 / 2018
pp. 31-42
DOI 10.5755/j01.erem.74.4.20723

Integrated Approach for Development of Environmental Biotechnologies for Treatment of Solid Organic Waste and Obtainment of Biohydrogen and a Lignocellulosic Substrates: Challenges Related to Hazardous Substances

Received 2018/05 | Accepted after revision 2018/10

http://dx.doi.org/10.5755/j01.erem.74.4.20723

Integrated Approach for Development of Environmental Biotechnologies for Treatment of Solid Organic Waste and Obtainment of Biohydrogen and a Lignocellulosic Substrate

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Solid food waste is a significant threat to the environment. Thermodynamic calculations allow determining theoretically possible metabolic pathways for degradation of organic compounds by microorganisms, and to select the optimal one to increase efficiency of food waste recycling. The purpose of our work was application of thermodynamic calculations to find out suitable fermentation parameters for regulation of microbial metabolism

to ensure a high rate of waste decomposition and formation of valuable products. The following methods were used: colorimetric and potentiometric for pH and oxidation-reduction potential (ORP) measurement, volumetric and chromatographic for the study of volume and composition of synthesised gas, and mathematical for fermentation parameters calculation. Fermentation of multicomponent kitchen food waste under theoretically calculated optimal parameters pH = 7.0 and Eh in the range form –250 to –350 mV provided extremely high metabolic activity of a hydrogen-producing microbial community, which resulted in a decrease in duration of batch fermentation to three days and an increase in hydrogen yield from 16 to 80–115 L/kg of dry waste. The coefficient of waste destruction (Kd), i.e., the ratio of initial and final weight of waste, reached 91. Obtained after fermentation, an unfermented lignocellulosic substrate was shown to be applied as plant probiotics and to supply mineral nitrogen for plant nutrition in an arid condition. Thus, high efficiency of application of a thermodynamic prognosis method of microbial interaction with organic compounds was shown to become the base for biotechnology of destruction of environmentally hazardous solid food waste with simultaneous obtainment of valuable products: environmentally friendly energy carrier – molecular hydrogen, as well as a lignocellulosic substrate to increase crop yields.

Keywords: thermodynamic prognosis, biohydrogen, dark fermentation, environmental biotechnologies, plant probiotics.

Introduction

Solid food waste is a significant threat to the environment (Pires et al., 2011; Guerrero et al., 2013). The rapid accumulation of multicomponent food waste (MFW) occurs both in developed countries (Pires et al., 2011) and in countries that are developing (Guerrero et al., 2013). Currently existing methods for recycling of multicomponent municipal and kitchen food waste do not ensure their complete disposal. It takes place due to the low efficiency of physical, chemical, and biological recycling technologies as well as their combination (Levin et al., 2004; Show et al., 2012). As a result, an accumulation of environmentally hazardous organic waste widely occurrs in the majority of countries, which leads to the poisoning of the environment by the products of their decomposition.

At the same time, MFW is a substrate for biotechnologies for obtaining valuable products, including biomethane and biohydrogen (Mata-Alvarez et al., 2000; Li et al., 2011). Rapidly rising costs associated with energy supply and waste transportation to landfills and increasing public concerns with environment degradation lead to an increasing interest of society to convert food wastes to energy (Zhang et al., 2007a). Modern waste utilisation technologies (Levin et al., 2004; Show et al., 2012) do not fully cope with the excessively high rate of their accumulation in megacities (Sharholy et al., 2008; Grimm et al., 2008).

A possible reason for low technological efficiency is the lack of a theoretical justification for the optimal method to process organic waste. All biotechnologies for the decomposition of organic wastes are based on empirical data.

In connection with the foregoing, it is obvious that a new methodological approach is needed to theoretically substantiate the optimal metabolic pathway of microbial destruction of multicomponent food waste. Thus, the purpose of our work was application of thermodynamic calculations to find out suitable fermentation parameters for regulation of microbial metabolism to ensure a high rate of waste decomposition and formation of valuable products.

Methods

Thermodynamic calculations

To optimise hydrogen fermentation, the diagrams of Purbe and the equations of state of H^+, H_2 and H_2O in the pH-Eh (ORP) coordinates were used (**Pourbaix, 1963**).

Lab-scale fermentation of multicomponent food waste

Experimental confirmation of theoretical considerations was carried out during lab-scale fermentation of multicomponent food waste in the batch reactor with optimised construction and 20 L volume (Tashyreva et al., 2017). The reactor was connected to a gas holder through a gas controller.

The model mixture of food waste was used: raw and boiled potatoes, raw tomatoes, cucumbers, zucchini, cabbage, carrots, apples, parsley, chicken fillet, boiled pasta, and bread in equal weight ratio. Hydrogen-producing microbial community was used for fermentation of organic waste. The pH buffer (Na_2CO_3) was used for prevention of medium acidification. The fermentation mixture loaded into the reactor consisted of 2.0 kg of pasteurised model waste (10 min), 6 L of tap water boiled during 10 min, and cooled to 35°C, and 1% of microbial inoculum. The solution of Na_2CO_3 was added periodically to the reactor during fermentation to provide optimal values of pH if needed.

The following fermentation mode was used for decomposition of waste (Tashyreva et al., 2017): 24 rpm (the rate of waste mixing, i.e., the rotation rate of blades in the reactor); 10 min of mixing / 20 min of pause (frequency of waste mixing, i.e., the ratio of duration of mixing to pause).

The following parameters were controlled. The values of pH and ORP (Eh) of the medium were measured by the potentiometric method. The content of gas was analysed using the standard procedure by gas chromatograph LHM-8-MD. The content of gas was calculated by the square of peaks of its components. The volume of synthesised gas was measured by summarising the volume of water squeezed out of the gas holder into the receiving collector under the pressure of synthesised gas (Tashyreva et al., 2017).

The operating period of fermentation was 7 days at a temperature of 30°C. Visual assessment of the degree of waste particles destruction, stabilisation of pH, an increase in Eh values of the medium from –250...–350 mV to 0...–100 mV, termination of gas synthesis, and a decrease in concentration of hydrogen in the gas mixture evidenced completion of the fermentation cycle.

