BIOTRANSFORMATION OF VEGETABLE WASTE USING MODERN EM-TECHNOLOGIES: EUROPEAN EXPERIENCE AND UKRAINIAN REALITIES

Iryna Korniienko[1*], Olena Kuznietsova[1], Valeriia Kuskova[1],
Vitalii Gulyaev[2], Andrii Anatskyi[2], Yurii Korniienko[2]
[1]*National Aviation University, Kyiv, Ukraine;*
[2] *Dnipro State Technical University, Kamianske, Ukraine*
**Corresponding author: kornienko.1979@gmail.com*

Abstract. *Accumulation, processing and disposal of organic waste, in particular, vegetable waste, is considered an urgent problem today for Ukraine and the EU countries. Currently, one of the promising ways to solve the problematic issue is the application of modern biotechnologies of organic waste processing with the use of a specific consortium of microorganisms, which allow in the process of bioconversion of beet, carrot and potato waste to obtain digestate and biohydrogen in 6 days of fermentation at a temperature of 37 ^{0}C. The effectiveness of using additional substrates, such as expired bread and probiotic lactulose, to activate the fermentation process of organic waste has been proven. The experiments confirmed the absence of pathogenic cultures in the digestate, which is explained by the high titer of viable cultures of antagonistic microorganisms (lactic acid bacteria, Bacillus subtilis, Paenibacillus polymyxa). The obtained research results show that in all samples (1–7) there is an intensification of the fermentation process on the 2nd, 3rd, and 4th day of fermentation, which is evidenced by changes in the redox potential index, which ranges for samples (1–7) from -100 to - 200 mV. The release of biohydrogen intensively began on the 2nd day of fermentation and lasted for 6 days of the experiment. In order to intensify the process of transformation of plant waste, the expediency of using additional sources of carbon, namely expired bread in the amount of 10% and lactulose in the amount of 2 and 4% of the mass of waste, was substantiated for the first time.*

DOI: 10.24263/EDSD-2023-5-18

Introduction. According to UN data, during the monitoring of the amount of accumulated waste in 152 countries of the world, spoilage of food products during their storage and transportation was recorded, namely, their losses were determined: 47% of root crops, 44% of fruits, 28% of vegetables, 23% of cereals and legumes and 12% dairy products. At the same time, there is a shortage of food products in the world, the depletion of soils due to the use of mineral fertilizers and pesticides is recorded, the amount of energy resources is sharply reduced, which confirms the relevance of the mentioned problem, which needs an immediate solution (Makovetska, 2015). Since Ukraine acquired the status of a candidate member of the EU on June 27, 2022 (European Council conclusions, 2022), the state must fulfill certain obligations regarding the adoption of technical standards and legal norms of the legislative framework regarding waste management and methods of their processing , which will be directed to obtaining renewable sources of energy and biofertilizers through the bioconversion of organic biomass using modern biotechnologies (Directive 2009/28/EC, 2009); European Commission: 2022, 2020a).

For EU member states, these documents highlight targets for a certain period of time that must be met. For example, Latvia has waste recycling targets to be implemented by 2025 (European Commission, 2023b).

The transformation of waste into resources is key to the transition to a cleaner, climate-neutral and circular economy. The reuse and recycling of waste leads to the creation of local jobs, stimulation of innovation, mitigation of negative impact on the environment, as well as reduction of dependence on imported raw materials (European Commission, 2023c).

The document states that the Commission has published an early warning report on the progress of EU Member States in meeting the 2025 waste reuse and recycling targets and the 2035 municipal waste landfill reduction targets. Achieving these goals is essential for the creation of well-functioning markets for secondary raw materials in the EU (European Commission, 2023d).

In the report, the Commission identifies those Member States that are at risk of missing the targets for reuse and recycling of municipal waste and all packaging waste by 2025, as well as the targets for landfilling by 2035. Nine member states are on track to meet the 2025 targets: Austria, Belgium, the Czech Republic, Denmark, Germany, Italy, Luxembourg, the Netherlands and Slovenia (European Commission, 2023e).

The Commission supports Member States in improving the efficiency of waste management with the help of European funds, it provides technical assistance, guidance and other tools (European Commission, 2023f).

According to the analysis, starting from 2015, 120-140 million tons of food waste is generated in the EU every year (Caldeira, De Laurentiis, Corrado, van Holsteijn & Sala, 2019a)

This is 20-25% of the total amount of food. Vegetables, fruits and grains are the food groups that generate the most food waste. The largest amount of food waste is generated during the consumption stage (46%), followed by primary production (25%), and processing and manufacturing (24%) (Caldeira, De Laurentiis & Sala, 2019b; Caldeira, Vlysidis, Fiore, De Laurentiis, Vignali & Sala, 2020).

Incidentally, 44% of the world's largest food companies have begun to count and record food losses and waste in their operations, and 30% of companies measure and report their losses in the absence of their processing into biofertilizer and biogas (Champions 12.3. 2019. SDG target 12.3 on food loss and waste, 2019; Corrado, Ardente, Sala & Saouter, 2017).

Countries that account for only 15% of the world's population are taking massive action to reduce the loss of food that must be processed as waste. But no country has yet announced that it has halved food waste with recycling, as the lack of quantification at the national level prevents tracking of this milestone. Although one-third of the world's 50 largest food companies have implemented programs to reduce food loss and waste. (De Laurentiis, Caldeira &Sala, 2020; Commission Notice (2018/C133/02), 2018b).

To date, the goals of the bioeconomy of the EU member states have been formed, which cover all sectors and systems that depend on biological resources (animals, plants, microorganisms and derived biomass, including organic waste), their functions and principles. They include all primary production sectors that use and produce biological resources (agriculture, forestry, fisheries and aquaculture) and all economic and industrial sectors that use biological resources and processes to produce food, feed, bioproducts, energy and services . To be successful, the European bioeconomy must be based on sustainability and circularity. This will contribute to the renewal of leading industries, modernization of primary production systems, environmental protection and increase in biodiversity (Commission Communication (COM/2018/673), 2019; Commission Delegated Decision (C(2019)3211), 2020).

The Climate Law sets the goal of achieving climate neutrality in the EU by 2050. In the EU, it is planned to reduce greenhouse gas emissions by 55% by 2030 compared to the levels of emissions in 1990. The Farm to Fork Strategy sets out a new approach to ensure that agriculture, fisheries and aquaculture development and the food value chain contribute appropriately to this process. Considering the issue of climate change, the basics of the circular economy and the principles of the bioeconomy, it becomes clear that the vegetable waste generated at the processing stage has a high potential for valorization, since food waste streams are present in large, concentrated and homogeneous quantities. Food waste can be transformed into a number of value-added products through several valorization pathways. It is proposed to use modern biotechnology for their processing. Actions to combat organic waste require an evaluation system that includes SMART goals and key performance indicators for tracking (Commission Communication (COM/2020/381), 2020b; Commission Communication (COM/2020/28), 2020c; Commission Communication (COM/2020/380), 2008).

Directive 2008/98/EC of the European Parliament and of the Council of 19 November 2008 on waste and repealing certain Directives is a normative act that regulates the procedure for the collection, processing and utilization of waste on the territory of the European Union. It aims at establishing the basic rules of waste management, which will allow to reduce the landfill of waste and ensure the saving of resources due to the reuse and processing of secondary raw materials. This Directive introduces a hierarchy of waste management priorities (Directive 2008/98/EC, 2008; Directive (EU) 2018/851, 2020; FLW Protocol, 2016).

From December 2023, separate collection of organic waste will become mandatory for all EU member states. Separate collection and processing of organic waste contribute to the reduction of greenhouse gas emissions, including methane. Separate waste collection systems differ among the 27 EU member states. The introduction of mandatory separate collection of organic waste followed by bioconversion of food and vegetable waste is based on the principles of circular economy (Manfredi et al., 2015)

Considering the urgency of the problem of processing organic waste and reducing its quantity in accordance with the principles of the circular economy and EU Directives, it becomes clear the need to develop and improve modern biotechnologies for the bioconversion of organic waste into biofertilizer and biogas (Prokaieva, 2021).

The issue of organic waste processing is considered in the paper, which shows the results of evaluating the potential of processing fruit and vegetable waste with sawdust in various combinations and establishes the relationship between microorganisms and physicochemical parameters: C/N in relation to the bioconversion of fruit waste (apple, banana peel , oranges and kiwi) and vegetable waste (cabbage leaves, potato and carrot peels). The total amount of fruit and vegetable waste was approximately 2 kg in each sample, to which different amounts of sawdust (1.23, 0.14 and 0.203 kg) were added to obtain the suggested C/N ratio and limit odor. The results showed that in the first week, the pH is acidic and the conductivity values are high for all three samples, and then the pH values increase during the composting process, while the conductivity values decrease, which is also noted in our presented research results. Taking into account such changes, we recommend adjusting the pH value in order to obtain a biofertilizer with a pH within the neutral range (Ghinea, Leahu, 2020).

The authors proposed a food waste processing technology that is easy to maintain, fast, economical and affordable, and most importantly - ecologically safe and socially acceptable. It is proposed to carry out bioconversion of organic waste based on the principle of biological decomposition with the participation of biodegrading microorganisms, thanks to which it was possible to obtain high-quality biofertilizer, which confirms the relevance of the biotechnological approaches presented in this article (Oktiawan, Hadiwidodo, Priyambada & Purwono, 2019).

Taking into account the problematic issues formed above, which are relevant for the whole world and need to be solved, it is proposed to investigate the process of biotransformation of organic waste according to modern biotechnologies (Prokaieva, 2021). The author discussed the accepted strategies of bioconversion, namely the processing of waste with minimization of the impact on the surrounding natural environment (Reshmy et.al., 2021). The work proved the positive effect of sugars contained in the skins of vegetable waste on the intensity of waste fermentation and on the concentration of organic acids and alcohols in the culture liquid (Kandari et.al., 2012). Also, the optimal fermentation time of vegetable waste was investigated, which is 5-6 days at a temperature of 37 ^{0}C (Kudri, 2020). During the bioconversion of vegetable waste, one of the main techniques is the process of homogenization and mixing of waste (Wang et.al., 2012).

Experiments have proven that the hydrolysis stage has a high impact on the subsequent stages of gas biosynthesis. Namely, the successful passage of the hydrolysis process will help increase the yield of biohydrogen by 1.5-3.0 times (Mishra et.al., 2021). However, accelerating the stage of hydrolysis can lead to an excessive increase in the concentration of acids in the culture liquid, which will inhibit the development of hydrogen-synthesizing microorganisms, so it is necessary to regularly adjust the pH of the medium using buffer solutions (Geletuha and Zhelyezna, 2014). The works studied the role of lactic acid bacteria (LAC) and lactulose prebiotics on the process of hydrolysis and gas biosynthesis. Lactulose is selectively metabolized by microbiota, forming SCFAs, gas (hydrogen, carbon dioxide, and methane), which leads to an increase in bacterial biomass. *Bacteroides oralis*, *Bacteroides vulgatus*, *Bifidobacterium bifidum*, *Clostridium perfingens*, *Lactobacillus casei* strains showed high efficiency in lactulose metabolism (> 20%). Also, it was established that during the first day of fermentation, the pH can decrease by 2.0-2.5 units (Karakan et.al., 2021).

The author investigated the effectiveness of using the solid fraction of digestate for agricultural purposes in order to fertilize agricultural soils (Ratushniak, Koshcheiev, 2011).

Taking into account the above aspects, the objective of the study consists in researching the efficiency of bioconversion of vegetable waste (beetroot, carrot and potato) into digestate and biohydrogen using modern EM technologies.

To achieve the objective, the following tasks were formed:

1) determine the optimal fermentation time of vegetable waste with the addition of EM preparations "Baikal-EM-1" and "Organic-balance";
2) establish the feasibility of using expired bread in the amount of 10% and lactulose prebiotic in the amount of 2 and 4% as additional substrates in the process of biotransformation of vegetable waste;
3) evaluate the quality of the obtained digestate according to the main physicochemical and microbiological indicators (pH, redox potential, content of biogenic elements, titer of viable cells of indicator microorganisms).