The following fermentation parameters were determined:

1. duration of the operating period (T, days), i.e., the time until the moment of maximum waste destruction and termination of gas synthesis;
2. molecular hydrogen yield, i.e., the amount of synthesised H_2 (in litres) from 1 kg of waste (calculated to absolutely dry weight or ADW);
3. coefficient of waste destruction (Kd), i.e., the ratio of initial and final dry weight of solid waste.

Treatment of lignocellulosic residues

The end products of multicomponent food waste fermentation consisting of lignocellulose were treated to become useful to increase the crop yield. For this purpose, lignocellulosic residues were loaded into the reactor where aerobic oxidation of fatty acids and alcohols of anaerobic fermentation was carried out. Microbial community used for aerobic oxidation of organic matter and colonisation of lignocellulose included soil facultative anaerobic microorganisms. The end of the process was indicated by a decrease in the concentration of soluble organics of anaerobic fermentation in the fermentation mixture, the total content of which was determined by the permanganate oxidation method (Suslova et al., 2014).

Then lignocellulosic residues were washed by tap water. The amount of ammonifying and diazotrophic bacteria in lignocellulose was determined. For this purpose, 1 g of residues dried to constant weight was grinded to a powdery state in a porcelain mortar under sterile conditions. The powder was added to a flask with 100 mL of sterile saline (0.8% NaCl) and standard ten-fold dilutions were prepared. From each dilution, 100 µL of the suspension was seeded onto agarised selective media in Petri dishes (in triplicates). The Ashby medium was used to determine diazotrophic bacteria (Zenova et al., 2002).

To count the total number of ammonifying microorganisms, the protein medium (Nutrient Agar, HiMedia) with indicator bromothymol blue was used. Ammonifying microorganisms caused alkalinisation of the medium with ammonia or NH_4^+, formed due to the cleavage of the amino group of protein compounds. It

was visually detected by the appearance of blue staining of the nutrient medium around microbial colonies. The remaining heterotrophic bacteria did not cause changes in the medium colour (i.e., did not belong to ammonifying bacteria) (Zenova et al., 2002).

Testing of a lignocellulose substrate as biofertiliser

Treated lignocellulosic residues colonsed by ammonifying and diazotrophic microorganisms were tested to increase the efficiency of plant growth. A lignocellulose substrate was added to sand in the concentration of 33 g/m^2 of sand. The lignocellulose substrate (LCS) was mixed with the sand at the depth of 0.1 m. Soil mixture "Universal substrate for vegetables and flowers" produced by "Agrosvit" and sand without an addition of a lignocellulose substrate were used as the control. *Raphanus sativus* seeds were used to determine the role of the lignocellulose substrate in the crop yield. They were cultivated in a photobox during 9 days at 20°C and 10 h photoperiod. The experiments were performed in triplicate. Growth effectiveness was characterised by germination energy of seeds (Czabator, 1962).

Results and Discussion

Thermodynamic rationale and selection of the optimal metabolic pathway for organic matter destruction

Modern physico-chemical, biological and combined waste utilisation technologies do not fully cope with the excessively high rate of their accumulation in megacities (Sharholy et al., 2008; Grimm et al., 2008). Our methodological approach will allow rapid and effective destruction of huge amounts of food waste. We use the method of thermodynamic prognosis of microbial degradation of organic compounds, and in particular multicomponent food wastes. We consider the destruction of organic compounds as linked binary redox reactions. In these reactions, organic compounds of food waste are electron donors, and O_2, NO_3^-, SO_4^{2-}, H^+, and CO_2 are acceptors. Microorganisms act as a biocatalyst in the donor-acceptor reaction (Noike and Mizuno, 2000; Hawkes et al., 2002; Kapdan and Kargi, 2006; Ren et al., 2006). The rate and efficiency of microbial degradation of organic compounds in general and food waste in particular are determined by the release of free energy ΔG in the coupled donor-acceptor reaction (Finley et al., 2009; Bethke et al., 2011). In addition, the criterion for destruction efficiency of organic compounds is the balanced stoichiometric ratio of donors and acceptors of the redox reaction (Bethke et al., 2011). The method of thermodynamic prognosis provides a possibility to evaluate the efficiency of a particular metabolic pathway for the decomposition of organic compounds by microorganisms.

The degradation of organic compounds is carried out by microorganisms using such metabolic pathways as:

1 aerobic (Gottschalk, 1979; Kleidon and Lorenz, 2005; Kekacs et al., 2015):

$C_6H_{12}O_6 + 6O_2 = 6CO_2 + 6H_2O$, $\Delta G_o = -2877$ kJ/M;

2 nitrate reduction (Gottschalk, 1979):

$C_6H_{12}O_6 + 4.8\ NO^-_3 + 4.8H^+ = 6CO_2 + 2.4\ N_2 + 8.4H_2O$, $\Delta G_o = -2669$ kJ/M;

3 methanogenesis (Gottschalk, 1979; Thauer, 1998):

$HCO^-_3 + H^+ + 4H_2 = CH_4 + 3H_2O$, $\Delta G_o = -135{,}6$ kJ/M;

4 hydrogen dark fermentation (Noike and Mizuno, 2000; Wen-Hsing Chen, 2006):

$C_6H_{12}O_6 + 2H_2O = 2CH_3COOH + 2CO_2 + 4H_2$, $\Delta G_o = -184$ kJ/M.

Positive and negative aspects of each of the metabolic pathways are the following.

1 Aerobic degradation is not suitable in principle due to imbalance in the ratio of the electron donor (organic compounds) and the acceptor (molecular oxygen) (Gottschalk, 1979). First, the terminal acceptor O_2 is a gas. Second, its concentration in air is only 21%. Third, the rate of oxygen diffusion into the solution is significantly lower than the rate of its consumption by microorganisms in the liquid phase. For these reasons, O_2 as the terminal electron acceptor cannot ensure complete aerobic oxidation of organic compounds by microorganisms if the concentration of organics counting to total carbon exceeds 5,000 ppm. The imbalance between the donor and the acceptor is so great that even the use of pure oxygen in oxytanks with intensive mixing does not compensate the lack of oxygen molecules.