Materials and Methods. The method of the experiment consisted in the implementation of bioconversion processes of vegetable waste - beet, carrot and potato, which were fermented both separately and in the form of a mixture with the addition of EM preparations "Baikal EM 1", "Organic-Balance" and additional substrates (expired bread - 10 %, prebiotic lactulose in the amount of 2 and 4% relative to the total mass of waste). Vegetable waste before bioconversion was not subject to heat treatment and disinfection, it contained the remains of the soil, which contains accompanying microflora. Before the start of the fermentation process, the vegetable waste was crushed to a particle size of 0.3-0.5 cm in order to intensify the fermentation process. Unprocessed, crushed vegetable waste was subjected to fermentation in a laboratory setup (Fig. 1), which consists of 2 flasks and a gas outlet tube. Biotransformation of waste took place in thermostatic conditions at a temperature of 37 ºC for 6 days. As a result of the acidification of the fermentation medium by lactic acid bacteria contained in the EM preparation, the pH value of the culture liquid was adjusted daily within optimal limits (pH 6.0-6.5) due to the introduction of a buffer solution (sodium hydroxide). Biopreparation "Baikal - EM 1" consists of a consortium of effective microorganisms that are in symbiosis with each other. Effective microorganisms include lactic acid, photosynthesizing, nitrogen-fixing bacteria and yeast (*Lactococcus lactis*, *Lactobacillus casei*, *Rhodopseudomonas palustris*, *Saccharomyces cerevisiae*). This preparation, according to the instructions, is recommended to be used as a biofertilizer, to protect plants from various diseases, to deodorize and disinfect cesspools. The preparation "Organic-Balance" contains organic substances - stabilizers, biologically active substances, vitamins, enzymes for decomposition of residues, as well as a concentrate of viable microorganisms: antagonistic cultures - *Bacillus subtilis*, *Paenibacillus polymyxa*, and nitrogen fixers *Azotobacter chroococcum*. The titer of cultures in both preparations ranges from $1x10^8$ to $1x10^9$ CFU/cm^3. To prepare a working solution (for effective fermentation of waste) from a mixture of two EM preparations, the following sequence was performed: EM preparations "Baikal EM 1" and "Organic-Balance" were added to a flask with prepared water (distilled and sterile) in concentrations of 10 and 2 ml/l, respectively, sucrose in the amount of 20 g/l. The activation temperature was 37 °C. The prepared working solution was kept in thermostatic conditions for 3.5 hours to activate microorganisms, after which it was used in a ratio of 1:4 relative to the crushed mass of vegetable waste. The following samples were obtained: No. 1 – vegetable mixture, working solution, expired bread (10% relative to the mass of vegetable waste), No. 2 – vegetable mixture, working solution, No. 3 – potato waste, working solution, No. 4 – carrot waste, working solution , No. 5 – beet waste, working solution, No. 6 – vegetable mixture, working solution, 2% lactulose, No. 7 – vegetable waste mixture, working solution, 4% lactulose, No. 8 – vegetable waste mixture, prepared water (without the addition of a biological preparation, control). During the experiment, physico-chemical and microbiological quality control of the digestate was carried out according to the main parameters: redox potential, pH, concentration of biogenic elements (ammonium nitrogen, nitrite nitrogen, nitrate nitrogen, phosphates), titer of viable cells of lactic acid bacteria (*Lactococcus lactis*, *Lactobacillus casei*), yeast (*Saccharomyces cerevisiae*), nitrogen-fixing and hydrogen-synthesizing bacteria on the example of *Paenibacillus polymyxa*, enethorobacteria (*E.Coli*). Indicators were determined daily in order to establish the intensity and depth of the fermentation process, as well as to identify the optimal fermentation time (according to the maximum negative value of the redox potential). The quality indicators of the digestate were evaluated in all experimental samples during the duration of the fermentation process (6 days) and the post-fermentation period (10th, 20th, 30th days) during the recommended storage period of the digestate at a temperature of 4 ^{0}C.

Experimental methods of researching the quality of biofertilizer (digestate) were carried out in accordance with the list of methods for measuring (determining) the composition and properties of samples of environmental objects, emissions, waste and discharges, temporarily allowed for use, approved by the Ministry of Environmental Protection of Ukraine (materials of the electronic system "Zakon", 2007).

a) b) c)

Fig.1. Laboratory installation for the bioconversion of vegetable waste (a) and samples of digestate: b) after separation of the solid fraction at the end of fermentation (after storage); c) without separation of the solid fraction from the liquid at the end of fermentation (after storage)

Indicators of redox potential and concentration of hydrogen ions were measured using a standard certified pH meter (millivoltmeter pH-150 mA), the pH indicator was determined according to ISO 4077-2001. The content of biogenic elements was determined by the photocolorimetric method, using standard research methods. Ammonium nitrogen was measured according to Nesler. The essence of the method is the interaction of the ammonium ion with the Nesler reagent, in which case yellow mercurammonium iodide is formed. Nitrites were determined by the ability of nitrogen nitrites in an acidic medium to form diazo compounds with aromatic amines. During the measurement of ammonium and nitrite nitrogen, the photocalorimeter was set to a green light filter. Nitrate content was determined using salicylic acid using a photoelectrocalorimeter with a blue light filter. Phosphates were determined using a photoelectrocalorimeter with a red light filter. In a dilute solution of phosphates, ammonium molybdate reacts in an acidic environment with the formation of molybdic acid, which is reduced by tin to an intense blue color. During fermentation of the substrate, the volume of gas was measured after determining the composition of the gas phase by the gas chromatographic method. A hermetic gas holder was used to determine the gas volume. After each measurement, the gas holder was completely filled with water in order to avoid errors when calculating the composition of the gas phase. Sampling of gases and culture liquid was carried out with plastic sterile syringes with a volume of 2.5 ml. Samples were taken by piercing the rubber stopper of the bottle with a syringe needle. Before that, the stoppers and needle of the syringes were carefully sterilized by wiping with alcohol and heating in the flame of the burner. The volume of synthesized gas was measured on the scale of the syringe (extrusion of the syringe piston by excess gas pressure). The composition of the gas phase was determined according to the standard method on a LHM-8-MD gas chromatograph. To grow bacteria and fungi in laboratory conditions, a study of their various properties was carried out, and the following generally accepted nutrient media were used: meat-peptone broth, Saburo, Chapek, Endo, MPA, MRS, which correspond to certain standards, creating optimal conditions for the growth, reproduction and vital activity of microorganisms. A nutrient medium with the following composition, mg/l, was used for isolation from the digestate of *R. polymyxa*: molasses -40, corn extract - 20, vermiculite - 0.4, K_2HPO_4 - 0.5, $MgSO_4$ - 0.5, NaCl - 0.5, $CaCO_3$ - 10.5. The technique of tenfold dilutions was used for microbiological studies. Microorganisms isolated from the digestate were cultivated in a thermostat (dry-air thermostat TS-1/80 SPU). The number of viable cells was determined using a DEN-1 densitometer and the direct counting method. Bacterial cell samples were stained according to the Gram staining method, fungi were stained with lactofuxin. Microscopy of test samples was carried out using a microscope (Microscope XS-5520 biological).

The study was carried out with eight samples of prepared organic waste, in 3 repetitions with each. The relative error of experimental studies was defined as the difference between the measured and the true value of the measured value and was within the limits outlined in the above research methods. The relative error did not exceed 5% for all determined indicators. Statistical processing of the obtained research results was carried out using the Microsoft Excel software program.

Results and Discussion. Modern agricultural production in the EU countries is based on the basis of extended reproduction of soil fertility under the conditions of compliance with the safety of the environment and grown products. The rational use of biofertilizers and biostimulants ensures the production of environmentally safe products, the accumulation of soil organic matter, reducing soil fatigue, improving its structure, and as a result, increasing soil fertility. Based on the above, we proposed to carry out bioconversion of vegetable waste with the participation of effective microorganisms of the complex preparation "Baikal EM 1" and "Organic-Balance" in order to obtain high-quality digestate and biohydrogen (the preparation contains cultures of *P.polymyxa* and *Bacillus subtilis*, which belong to hydrogen synthesizing bacteria).

Fermentation of vegetable waste is the metabolism of sugars (substrates), during which energy is obtained as a result of partial oxidation of an organic compound using organic intermediates as electron donors and acceptors. Carbohydrates are the main source of carbon and energy that form the necessary components in the environment. The concentration of sugars affects the growth and functionality of microorganisms, therefore, in order to accelerate the rate of the initial phase of bioconversion, we added additional substrates to the samples - expired bread in the amount of 10% relative to the mass of waste and lactulose - a prebiotic that increases the number of viable LAB that participate in the process hydrolysis of vegetable waste.

During the process of fermentation and storage of digestate, the concentration of hydrogen ions and the redox potential were measured, which indicate changes in the environment, and the stabilization of the redox potential indicator indicates the cessation of fermentation processes in the system, i.e., the possibility of the end of the fermentation process of vegetable waste (Figs. 2, 3).

Analyzing the presented research results (Fig. 2), it is possible to assert the beginning of the stage of acidogenesis at the end of the 1st day of the experiment, especially for samples 1, 6, 7, to which additional substrates were added. Acidification of the fermentation medium was recorded for 1 day of the experiment, which is explained by an increase in the titer of viable cells of the LAB.

The dynamics of fermentation of organic waste was studied according to the following parameters - pH (Fig. 2), Eh (Fig. 3) and intensity of gas formation (volume of isolated biogas). Completion of the fermentation process was determined by visual assessment, i.e. cessation of gas formation, reduction of hydrogen concentration in the gas mixture. The parameters were monitored once a day during fermentation (1-6 days).

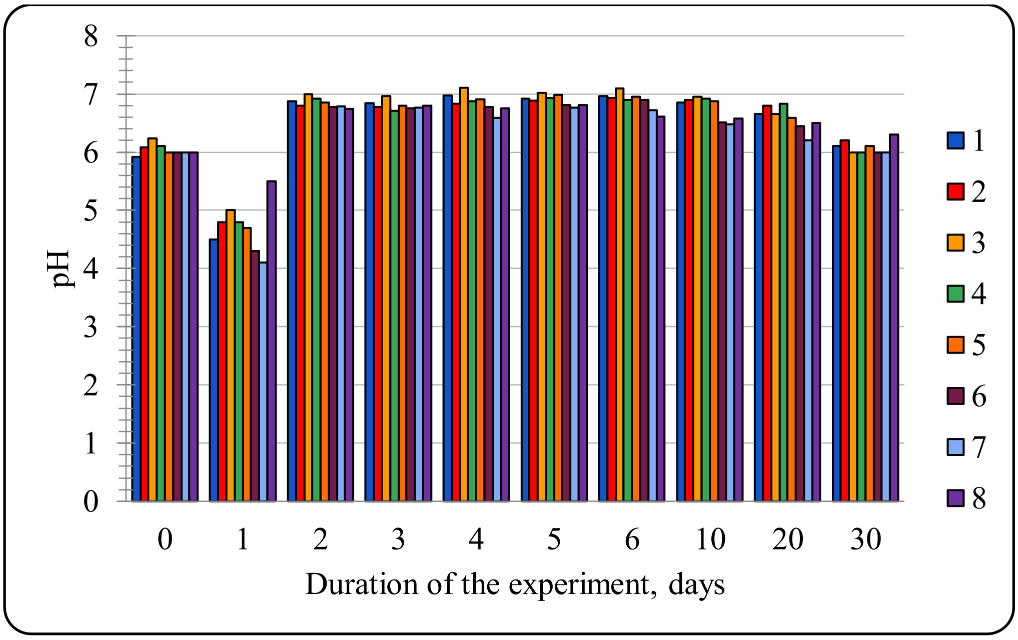


Fig. 2. The dynamics of changes in the concentration of hydrogen ions in the experimental samples during fermentation (1–6 days) and storage (30 days): No. 1 – vegetable mixture, working solution, expired bread (10% relative to the mass of vegetable waste), No. 2 – vegetable mixture, working solution solution, No. 3 – potato waste, working solution, No. 4 – carrot waste, working solution, No. 5 – beet waste, working solution, No. 6 – vegetable mixture, working solution, 2% lactulose, No. 7 – vegetable waste mixture, working solution, 4% lactulose, No. 8 - vegetable mixture of waste, prepared water (without the addition of biological preparations, control)

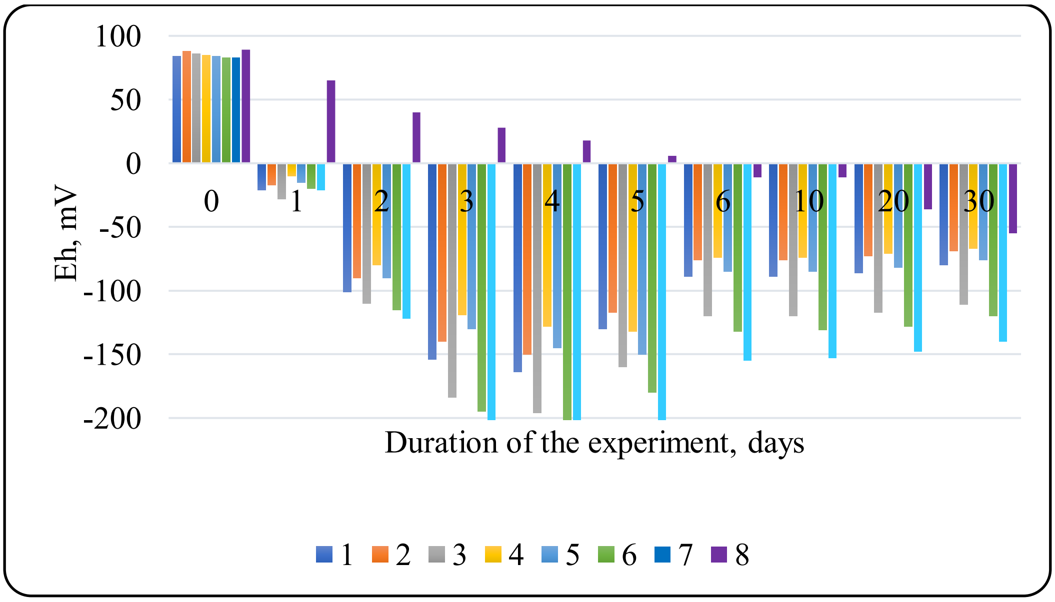


Fig. 3. Dynamics of changes in the redox potential (Eh) in experimental samples during fermentation (1–6 days) and storage (30 days): No. 1 – vegetable mixture, working solution, expired bread (10% relative to the mass of vegetable waste), No. 2 – vegetable mixture, working solution, No. 3 – potato waste, working solution, No. 4 – carrot waste, working solution, No. 5 – beet waste, working solution, No. 6 – vegetable mixture, working solution, 2% lactulose, No. 7 – vegetable mixture waste, working solution, 4% lactulose, No. 8 - vegetable mixture of waste, prepared water (without adding biological preparations, control)