2 The destruction of organic compounds by dissimilatory nitrate reduction is also not effective. In fact, it is a high-potential metabolic pathway (form +300 to +400 mV) (Gottschalk, 1979). Nitrate-reducing microorganisms preferably use low-molecular organic acids (citrate, acetate) as a substrate (electron donor). High molecular weight soluble compounds and insoluble polymers are inaccessible to nitrate reducers (Moreno-Vivián et al., 1999). Finally, for the destruction of concentrated organic compounds, a large amount of nitrates must be added to achieve the optimal stoichiometric ratio (Gottschalk, 1979).

3 Methanogenesis is a complex three-stage metabolic process, based on the phenomenon of synthrophy. Syntrophy is a metabolically dependent interaction of microorganisms, in which they can exist in a medium that is inaccessible to any of these species separately (Morris et al., 2013). At the first stage, aerobic and facultative anaerobic microorganisms consume oxygen from the culture medium and reduce the ORP to the values in the range from –150 to –200 mV. At the beginning of the second stage, the so-called primary anaerobes destroy high-molecular polymer compounds to low-molecular fatty acids, alcohols, H_2 and CO_2. At the end of the stage, low-molecular compounds are degraded to precursors of methanogenesis (single-molecular formate, methanol, methylamine, etc.). At the third stage, methanogenic microorganisms produce methane from hydrogen/carbon dioxide or degrade single-molecular compounds to methane and carbon dioxide (Thauer, 1998; Ferry, 1999):

$4H_2 + CO_2 \rightarrow CH_4 + 2H_2O$;

$4CH_3OH + 2H_2O \rightarrow 3CH_4 + CO_2 + 4H_2O$;

$4HCOO^- + 4H^+ \rightarrow CH_4 + 3CO_2 + 2H_2O$;

$4NH_2CH_3 + 2H_2O \rightarrow NH_3 + 3CH_4 + CO_2$.

At present, methane fermentation is considered the most effective pathway of degradation of solid food and other polymeric organic wastes (Ike et al., 2010; Kondusamy and Kalamdhad, 2014). Throughout the world, industrial-scale methane digesters are used for recycling food and agricultural waste, manure and aerobic sludge (the working volume is 5,000–6,000 m^3). It is believed that the synthesis of biomethane is an economically viable way of energy production (Budzianowski and Budzianowskaa, 2015). However, biomethane-based digestion of organic waste has a number of significant disadvantages.

The negative aspects of methanogenic pathway include the following.

- The process is three-stage and, therefore, time-consuming (Ziemiński and Frac, 2012). The first stage takes about 3–5 days. The second stage (acid hydrolysis) lasts 7–10 days. Methane production phase lasts on average 20 days. In general, methane fermentation lasts no less than 30 days.
- The second phase (hydrolysis of polymers) is accompanied by a strong medium acidification to pH = 3.0–4.0. For methanogens, optimal pH values are in the range of pH 7.0–7.6. Therefore, methane production is significantly inhibited at non-optimal pH values (Lay et al., 1997).
- Metabolism of methanogenic microorganisms and sulphate-reducing bacteria (SRB) is inextricably linked (Muyzer and Stam, 2008; Ziemiński and Frac, 2012). Thus, methanogens are not able to reduce SO_4^{2-} to S^{2-} and, therefore, can grow only in the presence of SRB, which reduce sulphate to sulphide. On the other hand, SRB need cobalt for normal metabolism. Methane-reducing bacteria supply SRB with cobalt in the form of methylcobalamin (vitamin B_{12}) (Mazumder et al., 1987; Martens et al, 2002). That is why the methane synthesis process is vulnerable and easily can shift to hydrogen sulphide production. High S^{2-} concentrations lead to the inhibition and sometimes to the death of methanogens. For example, methanogens are highly sensitive to toxic metals (Cu^{2+}, Co^{2+}, Hg^{2+}, etc.), while SRB show a higher resistance. In addition, an increase in the concentration of sulphates in solution leads to an intensive synthesis of hydrogen sulphide, which is also toxic for methanogens (Karhadkar et al., 1987).
- The strategic direction – biomethane production – in fact is economically inefficient and environmentally hazardous (Poeschi et al., 2012). First, the rate of waste recycling is much lower than the rate of their formation. This leads to an inevitable accumulation of rotting waste in landfills. Second, produced biogas can be low-quality and even dangerous for use. Nor-

mally, biogas consists of 60–70% of CH_4 and 30–40% of CO_2. Low methane content in the mixture does not allow using it without preliminary purification (CO_2 removal). Finally, methane fermentation is associated with formation of toxic products, i.e., hydrogen sulphide (H_2S) and mercaptans (sulphur-containing organic molecules) (Ziemiński and Frac, 2012).

Due to the listed disadvantages, the methanogenic process does not provide a high rate of food waste recycling as well as high biogas yield of sufficient quality. Therefore, alternative approaches with strong theoretical background are required to achieve fast and cost-efficient process.

In our opinion, a successful solution could be hydrogen dark fermentation under strict maintenance of the optimal parameters.

4 Hydrogen dark fermentation of solid food waste is more effective than any other known metabolic pathway (including methane fermentation). First, it should be noted that the decomposition of solid compounds (for example, starch) to the final products (H_2, CO_2 and fatty acids) is a single-step and short-term process (Zhang et al., 2007b). An undeniable advantage is also the possibility of effective fermentation of waste by one physiological group of microorganisms (primary anaerobes) or even one strain. In contrast, methane fermentation requires involving at least three physiological groups of microorganisms (Ziemiński and Frac, 2012).

Hydrogen fermentation provides much higher energy yield compared with methanogenesis.

Hydrolysis of 1 M of glucose leads to the formation of 4 M (Cavalcante de Amorima et al., 2009):

$C_6H_{12}O_6 + 2H_2O \rightarrow 2CH_3COOH + 4H_2 + 2CO_2$.

Methane fermentation results in the formation only of 1 M of methane from 4 M of hydrogen (Thauer, 1998; Ferry, 1999):

$4H_2 + CO_2 \rightarrow CH_4 + 2H_2O$.

Since both gases are suitable for direct combustion, the use of hydrogen for microbial methane production is unnecessary. Finally, the specific heat of hydrogen combustion (120 MJ/kg) is 2.8 times higher compared with methane (50 MJ/kg) (OECD/IEA, 2008). Thus, hydrogen dark fermentation of food waste provides a faster recycling process of higher biogas quality.