The obtained research results show (Fig. 3) that in all samples (1–7) there is an intensification of the fermentation process on the 2nd, 3rd, and 4th days of fermentation, as evidenced by changes in the redox potential index, which fluctuates within the limits for samples (1 -7) from -100 to -200 mV. The lowest indicator of redox potential was recorded for sample No. 3, which contained only potato waste, as well as for samples No. 6, 7, to which lactulose was added. Therefore, in the biotechnology of directed biosynthesis of hydrogen, it is better to use potato waste, but considering that in small households a mixture of vegetable waste is formed and there is only a need to obtain biofertilizer for own needs, it is better to use a mixture of vegetable waste, which in terms of the content of vitamins and micro- and macroelements will be higher than the digestate obtained from such monowaste as potatoes. Also, if expired bread (experimental sample No. 1) and lactulose (experimental samples No. 6 and 7) are added to the vegetable mixture during fermentation instead of sucrose at the microflora activation stage, the fermentation process can be intensified, as well as the time of the hydrolysis stage and acid accumulation can be shortened. As for samples No. 6 and 7, to which the prebiotic lactulose was added, which contributes only to the increase of the LAB titer, we note the acceleration of the acid accumulation stage, which is confirmed by the results of microbiological studies to determine the LAB titer, which have antagonistic properties in relation to pathogenic bacteria and fungi. Considering the fact that the concentration of fatty acids increases thanks to LAB, at the stage of acetogenesis, they are transformed into hydrogen and then into methane. Experiments proved that in sample No. 8 (control) the waste transformation process is slowed down, which is associated with insufficient reproduction of accompanying microflora, which entered the fermentation medium together with untreated waste, which is confirmed by insignificant changes in pH and redox potential. Therefore, to accelerate the fermentation process and prevent the development of pathogenic wild plants, it is necessary to use EM preparations (Levin, 2014).

The release of biogas (H_2) began intensively on the 2nd day of fermentation, especially for samples No. 3, 6, and 7. This is explained by the fact that potato waste is starch-containing and susceptible to the development of hydrogen-synthesizing microorganisms, especially Bacillus subtilis. In samples No. 6 and 7, to which the substrate lactulose was added, the release of biohydrogen also intensifies, which is explained by the fact that lactulose is a disaccharide consisting of fructose and galactose, it decomposes into lactic acid, acetic acid, and then transforms into hydrogen by with the participation of hydrogen-synthesizing microorganisms.

The hydrogen content for samples No. 3, 6, and 7 at the end of the 2nd day of fermentation was 10.1–11.4 ±2.0%, and on the 4th and 5th day of the experiment it was 32–36, ±2.0%. On the 2nd-3rd day, foam and gas bubbles accumulated on the surface of the fermentation medium, on the 5th-6th day, a gradual destruction of vegetable waste was observed with the deposition of highly dispersed detritus at the bottom of the flask, the amount of fermented substrate (vegetable waste) decreased to approximately 25% in 6 days of cultivation. Therefore, it can be assumed that the full process of waste destruction takes place within 6 days.

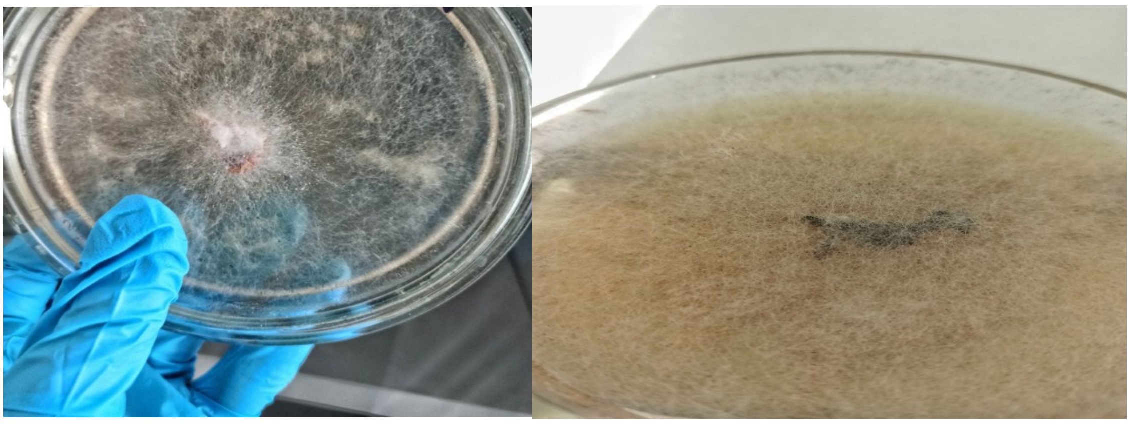
We note that during the experiment there is an active transformation of carbohydrates of vegetable waste by the presented microflora, which was introduced together with the working solutions of two EM preparations. We consider pH=6.3-7.0 and Eh= –100...–200 mV to be the optimal conditions for the biotransformation process. The duration of destruction is 6 days, which also correlates with similar studies (Jarunglumlert, 2018).

According to the generally accepted methods of determining the content of biogenic elements, we determined the quality of the digestate at the end of the 6th day of fermentation, which varies in the range for samples 1–7, mg/l: ammonium nitrogen from 86 to 120, nitrite nitrogen from 10 to 12, nitrate nitrogen from 430 to 480 mg/l, phosphates from 430 to 480, respectively. The obtained results of research indicate the expediency of using digestate as a biofertilizer, since the content of biogenic elements is high enough and needs to be diluted with water in a ratio of 1:2.5.

The next stage is conducting microbiological studies to determine the safety of the digestate from the point of view of the content of *E. coli* and mold fungi, since non-liquid vegetable waste was used, which could not be cleaned, washed and sterilized, and contained soil residues. The use of digestate containing pathogenic cultures is not an acceptable factor in agricultural technologies. In order to identify bacteria of the *Escherichia coli* group, the studied samples were sown on Endo's medium. Due to the use of a working solution with the addition of two EM preparations, which contain a symbiosis of antagonistic bacteria: LAB (*Lactococcus lactis*, *Lactobacillus casei*), *Bacillus subtilis*, *Paenibacillus polymyxa*, no enterobacteria were found in samples 1-7. In sample No. 8 (control, without the addition of EM preparations), the presence of *E. Coli* with a titer of 150 CFU/ml was noted. Unfortunately, the presence of white and green mold was noted in sample No. 8, to which EM preparations were not added (Figs. 4, 5).

a) b)

Fig. 4. Light photo: a) colonies of white mold on carrot waste; b) gray and fusarium fungal disease colonies on beet waste



a) b)

Fig. 5. Light photo: a) aerial mycelium of the causative agent of white fungal disease of carrots on a nutrient medium
b) aerial mycelium of the causative agent of gray and fusarium disease on a nutrient

In the course of the experiment, it was found that white mold got into the vegetable mixture together with illiquid carrots, and gray and fusarium rot - together with beet waste. Potato waste was not affected by pathogens of root crops.

In this study, it was proved that the causative agents of fungal diseases of carrots and beets (*Fusarium Link*, *Sclerotinia sclerotiorum*, *Botrytis cinerea*) are inhibited by the symbiosis of LAB, *Bacillus subtilis*, *Paenibacillus polymyxa*, which are presented in EM preparations, since these representatives have antimicrobial and antifungal effects.

The yeast *Saccharomyces cerevisiae* was detected in Saburo's medium in small quantities (from 2×10^2 to 3×10^3 cells/l), which was isolated from the culture liquid of experimental samples (1-8).