It is also necessary to note the following advantages of hydrogen fermentation of solid food waste. Hydrogen-producing spore-forming anaerobes are most promising for rapid and effective degradation of multicomponent food wastes consisting of solid polymers of animal and vegetable origin. Anaerobic hydrogen producers, as a rule, are capable of simultaneous fermentation of a wide range of substrates belonging to different classes of organic compounds: starch, cellulose, proteins, which results in the production of H_2/CO_2 mixture (Kapdan and Kargi, 2006; Tashyreva et al., 2014). In addition, the hydrolysis of polymers with the formation of H_2 is carried out extremely fast within 3–5 days (Tashyreva et al., 2017). It follows that anaerobic hydrogen-producing bacteria provide a double positive effect – a rapid decrease in food waste volume and green energy production.

However, it is necessary to note the negative aspects of hydrogen fermentation, too. A high rate of polymer hydrolysis leads to the formation of a vast amount of volatile fatty acids (formate, acetate, propionate, etc.) (Khanal et al., 2004) and strong medium acidification, which causes inhibition of hydrogen-producing bacteria and a decrease in hydrogen yield (Ababouch et al., 1992). For this reason, laboratory and pilot-scale experiments do not show high values of hydrogen yield compared with theoretical calculations. Therefore, careful monitoring and adjustment of the pH value is essential at the initial stage of waste decomposition. We assume that theoretical consideration on metabolism of hydrogen-producing bacteria in terms of thermodynamics and electrochemistry will allow establishing optimal fermentation conditions.

Theoretical calculations on optimal parameters for dark fermentation hydrogen production and their experimental justification

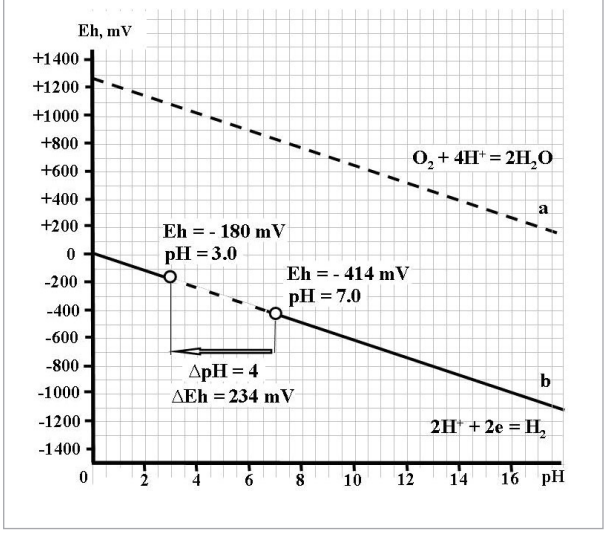
The pH and ORP are supposed to be the key factors influencing dark fermentation hydrogen production, and their adjustment can significantly improve the efficiency of food waste decomposition as well as increase hydrogen yield.

Earlier we showed that microbial metabolism was possible only in the zone of thermodynamic stability of water (Tashyrev et al., 2008). The upper limit of the zone is described by the equation for the reduction of molecular

hydrogen to water: $O_2 + 4H^+ = 2H_2O$, and the lower is described by equation $2H^+ + 2e = H_2$ (Fig. 1) Under standard conditions (equal concentrations of oxidised and reduced forms and pH = 7.0), the potential of the lower limit of water stability is –414 mV, and the upper one is +814 mV. With a decrease in pH, ORP increases from –414 mV at pH = 7.0 to 0 mV at pH = 0.0. For anaerobic microorganisms fermenting glucose with the formation of H_2, the optimal ORP value is in the range from –380 to –414 mV (Cavalcante de Amorima et al., 2009; Wang et al., 2012; Tashyreva et al., 2017). Fig. 1 shows that a decrease in pH from 7.0 to 3.0 leads to an ORP increase from –414 to –180 mV. Therefore, at pH = 3.0, the ORP value is by 234 mV higher than the optimal value for H_2 production. It is obvious that pH is a factor that significantly influences microbial metabolism. In neutral and slightly alkaline conditions, maximum hydrogen synthesis will occur, and a decrease in pH will be accompanied by an increase in ORP and, accordingly, by inhibition of hydrogen synthesis. Therefore, it is quite natural that in neutral and slightly alkaline conditions fermentation of carbohydrates with the maximum hydrogen yield will occur.

Fig. 1

Theoretical calculations on the effect of pH on ORP values in the fermentation medium: a (upper) and b (lower) – the limit of thermodynamic stability of water: a is described by equation $O_2+4H^+=2H_2O$ and $Eh = 1.228 - 0.0591 \cdot pH - 0.0295 \cdot lgPH_2$; b is described by the equation: $2H^+ + 2e=H_2$ and $Eh = 0.000 - 0.0591 \cdot pH - 0.0591 \cdot lgPH_2$



It is known that the accumulation of end products may inhibit a biochemical reaction (Herrero, 1983). Molecular hydrogen is gaseous metabolite, which leaves the reaction environment at atmospheric pressure, while organic acids stay in the fermentation medium and inhibit not only hydrogen synthesis, but also the growth of microorganisms in general. Under hydrogen dark fermentation, the starch contained in food waste is hydrolysed to glucose. Moreover, at the next stage, volatile fatty acids, which are the main product of glucose fermentation, are produced in high concentration and cause a significant acidification of the medium. Therefore, it is obvious that not only the synthesis of hydrogen, but also the growth of microorganisms is supressed by the mechanism of end product inhibition (Herrero, 1983; Sivagurunathana et al., 2014).

Consideration of the patterns of microbial degradation of food waste polymers with H_2 formation makes it possible to optimise the biotechnological process. From the point of view of thermodynamics, hydrogen fermentation of solid food waste can provide rapid decomposition of waste polymers to low-molecular soluble compounds and at the same time obtain a large amount of green fuel molecular hydrogen under permanent maintenance of optimal pH and ORP values.