Table 1 shows the number of viable cells of LAB and *Paenibacillus polymyxa*, which were found in the test samples (1-8).

Table 1

The amount of LAB and *Paenibacillus polymyxa* in digestate samples

The number of LAB and *Paenibacillus polymyxa* in samples 1-8	Experiment duration, day					
	2	4	6	10	20	30
	Titer of viable cells, cells/ml					
LAB:						
1	$3,2 \times 10^7$	$1,1 \times 10^7$	$7,1 \times 10^6$	$3,1 \times 10^6$	$8,1 \times 10^5$	$2,9 \times 10^5$
2	$2,4 \times 10^7$	$8,0 \times 10^6$	$4,0 \times 10^6$	$1,8 \times 10^6$	$7,9 \times 10^5$	$1,2 \times 10^5$
3	$1,2 \times 10^7$	$5,6 \times 10^6$	$2,1 \times 10^6$	$7,7 \times 10^5$	$1,7 \times 10^5$	$8,0 \times 10^4$
4	$2,7 \times 10^7$	$7,7 \times 10^6$	$5,2 \times 10^6$	$2,5 \times 10^6$	$6,4 \times 10^5$	$2,1 \times 10^5$
5	$3,0 \times 10^7$	$9,0 \times 10^6$	$6,8 \times 10^6$	$3,3 \times 10^6$	$7,0 \times 10^5$	$3,0 \times 10^5$
6	$5,8 \times 10^7$	$5,5 \times 10^7$	$4,5 \times 10^7$	$1,5 \times 10^7$	$3,5 \times 10^6$	$1,5 \times 10^6$
7	$7,0 \times 10^7$	$6,8 \times 10^7$	$5,9 \times 10^7$	$2,4 \times 10^7$	$5,0 \times 10^6$	$3,0 \times 10^6$
8	$5,0 \times 10^5$	$2,3 \times 10^5$	$2,0 \times 10^5$	$5,0 \times 10^4$	$1,9 \times 10^4$	$7,9 \times 10^3$
Paenibacillus polymyxa:						
1	$7,5 \times 10^5$	$5,5 \times 10^7$	$6,0 \times 10^7$	$3,5 \times 10^6$	$2,0 \times 10^6$	$8,9 \times 10^5$
2	$4,9 \times 10^5$	$4,9 \times 10^7$	$5,8 \times 10^7$	$3,4 \times 10^6$	$1,8 \times 10^6$	$7,5 \times 10^5$
3	$7,8 \times 10^5$	$6,2 \times 10^7$	$6,3 \times 10^7$	$4,0 \times 10^6$	$2,8 \times 10^6$	$8,8 \times 10^5$
4	$5,0 \times 10^5$	$9,3 \times 10^6$	$1,0 \times 10^7$	$6,5 \times 10^6$	$8,5 \times 10^5$	$2,1 \times 10^5$
5	$6,5 \times 10^5$	$1,5 \times 10^7$	$2,3 \times 10^7$	$8,5 \times 10^6$	$1,5 \times 10^6$	$6,9 \times 10^6$
6	$8,3 \times 10^5$	$6,8 \times 10^7$	$7,2 \times 10^7$	$2,5 \times 10^7$	$2,6 \times 10^6$	$9,5 \times 10^5$
7	$9,5 \times 10^5$	$7,7 \times 10^7$	$8,0 \times 10^7$	$4,3 \times 10^7$	$3,1 \times 10^6$	$1,0 \times 10^6$
8	not found	not found	not found	not found	not found	not found

The results presented in Table 1 show that in samples 1, 6, 7, to which additional substrates were added, an increase in the number of studied microorganisms was recorded. On the 2nd day, in samples 1-7, an increase in the titer of LAB, which participates in the hydrolysis process, is noted. After 4 days of fermentation, there is a decrease in the presence of LAB in all samples, which corresponds to the generally accepted provisions on the process of bioconversion of vegetable waste, since the stage of acetogenesis has begun. At the stage of hydrolysis, complex biopolymeric compounds (proteins, carbohydrates, cellulose, etc.) are biotransformed into simpler oligo- and monomers (amino acids, sugars, fatty acids, and water). At this stage, organic substances are fermented by extracellular enzymes of microorganisms (amylase, lipase, protease), as well as the transfer of dry substances from an insoluble state to a soluble state occurs.

In general, hydrolysis occurs very slowly, as it depends on exoenzymes (extracellular enzymes), which, attaching to the cell wall of bacteria, turn complex organic substances into small water-soluble molecules. Therefore, sometimes this process can limit the entire process of biohydrogen formation, since the next stages cannot begin without the end of hydrolysis. The process when the formed monomers are converted by fermenting microorganisms (lactic acid bacteria) into a number of simple compounds: fats, acids, alcohols, lactic acid, methanol, CO_2, H_2, NH_3 and H_2S. At this stage, anaerobic microorganisms utilize the remaining oxygen, creating conditions for the next two stages. At a pH close to neutral, unstable fatty acids (acetic, propionic, butyric, etc.), low-molecular alcohol and gases are synthesized, after which a sharp decrease in pH occurs, i.e., an oxidation process occurs. At the end of the second to sixth day, inclusive, the acetogenic stage of waste bioconversion takes place, namely, the process of converting previously formed products into acetate and a large amount of hydrogen. It is this stage that is key and final in the production of biohydrogen. The formation of a large amount of acetic acid, carbon and CO_2 is recorded. No *Paenibacillus polymyxa* was detected in the control sample No. 8, because we did not add the working solution.

The technology of biotransformation of vegetable waste proceeded according to the following stages: preparation and grinding of waste, the main fermentation process, separation of the liquid fraction from the solid fraction, storage of the liquid fraction of the digestate at a temperature of 4 ^{o}C without access to light. It is recommended to distribute the solid fraction (sediment) immediately to the fields.

Conclusions. An urgent problem today, both in Ukraine and in the EU countries, is the issue of accumulation and processing of organic waste, in particular, vegetable waste. Compressed under tons of garbage and soil, waste without the presence of specific microorganisms does not decompose. That is why, unfortunately, vegetable waste that is not properly prepared does not become compost by itself, therefore the processes of rotting lead to the deterioration of the sanitary condition of the soil and the pollution of groundwater. The process of bioconversion of vegetable waste (beetroot, carrot and potato) by a specific consortium of EM-preparations microorganisms - "Baikal-EM 1" and "Organic-balance" is proposed. The photocalorimetric method confirmed the sufficient content of biogenic elements in the liquid fraction of the digestate, which allows it to be recommended for use for soil fertilization. The novelty of the study consists in the use as additional carbon sources of expired bread in the amount of 10% and lactulose in the amount of 2 and 4% relative to the mass of waste. As a result of biotransformation of vegetable waste, digestate and biohydrogen were obtained in 6 days of fermentation. It has been proven that the quality of the digestate remains at a sufficiently high level in terms of microbiological and physicochemical indicators at a temperature of 4 ^{0}C for 30 days, provided that the solid fraction is separated from the liquid immediately after the fermentation process is finished. Soils usually lack many types of beneficial microorganisms for their recovery. Experiments have proven that the obtained digestate does not contain pathogenic cultures, on the contrary, it contains useful soil microorganisms, which are classified as nitrogen fixers and antagonists in relation to pathogenic cultures, especially molds.

The proposed method of decomposition of organic waste can be used not only in the conditions of small households, but also on large agricultural farms, which are engaged in the cultivation of vegetable crops, their storage throughout the year and conservation. At these agricultural enterprises, great attention is paid to the liquidity of products for further sale. Unfortunately, vegetable losses account for 30-40% of the harvest.. Therefore, the implementation of this method of bioconversion of vegetable waste is also relevant for large volumes of waste. An example of the European experience of using this practice of processing vegetable waste is the agricultural farm of Janis Winter (Latvia). On the farm, up to 75 tons of crushed organic vegetable waste is loaded into the bioreactor in one fermentation cycle. Non-liquid vegetables (carrots, beets, potatoes and other agricultural waste) or vegetables with signs of rotting are used as raw materials on this farm. Waste in the form of expired bakery products is used exclusively to intensify the fermentation process, that is, as an additional substrate. According to Directive 2008/98/EC, this farm implements a full technological waste-free process of growing and processing vegetable crops: growing vegetables in the fields and in greenhouses, their harvesting, preservation and storage throughout the year, processing of generated waste into biogas and biofertilizer and wastewater treatment, which together with the digestate are fed to the fields (Fig. 6).

a ***б*** ***в***

Fig. 6. An example of digestate (biofertilizer) storage in lagoons near agricultural fields: *a* – digestate storage lagoon; *b* – redistribution of digestate through pipelines; *c* – collection of the solid fraction of the digestate

The digestate formed after fermentation is separated into liquid and solid fractions. The liquid fraction contains 3% of the dry mass, it is stored in lagoons (Fig. 6), and thanks to the pipelines, it is distributed among nearby fields (within a radius of 20 km), whereas the solid fraction is sent to fields far from the farm. In the cold season (from November 15 to March 15), the lagoons must be completely emptied. The formed digestate (Fig. 6) is used as a biofertilizer in fields intended for growing vegetables. Today, more than 90% of Latvian biogas cogeneration plants, which enable households and farmer-entrepreneurs to more efficiently use agricultural by-products (manure, straw, vegetable waste and food waste), enable to obtain electrical energy, thanks to support and motivation from the leadership of Latvia and EU countries. As of today, 52 cogeneration stations are operating in Latvia, the capacity of which is about 0.83 MW with the production of electric energy of 6600 MW h/year.

Thanks to cogeneration biogas plants, the obtained biogas can be turned into electricity. According to the information provided by the management of the Janis Winter's "Līgo" farm, it is possible to obtain 1 MW of electricity from 450 m^3 of biogas, which contains 52% biomethane. This farm uses electricity for its own needs (heating greenhouses, lighting, hot water), and the surplus is sold.

Acknowledgements. The study was carried out within the framework of the implementation of the project of the EU program Erasmus+Jean Monnet "Clean Energy Technologies and Energy Efficiency: the EU Experience" (101047602 — EnergyC — ERASMUS-JMO-2021-HEI-TCH-RSCH) and exchange of experience (on the example of Janis Winter's "Līgo" farm in Latvia).

Co-funded by
the European Union

Funded by the European Union. Views and opinions expressed are however those of the author(s) only and do not necessarily reflect those of the European Union or European Education and Culture Executive Agency. Neither the European Union nor the granting authority can be held responsible for them.

References:

Caldeira, C., De Laurentiis, V., Corrado, S., van Holsteijn, F., Sala, S. (2019a). Quantification of food waste per product group along the food supply chain in the European Union: a Mass Flows Analysis. Resources, Conservation & Recycling. 149:479-488. Available at: https://doi.org/10.1016/j.resconrec.2019.06.011.

Caldeira, C., De Laurentiis, V., Sala, S. (2019b). Assessment of food waste prevention actions: development of an evaluation framework to assess the performance of food waste prevention actions, EUR 29901 EN; Luxembourg (Luxembourg): Publications Office of the European Union; 2019, ISBN 978-92-76-12388-0. Available at: https://doi:10.2760/9773.

Caldeira, C., Vlysidis, A., Fiore, G., De Laurentiis, V., Vignali, G., Sala, S. (2020). Sustainability of food waste biorefinery: a review on valorisation pathways, techno-economic constraints, and environmental assessment. *Bioresource Technology,* 312: 123-175. Available at: https://doi.org/10.1016/j.biortech.2020.123575.

Champions 12.3. 2019. SDG target 12.3 on food loss and waste: 2019 progress report. Available at: https://champions123.org/2019-progress-report/. Accessed: 07 April 2020.

Commission Communication COM/2018/673. A sustainable Bioeconomy for Europe: Strengthening the connection between economy, society and the environment. Available at: https://eurlex.europa.eu/legal-content/en/ALL/?uri=CELEX%3A52018DC0673. Accessed: 14 July 2020.

Commission Delegated Decision (EU) of 3.5.2019 supplementing Directive 2008/98/EC of the European Parliament and of the Council as regards a common methodology and minimum quality requirements for the uniform measurement of levels of food waste. C(2019)3211. Available at: https://eurlex.europa.eu/legal-content/en/TXT/?uri=CELEX:32019D1597. Accessed: 23 April 2020. EC, 2020.

Commission Notice — Guidelines for the Feed Use of Food No Longer Intended for Human Consumption (2018/C133/02). Available at: https://circulareconomy.europa.eu/platform/sites/default/files/feed_guidelines.pdf.

Corrado, S., Ardente, F., Sala, S., Saouter, E. (2017). Modelling of food loss within life cycle assessment: From current practice towards a systematisation. *Journal of Cleaner Production*. 140:847-859. Available at: https://doi.org/10.1016/j.jclepro.2016.06.050.

De Laurentiis, V., Caldeira, C., Sala, S. (2020). No time to waste: assessing the performance of food waste prevention actions. *Resources, Conservation & Recycling*. 161: 104946. Available at: https:///doi.org/10.1016/j.resconrec.2020.104946. EC, 2018.

Dincer I. (2012). Green methods for hydrogen production. *Hydrogen Energy,* 2, 1954-1971.

Directive 2008/98/EC of the European Parliament and of the Council of 19 November 2008 on waste and repealing certain Directives. Available at: https://eur-lex.europa.eu/legalcontent/EN/TXT/?uri=celex%3A32008L0098. Accessed: 23 April 2020.

Directive 2009/28/EC of the European Parliament and ofthe Council of 23 April 2009 on the promotion of the use of energy from renewable sources (2009). *Official Journal of the European Union*, 140 (52), 16–62. Available at: https://eurlex.europa.eu/legal-content/EN/TXT/PDF

EU (2018). Directive (EU) 2018/851 of the European Parliament and of the Council of 30 May amending Directive 2008/98/EC on waste. Available at: https://eur-lex.europa.eu/legalcontent/EN/TXT/?uri=celex:32018L0851. Accessed: 23 April 2020.