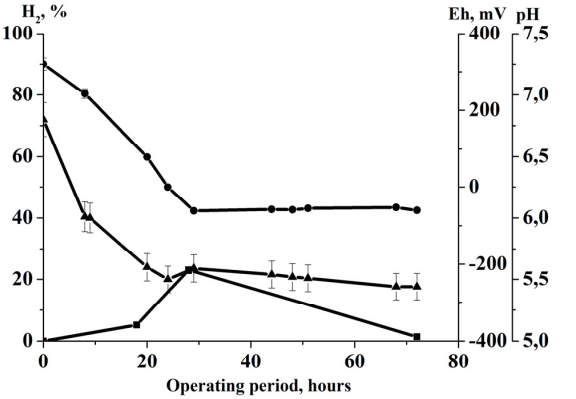
However, the existing patterns of two-stage hydrogen fermentation (first – acidic hydrolysis of polymers, second – synthesis of hydrogen itself) of solid waste lead to the inevitable inhibition of both the process of waste destruction and the synthesis of hydrogen due to strong acidification of the medium. It follows that in order to ensure a theoretically justified rapid and effective process of waste destruction with simultaneous maximum synthesis of hydrogen, control and permanent regulation of pH and ORP of the medium are necessary.

The possibility for optimisation of the hydrogen dark fermentation process was confirmed experimentally. Fig. 2 shows dynamics of fermentation of multicomponent food waste by an anaerobic hydrogen-producing microbial community without pH adjustment. The lack of pH and ORP regulation leads to inhibition of microbial growth.

As expected, a sharp decrease in pH was observed during the first 24 hours. After 9 hours, pH dropped

Fig. 2

Inhibition of hydrogen synthesis in the absence of regulation of pH and ORP



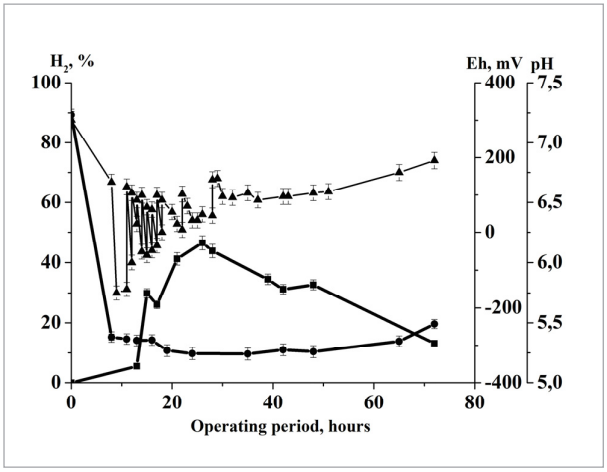
from 6.8 to 6.0, and after 24 hours to 5.5. Therefore, it is obvious that the acidification of the medium led to the inhibition of the growth of microbial community. This was evidenced by a decrease in ORP to the values non-optimal for hydrogen synthesis, from +320 mV to 0 mV during the first day of fermentation. The process of hydrogen production started after 18 hours, and its maximal content of 23% was recorded after 28 hours of fermentation. Quite naturally, under such non-optimal conditions, MFW fermentation was strongly inhibited. After three days of fermentation, the ORP value slightly decreased to –60 mV, and the hydrogen content in the gas phase made 1.4%. The coefficient of waste destruction (Kd), i.e., the ratio of their initial and final mass, was only 5. Hydrogen yield was very low and made 16 L/kg of waste in terms of absolutely dry weight. Obviously, the lack of mixing of MFW also had a negative impact. This occurs because fermentation of MFW is a heterophase process. Anaerobic microorganisms are immobilised on the surface of waste particles and, therefore, form a dense bacterial film. During acid hydrolysis of carbohydrates, organic acids accumulate around waste particles and suppress hydrogen production as well as further degradation process.

Previously we have shown that the absence of mixing significantly inhibited waste fermentation (Tashyreva, 2017). Fig. 3 shows dynamics of hydrogen production under maintenance of optimal pH and ORP values, and the optimal mixing mode.

To optimise the growth of obligate anaerobic microorganisms, low-potential reducing agents such as sodium sulphide (Hungate, 1969), titanium citrate (III), etc. are used (Zender, 1978). The high cost of reagents, as well as the complex technological operations necessary for addition of these reducing agents into industrial reactors, makes their use complicated. For rapid reduction of the ORP value at the initial stage of waste fermentation, we used concentrated biomass of aerobic bacteria as a biological reductant. To produce it, 100 g of food waste was mixed with 3 L of tap water and incubated during three days in aerobic conditions under intensive aeration. After three days, obtained biomass was concentrated by centrifugation and introduced into a reactor.

Fig. 3

High efficiency of multicomponent food waste fermentation during the regulation of pH and ORP of the medium



It is natural that aerobic microorganisms intensively consume oxygen and release exometabolites into the medium. The addition of microbial biomass provided a rapid setting of ORP values optimal for the growth of obligate anaerobic hydrogen-producing bacteria. After 8 hours, ORP decreased to –280 mV, and after 24 hours to –320 mV. Intensive growth of aerobic and anaerobic microorganisms was naturally accompanied by acidification of the medium. After 9 hours of fermentation, pH dropped from 7.2 to 5.75. After

pH adjustment with a saturated Na_2CO_3, its value increased to 6.6. It should be noted that optimisation of ORP values increased metabolic activity of the microbial community and intensified the acidification process. Despite frequent pH adjustment, the value did not exceed 6.6. Nevertheless, after 26 hours of fermentation, the hydrogen content in the gas phase was high and made 47%. In comparison with the control (Fig. 2), maintenance of neutral pH value during fermentation provided a 7.3 times higher hydrogen yield to increase the yield of hydrogen (up to 115 L/kg) and an 18.2 times higher coefficient of waste destruction (Kd) by (up to 91).

Effective food waste fermentation to produce an environmentally friendly energy carrier hydrogen is of interest to both researchers and the industry. However, modern biotechnologies for fermenting food waste have several disadvantages. One disadvantage is the low yield of hydrogen (Kim and Shin, 2008; Wang and Zhao, 2009); another is the low efficiency of waste destruction. Wang received only 65 L (kg^{-1}_{vs}) of H_2 during fermentation of food waste (Wang and Zhao, 2009). Besides, costly thermophilic modes of organic waste fermentation are often used to increase the efficiency of hydrogen synthesis. In this case, hydrogen yield increases insignificantly from 63 L/kg (mesophilic mode) to 81 L/kg of waste (Kim and Shin, 2008).

Much more hydrogen was synthesised after our complex optimisation of the process of food waste fermentation. Thus, by regulating pH, Eh, the mixing mode, etc., we provided a higher hydrogen yield up to 115 L/kg of waste.

It should be especially noted that large volumes of hydrogen were obtained during the fermentation of corn starch waste – 150 L/kg (Zhang et al., 2007b), and hexoses (monosaccharide) – 311 L/kg (Hawkes et al., 2007). Such a high yield of hydrogen is natural, since hexoses are completely fermented to hydrogen, and starch is also a carbohydrate polymer.

In our case, the amount of hexoses does not exceed 10%. We developed a model mixture of food waste, which is close to real household, restaurant and industrial food waste. It consisted of difficult-fermented fresh and rotten solid residues. Waste fermentation in such composition resulted in a hydrogen yield close to the yield obtained by fermentation of waste consisting only of corn starch. Thus, the amount obtained was only by 35 L of H_2 less compared with literature data (115 L/kg compared with 150 L/kg). Waste mixture used in our experiment also included a starch-containing substrate – potatoes. However, potatoes contain no more than 10–15% of starch (Káš et al., 2009). Based on this, our model waste contained about 6% of starch. The rest of vegetables contained no more than 4% of carbohydrates, according to the theoretical calculations. Therefore, model waste contained no more than 10% of carbohydrates, which is 10 times less than 100% hexose and cornstarch (Zhang et al., 2007b; Hawkes et al., 2007).

Recent studies also show low efficiency of food waste destruction, which ranges between 40–80% (Zhang et al., 2007b; Elbeshbishy et al., 2012) and corresponds to *Kd* = 2.5–5.0. In our study, a highly efficient waste destruction was obtained – 98% (Kd = 91), which is 18 times higher than the results obtained by Zhang (Zhang et al., 2007b).

Thus, on the basis of theoretical consideration of patterns of hydrogen dark fermentation of multicomponent food waste, we determined the optimal conditions necessary for efficient waste decomposition and a high hydrogen yield. The key factors influencing the hydrogen yield are adjustment of pH and ORP, as well as periodic mixing of solid food waste.

The use of unfermented residues of food waste as a lignocellulosic substrate for increasing crop yields

It should be noted that even at high efficiency of solid food waste decomposition (Kd = 91), recycling of each ton results in the formation of 10 kg of unfermented solid residues. As a rule, they consist of lignocellulose, which is inaccessible to most anaerobic hydrogen-producing microorganisms (Mussatto and Teixeira, 2010). The H_2 production from lignocellulosic materials can be performed by the anaerobic fermentation process. However, direct conversion of lignocellulosic biomass to hydrogen needs a previous treatment to hydrolyse cellulose crystalline structure (Mussatto and Teixeira, 2010). It is believed that products of anaerobic fermentation of organic compounds

are valuable fertilisers (Yu et al., 2010; Insam et al., 2015). However, we have shown that unfermented residues can be toxic and cause plant and seed death (Bielikova et al., 2017). Their toxicity may be connected with a high content of volatile fatty acids (Bolzonella et al., 2005; Prytula et al., 2011).

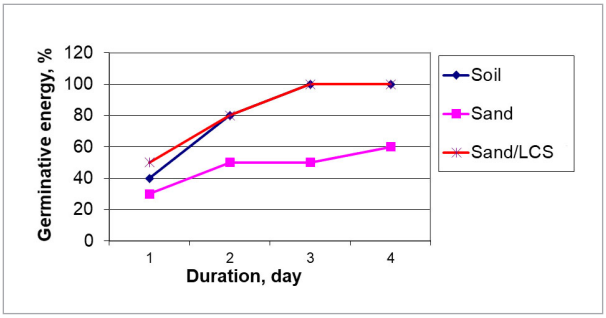
However, in our opinion, toxic solid residues can be converted into an effective biofertiliser. To eliminate toxic components, unfermented residues were aerobically treated to reduce the concentration of soluble organic compounds (shaking during 6 hours, 60 rpm), then rinsed in tap water, weighed and dried at a room temperature.

As a result of aerobic treatment, a lignocellulosic substrate was obtained. In a mix with plain sand, a lignocellulosic substrate showed a strong moisture-retaining capacity. In addition, a pure lignocellulosic substrate counted 7.2×107 CFU/g of ammonifying aerobic bacteria and 5.0×106 CFU/g of free-living aerobic/microaerophilic diazotrophs. The use of a lignocellulosic substrate in a concentration of 33 g/m^2 of sand increased the resistance of radish seedlings to drought stress and provided a crop yield similar to chernozem soil. Fig. 4 shows that the percent of germination of radish seeds in soil and sand/LCS mixture is almost identical.

Obtained preliminary data indicate the prospects of this direction, in particular, the conversion of lignocellulosic residues obtained after hydrogen dark fermentation of food waste into a biofertiliser.

Fig. 4

Percentage of germinated Raphanus sativus seeds grown on different substrates



Conclusions

It was shown that the use of thermodynamic calculations for the selection of the optimal microbial metabolic pathway could be an efficient approach to develop microbial processes of food waste recycling. Application of optimised hydrogen dark fermentation provides a high rate of food waste degradation and formation of valuable products, i.e., environmental friendly energy carrier molecular hydrogen and a lignocellulosic substrate, which acts as a plant probiotic. The obtained results are the basis for the establishment of novel environmental biotechnologies, which are perspective as a solution for the problem of multicomponent food waste accumulation.{Gurauskiene, 2006, Eco-design methodology for electrical and electronic equipment industry}

References

Ababouch L., Chaibi A. and Busta F. F. (1992) Inhibition of Bacterial Spore Growth by Fatty Acids and Their Sodium Salts. Journal of Food Protection. 55 (12): 980-984. https://doi.org/10.4315/0362-028X-55.12.980

Bethke C.M., Sanford R.A., Kirk M.F., Jin Q., Flynn T.M. (2011) The thermodynamic ladder in geomicrobiology American Journal of Science 311(3):183-210. https://doi.org/10.2475/03.2011.01

Bolzonella D., Fatone F., Pavan P. and Cecchi F. (2005) Anaerobic Fermentation of Organic Municipal Solid Wastes for the Production of Soluble Organic Compounds. Industrial Engineering Chemistry Research 44: 3412-3418. https://doi.org/10.1021/ie048937m

Budzianowski W. M., Budzianowskaa D. A. (2015) Economic analysis of biomethane and bioelectricity generation from biogas using different support schemes and plant configurations. Energy. 88: 658-666. https://doi.org/10.1016/j.energy.2015.05.104

Cavalcante de Amorima E. L., Barrosb A. R., Damianovicb M. H. R., Silvab E. L. (2009) Anaerobic fluidized bed reactor with expanded clay as support for hydrogen production through dark fermentation of glucose. International journal of hydrogen energy 34(2): 783–790. https://doi.org/10.1016/j.ijhydene.2008.11.007

Czabator F. J. (1962) Germination Value: An Index Combining Speed and Completeness of Pine Seed Germination. Forest Science 8(4): 386–396.