European Commission (2022). Proposal for a Regulation of the European Parliament and of the Council on packaging and packaging waste, amending Regulation (EU) 2019/1020 and Directive (EU) 2019/904, and repealing Directive 94/62/EC. Available at https://eur-lex.europa.eu/legal-content/EN/TXT/?uri=CELEX%3A52022PC0677

European Commission, Directorate-General for Communication (2020a). Circular economy action plan: for a cleaner and more competitive Europe, Publications Office of the European Union. Available at https://data.europa.eu/doi/10.2779/05068

European Commission, Directorate-General for Environment (2023b). Waste Early Warning Reports 2023 - country specific factsheets. Available at https://environment.ec.europa.eu/publications/waste-early-warning-reports-2023-country-specific-factsheets_en

European Commission, Directorate-General for Environment (2023c). Sweden, 2025 EU waste recycling targets: state of play, Publications Office of the European Union. Available at https://data.europa.eu/doi/10.2779/859809

European Commission, Directorate-General for Environment (2023d). Österreich, EU-Abfallrecyclingziele für 2025: Aktueller Stand, Publications Office of the European Union. Available at https://data.europa.eu/doi/10.2779/031453

European Commission, Directorate-General for Environment (2023e). Ireland, 2025 EU waste recycling targets: state of play, Publications Office of the European Union. Available at https://data.europa.eu/doi/10.2779/378751

European Commission, Directorate-General for Environment (2023f). Finland, 2025 EU waste recycling targets: state of play, Publications Office of the European Union. Available at https://data.europa).

European communication COM/2020/28. A new Circular Economy Action Plan For a cleaner and more competitive Europe. Available at: https://eur-lex.europa.eu/legalcontent/EN/TXT/?uri=COM%3A2020%3A98%3AFIN. Accessed: 14 July 2020. EC, 2020c.

European communication COM/2020/380. EU Biodiversity Strategy for 2030. Bringing nature back into our lives. Available at: https://eur-lex.europa.eu/legalcontent/EN/TXT/?qid=1590574123338&uri=CELEX:52020DC0380. Accessed: 14 July 2020. EU, 2008.

European communication COM/2020/381. A Farm to Fork Strategy for a fair, healthy and environmentally-friendly food system. Available at: https://eur-lex.europa.eu/legalcontent/EN/TXT/?uri=CELEX:52020DC0381. Accessed: 28 June 2020. EC, 2020b.

European Council conclusions, 23–24 June 2022. Available at: https://www.consilium.europa.eu/en/press/press-releases/

FLW Protocol, 2016. Food Loss and Waste Accounting and Reporting Standard. FLW Protocol, Washington DC. Available at: https://flwprotocol.org/flw-standard/. Accessed: 23 April 2020.

Geletuha, G.G. and Zhelyezna, T.A. (2014). The perspectives of the using of agricultural waste for energy production in Ukraine. *Analytic note of Bioenergy Association of Ukraine*, 7. Available at: https://www.uabio.org/img/files/docs/position-paper-uabio-7-ua.pdf.

Ghinea, C, Leahu, A.(2020). Monitoring of Fruit and Vegetable Waste Composting Process: Relationship between Microorganisms and Physico-Chemical Parameters. *Processes*. 8(3):302. Available at: https://doi.org/10.3390/pr8030302

Jarunglumlert, T. (2018). Scaling-up bio-hydrogen production from food waste: Feasibilities and challenges. *International journal of hydrogen energy*, 43, 634-648.

Kandari, V., Gupta, S. (2012). Bioconversion of vegetable and fruit peel wastes inviable product. " *Journal of Microbiology and biotechnology research*, 2, 308-312.

Karakan, T., Tuohy, K., Solingen, G. (2021). Low-Dose Lactulose as a Prebiotic for Improved Gut Health and Enhanced Mineral Absorption. Front. Nutr., 8, 672-925. Available at: https://doi: 10.3389/fnut.2021.672925.

Kudri S. O. (2020). Vidnovliuvani dzherela enerhii: monohrafiia. *Instytut vidnovliuvalnoi enerhetyky NANU*, 392.

Levin, D. (2014). Biohydrogen production: prospects and limitations to practical application. *International journal of hydrogen energy Energy*, 29, 173–185.

Makovetska, Y. (2015). Analiz osoblyvostei utvorennia ta povodzhennia z vidkhodamy na silskykh terytoriiakh. *Efektyvna ekonomika*, 12. Available at: http://www.economy.nayka.com.ua/?op=1&z=4684.

Manfredi, S., [et al.]. (2015). Improving Sustainability and Circularity of European Food Waste Management with a Life Cycle Approach; EUR 27657 EN. Available at: https://doi:10.2788/182997.

Mishra, M., Chauhan, S., Velramar, B. [et al..] (2021). Facile bioconversion of vegetable food waste into valuable organic acids and green fuels using synthetic microbial consortium. *Korean J. Chem. Eng.*, 38, 833–842. Available at: https://doi.org/10.1007/s11814-020-0735-7.

Oktiawan Wiharyanto, Hadiwidodo Mochtar, Bagus Priyambada Ika and Purwono Purwono. (2018). Decomposition of food waste using bulking agent and bio-drying technology. *E3S Web of Conferences.* Available at: https://www.e3s-conferences.org/articles/e3sconf/pdf/2018/48/e3sconf_icenis18_05013.pdf)

Perelik metodyk vykonannia vymiriuvan (vyznachen) skladu ta vlastyvostei prob obiektiv dovkillia, vykydiv, vidkhodiv i skydiv, tymchasovo dopushchenykh do vykorystannia Minpryrody. (2007). *Ministerstvo okhorony navkolyshnoho pryrodnoho seredovyshcha Ukrainy*. 175.

Prokaieva A. (2021). Suchasne keruvannia vidkhodamy vidpovidno do pryntsypiv tsyrkuliarnoi ekonomiky. *Navchalnyi posibnyk kursu ZWA deep level*, 140. URL: https://zerowastekharkiv.org.ua/wpcontent/uploads/2021/12/posybnic-lekciyebook-5.pdf.

Ratushniak, H. S., Koshcheiev, I. A. (2011). Intensyfikatsiia biokonversii shliakhom vykorystannia vidnovliuvalnykh dzherel enerhii. *Suchasni tekhnolohii, materialy i konstruktsii v budivnytstvi,* 11(2), 157–160.

Reshmy, R. [et al.]. (2021). Potential Utilisation of Fruit and Vegetable Waste: An Overview. Sustainable Bioconversion of Waste to Value Added Products. *Advances in Science, Technology & Innovation. Springer, Cham.* Available at: https://doi.org/10.1007/978-3-030-61837-7_11.

Wang A.-J. *et al.* (2012). Biohydrogen production from anaerobic fermentation. *Biochem. Engin./Biotechnol,* 128, 143–163.