Elbeshbishy E., Nakhla G., Hafez H. (2012) Biochemical methane potential (BMP) of food waste and primary sludge: In-

fluence of inoculum pre-incubation and inoculum source. Bioresource Technology 110: 18-25. https://doi.org/10.1016/j.biortech.2012.01.025

Ferry J. G. (1999) Enzymology of one-carbon metabolism in methanogenic pathways. FEMS Microbiology Reviews 23: 13-38. https://doi.org/10.1111/j.1574-6976.1999.tb00390.x

Finley S. D., Broadbelt L. J., and Hatzimanikatis V. (2009) Thermodynamic Analysis of Biodegradation Pathways Biotechnol Bioeng. 103(3): 532–541.

Gottschalk G. (1979) Bacterial Metabolism. Springer-Verlag New York XIII, 359 p. https://doi.org/10.1007/978-1-4684-0465-4

Grimm N. B., Faeth S. H., Golubiewski N. E., Redman C. L., Wu J., Bai X., Briggs J. M. (2008) Global Change and the Ecology of Cities. Science 319: 756-760. https://doi.org/10.1126/science.1150195

Guerrero L. A., Maas G., Hogland W. (2013) Solid waste management challenges for cities in developing countries. Waste Management 33: 220-232. https://doi.org/10.1016/j.wasman.2012.09.008

Hawkes F. R., Dinsdale R., Hawkes D. L., Hussy I. (2002). Sustainable fermentative hydrogen production: challenges for process optimisation. International Journal of Hydrogen Energy, 27(11–12), 1339–1347. https://doi.org/10.1016/S0360-3199(02)00090-3

Hawkes F.R., Hussy I., Kyazze G., Dinsdale R., Hawkes D.L. (2007) Continuous dark fermentative hydrogen production by mesophilic microflora: principles and progress. International Journal of Hydrogen Energy 32(2): 172e84. https://doi.org/10.1016/j.ijhydene.2006.08.014

Herrero A. A. (1983) End-product inhibition in anaerobic fermentations. Trends in biothechnology 1(2): 49-53. https://doi.org/10.1016/0167-7799(83)90069-0

Hungate R. E. (1969) A Roll Tube Method for Cultivation of Strict Anaerobes. Methods in Microbiology 3(B): 117-132. https://doi.org/10.1016/S0580-9517(08)70503-8

Ike M., Inoue D., Miyano T., Liu T.T., Sei K., Soda S., Kadoshin S. (2010) Microbial population dynamics during startup of a full-scale anaerobic digester treating industrial food waste in Kyoto eco-energy project. Bioresource Technology 101: 3952–3957. https://doi.org/10.1016/j.biortech.2010.01.028

Insam H., Gómez-Brandón M., Ascher J. (2015) Manure-based biogas fermentation residues—friend or foe of soil fertility? Soil Biology and Biochemistry 84: 1–14. https://doi.org/10.1016/j.soilbio.2015.02.006

Kapdan, I. K., Kargi, F. (2006). Review: Bio-hydrogen production from waste materials. Enzyme and Microbial Technology, 38(5), 569–582. https://doi.org/10.1016/j.enzmictec.2005.09.015

Karhadkar P.P., Audic J.M., Faup G.M., Khanna P. (1987) Sulfide and sulfate inhibition of methanogenesis. Water Research 21 (9): 1061-1066. https://doi.org/10.1016/0043-1354(87)90027-3

Káš M., Matějková Š., Diviš J. (2009) Effect of preceding crops on potato yield in organic and conventional farming. Journal of Agrobiology 26(2): 61-67.

Kekacs D., Drollette B.D., Brooker M., Plata D.L., Mouser P.J. (2015) Aerobic biodegradation of organic compounds in hydraulic fracturing fluids.Biodegradation 26(4):271-87. https://doi.org/10.1007/s10532-015-9733-6

Kim S.H., Shin H.S. (2008) Effects of base-pretreatment on continuous enriched culture for hydrogen production from food waste. International Journal of Hydrogen Energy 33(19): 5266-5274. https://doi.org/10.1016/j.ijhydene.2008.05.010

Khanal S.K., Chen W.H., Li L., Sung S. (2004) Biological hydrogen production: effects of pH and intermediate products. International Journal of Hydrogen Energy 29: 1123–1131.

Kondusamy D., Kalamdhad A. S. (2014) Pre-treatment and anaerobic digestion of food waste for high rate methane production – A review. Journal of Environmental Chemical Engineering 2(3): 1821-1830. https://doi.org/10.1016/j.jece.2014.07.024

Lay J.J., Li Y.Y. and Noike T. (1997) Influences of pH and moisture content on the methane production in high-solids sludge digestion. Water Research 31(6): 1518-1524. https://doi.org/10.1016/S0043-1354(96)00413-7

Levin D. B, Pitt L. and Love M. (2004) Biohydrogen production: prospects and limitations to practical application. International Journal of Hydrogen Energy 29(2): 173-185. https://doi.org/10.1016/S0360-3199(03)00094-6

Li Y., Park S.Y., Zhu J. (2011) Solid-state anaerobic digestion for methane production from organic waste. Renewable and Sustainable Energy Reviews 15: 821-826. https://doi.org/10.1016/j.rser.2010.07.042

Martens J.H., Barg H., Warren M.J., Jahn D. (2002) Microbial production of vitamin B12. Applied Microbiology and Biotechnology 58:275–285. https://doi.org/10.1007/s00253-001-0902-7

Mata-Alvarez J., Mace S., Llabres P. (2000) Anaerobic digestion of organic solid wastes. An overview of research achievements and perspectives. Bioresource Technology 74: 3-16. https://doi.org/10.1016/S0960-8524(00)00023-7

Mazumder T.K., Nishio N., Fukuzaki S., Nagai S. (1987) Production of extracellular vitamin B-12 compounds from methanol by Methanosarcina barkeri. Applied Microbiology and Biotechnology 26: 511-516. https://doi.org/10.1007/BF00253023

Moreno-Vivián C., Cabello P., Martínez -Luque M., Blasco R., and Castillo F. (1999) Prokaryotic Nitrate Reduction: Molecular Properties and Functional Distinction among Bacterial Nitrate Reductases. Journal Bacteriology 181 (21): 6573-6584.

Morris E.L., Henneberger R., Huber H., Moissl-Eichinger C. (2013) Microbial syntrophy: interaction for the common good Brandon. Microbiology Reviews 37: 384–406. https://doi.org/10.1111/1574-6976.12019

Muyzer G., Stam A. J. (2008) The ecology and biotechnology of sulphate-reducing bacteria. Nature Reviews Microbiology 6(6): 441-454. https://doi.org/10.1038/nrmicro1892

Mussatto SI, Teixeira JA. (2010) Lignocellulose as raw material in fermentation processes. Mendez-Vilas A (editors). Current research technology and microbial biotechnology: 897-907.

Noike T., Mizuno O. (2000) Hydrogen fermentation of organic municipal wastes.. Water Science and Technology 42(12): 155-162. https://doi.org/10.2166/wst.2000.0261

OECD/IEA Electricity Information (2008) for coal; Australian Energy Consumption and Production, historical trends and projections, ABARE Research Report 1999.

Pires A., Martinhoa G., Chang N.B. (2011) Solid waste management in European countries: A review of systems analysis techniques. Journal of Environmental Management 92: 1033-1050. https://doi.org/10.1016/j.jenvman.2010.11.024

Pourbaix M. (1966) Atlas of Electrochemical Equilibria in Aqueous Solutions. Pergamon Press, London 177-190

Ren N. Q., Li Y. F., Wang A. J., Li J. Z., Ding J., & Zadsa, M. (2006). Hydrogen production by fermentation: Review of a new approach to environmentally safe energy production. Aquatic Ecosystem Health & Management, 9(1), 39–42. https://doi.org/10.1080/14634980500536030

Sharholy M., Ahmad K., Mahmood G., Trivedi R.C. (2008) Municipal solid waste management in Indian cities – A review. Waste Management 28: 459-467. https://doi.org/10.1016/j.wasman.2007.02.008

Show K.Y., Lee D.J.,Tay J.H., Lin C.Y. Chang J.S (2012) Biohydrogen production: Current perspectives and the way forward. International Journal of Hydrogen Energy 37(20): 15616-15631. https://doi.org/10.1016/j.ijhydene.2012.04.109

Sivagurunathana P., Senabc B., Lin C.Y. (2014) Overcoming propionic acid inhibition of hydrogen fermentation by temperature shift strategy. International Journal of Hydrogen Energy. 39(33): 19232-19241. https://doi.org/10.1016/j.ijhydene.2014.03.260

Tashyreva H., Tashyrev O., Govorukha V., Havryliuk O. (2017) The effect of mixing modes on biohydrogen yield and spatial ph gradient at dark fermentation of solid food waste. Ecological Engineering and Environment Protection 2: 53-62.

Tashyrev O., Galinker E., Andreyuk E. (2008) Thermodynamic prediction of the redox interaction of microorganisms with oxidizing metals (Hg2+, CrO42- and Cu2+). Reports of the National Academy of Sciences of Ukraine, 4: 166-172.

Tashyreva A. Tashyrev O., Prytula I. (2014) The novel comprehensive approach for non-food agricultural and landfill biomass microbial fermentation and biogas production. Biotechnology and Plant Breeding Perspectives; Eds R.K. Behl, E. Arseniuk. Agrobios (International) Publishers: 347–356.

Thauer R. K. (1998) Biochemistry of methanogenesis: a tribute to Marjory Stephenson. Society for General Microbiology 144: 2377-2406.

Wang S., Zhu Y., Zhang Y., Li Y. (2012) Controlling the oxidoreduction potential of the culture of Clostridium acetobutylicum leads to an earlier initiation of solventogenesis, thus increasing solvent productivity. Applied Microbiology Biotechnology 93(3): 1021-1030. https://doi.org/10.1007/s00253-011-3570-2

Wang X, Zhao Y. A. (2009) A bench scale study of fermentative hydrogen and methane production from food waste in integrated two-stage process. International Journal of Hydrogen Energy 34(1): 245-254. https://doi.org/10.1016/j.ijhydene.2008.09.100

Wen-Hsing Chen (2006) Biological hydrogen production by anaerobic fermentation Retrospective Theses and Dissertations. 1863. http://lib.dr.iastate.edu/rtd/1863.

Yu F.B., Luo X.P., Song C.F., Zhang M. X., Shan S. D. (2010) Concentrated biogas slurry enhanced soil fertility and tomato quality. Acta Agriculturae Scandinavica, Section B — Soil & Plant Science 60(3): 262-268.

Zehnder A. J. B. (1978) Ecology of methane formation. Water pollution Microbiology 2: 349-376.

Zenova G., Stepanov A., Likhacheva A., Manucharova N. (2002) Workshop of Soil Biology. Publisher MSU, 2002, 120 p. ISBN 5-211-04657-9.

Zhang M.L., Fan Y., Xing Y., Pan C., Zhang G., Lay J. (2007) Enhanced biohydrogen production from cornstalk wastes with acidification pretreatment by mixed anaerobic cultures. International Journal of Hydrogen Energy 31(4): 250-4. https://doi.org/10.1016/j.biombioe.2006.08.004

Zhang R., El-Mashad H. M., Hartman K., Wang F., Liu G., Choate C., Gamble P. (2007) Characterization of food waste as feedstock for anaerobic digestion. Bioresource Technology 98: 929-935. https://doi.org/10.1016/j.biortech.2006.02.039

Ziemiński K., Frąc M. (2012) Methane fermentation process as anaerobic digestion of biomass: Transformations, stages and microorganisms. African Journal of Biotechnology 11(18): 4127-4139.