Ontological Tools in Anaerobic Fermentation Technologies:
Bioinformation Database Applications

Shulipa Ye. O.[1][0000-0002-4078-4124], Chernysh Ye. Yu.[1*][0000-0003-4103-4306], Plyatsuk L. D.[1][0000-0003-0095-5846], Fukui M.[2][0000-0002-3563-1262]

[1] Sumy State University, 2 Rymskogo-Korsakova St., 40007 Sumy, Ukraine;
[2] Institute of Low Temperature Science, Hokkaido University, 8 Chome Kita St., 060-0819 Hokkaido, Japan

Abstract. An important direction of forming an effective system for recycling waste of various genesis is to optimize the processes of their treatment using the latest information resources. The paper deals with theoretical studies of directions for the systematization and optimization of anaerobic waste processing technologies using ontological tools based on information resources. Significant scientific support to biochemical research is provided by electronic bioinformatics databases such as KEGG, BacDive, and EAWAG-BBD, etc., which provide access to a collection of graphical representations and text descriptions of metabolic or signal pathways, schemes of regulation of biological processes, information about the organism. They cover various aspects of bacterial and archaic biodiversity, information on microbial biocatalytic reactions and biodegradation pathways of mainly xenobiotic chemical compounds. Also, in the course of work based on analytical data of electronic databases of bioinformatics, in particular, the interaction of necessary ecological and trophic groups of microorganisms, biochemical simulation of anaerobic waste processing with biofuel production was carried out.

Keywords: waste disposal, bioinformatics database, anaerobic fermentation, biofuel.

1 Introduction

An important direction of the formation of an effective system of waste disposal of different genesis is to optimize the processes of their recycling using the latest information resources, domestic and foreign electronic databases.

As indicated in [1], the ontological system of perceptions of prior information of scientific research in the field of biogas production is formed based on a structured view of microbiological aspects, aspects of biogas production, previous scientific studies.

Conversion of organic wastes using anaerobic technologies has significant prospects for the application of ontological instruments, which will allow determining the directions of further optimization of their utilization processes in environmental technologies.

2 Literature Review

In Ukraine, the volume of organic waste is about 40 % of the total amount of household waste. Given that 92 % of household waste goes to landfills, which leads to bacteriological contamination of water, soils, the formation of gases that have an unpleasant odor and enhance the greenhouse effect, are explosive and fire hazardous. They also create a health hazard and are a source of infectious disease. Besides, other types of organic waste from industry and agro-complex are also required.

Today, biological, chemical, and physical methods are used to dispose of organic waste in the world. In this regard, we will look at the directions of systematization of bioenergy methods of organic waste processing.

The efficiency of its use, including in waste fermentation technologies, depends on the optimal definition and dynamics of the formation of hierarchies of the interaction of components of the operating environment of information-analytical systems (IAS). Therefore, it is essential to have specific tools that can be used to effectively design and implement mechanisms for managing a hierarchy that reflects the interaction of all IAS components.

The ontology (O) of some operating environment in the general case is formally represented by an ordered three [2]:

$$O = < X, R, F >,$$

where *X* is the concepts (terms) of the subject area (SA), based on which the subject component of the IAS operating environment is formed; *R* – relations and properties between them (we will assume that properties are the interpretation of relations, that is, there is a transformation that establishes a relation for each relation of a certain property); *F* – the interpretation and definition functions of *X* and/or *R* that make up the functional part of the IAS operating environment.

The novelty and distinctive feature of the ontology-based approach to creating an ontology interface, compared to existing ontology-based WEB-Semantic tools, is that automation methods are based on the knowledge that is always in motion. Therefore, through the use of SA ontologies, the knowledge gained in the process of interaction is expanded without changing the source code of the tool, which, besides, improves the quality of the interface by taking into account a set of ergonomic and technological factors [3].

The semantic characteristics of scientific studies on the production of biogas from chicken manure have been highlighted in [1] for the construction of the ranking system of previous studies. Such semantic characteristics include temperature, reactor volume, content of chicken manure, moisture content, content of activated sludge, final solids content, biogas and methane production, methane content, year, ammonium nitrogen content, volatile fatty acids (VFA), pH final, pH initial, pH is minimum and pH is maximum.

Due to the exacerbation of environmental problems associated with environmental pollution, the problem of the disposal of organic industrial, household and agricultural waste, i.e. the use of secondary material or energy resources, is quite urgent.

The graph (Figure 1), which was built based on the Scopus database, shows a gradual increase in publications whose main areas of research are anaerobic fermentation of waste.
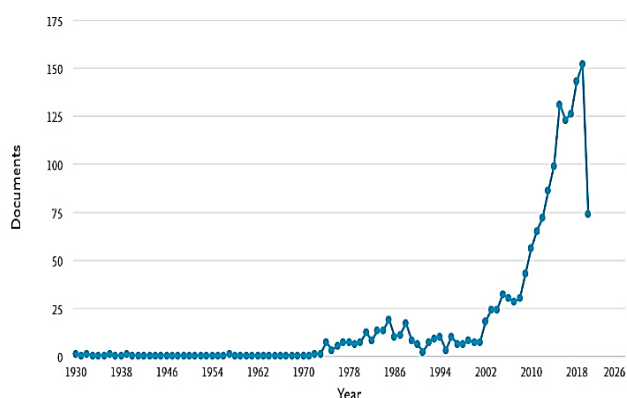

Figure 1 – Different type of publications by year according to keywords "anaerobic fermentation" and "waste"

Since biological methods of organic waste processing are recognized as cost-effective and environmentally sound, the degree of their study must also increase.

Anaerobic fermentation can be waste from the agricultural industry containing 22–45 % of the cellulose associated with hemicellulose (11–44 %) and lignin (11–28 %).

In [4], a mixture of corn waste and sunflower was used as the substrate. To increase the rate of hydrogen formation and the availability of nutrients, the waste was pre-ground to a size of 3-5 mm and treated with alkali solution, which allowed the process of biomass delignification and significantly increased hydrogen yield.

In research [5] the use of anaerobic fermentation processes in the development of a method of utilization of by-products of the fractionation of the vegetative mass of legumes by bioconversion, which allows obtaining feed for farm animals. It has been found that in anaerobic fermentation, the oxidation processes of unsaturated fatty acids and phenols are less pronounced than when using other preservative agents. In the process of fermentation of plant biomass, such undesirable components as the trypsin inhibitor, saponins, chlorophyll decay products are inactivated. This has a positive effect on the quality and taste of the resulting product.

In work [6], the fermentation of components of municipal solid waste (MSW), which are not allowed to be mixed with the total flow and are the raw material for biochemical disposal, was investigated. It is 100 % of food, garden and park waste and wood, as well as substandard waste paper (15 %). The method of biochemical utilization is proposed, in which the organic part of MSW is subjected to sequential treatment: anaerobic fermentation to produce biogas and solid product (1st stage), and the resulting solid product is subjected to aerobic composting (2nd stage). The results show the perspective of the proposed method, as the obtained marketable products allow to return of carbon and biogenic elements with MSW to the environment and include them in the natural cycle.

The paper focused on theoretical research on the directions of systematization and optimization of anaerobic waste processing technologies with the involvement of ontological tools based on information resources. To achieve the aim, the following tasks were set:
- research of directions of application of electronic bioinformation databases for anaerobic fermentation technologies;
- biochemical modeling in the ontological system of anaerobic fermentation.

3 Research Methodology

3.1 The use of bioinformation electronic database KEGG database

In this work, KEGG databases, BacDive, and EAWAG-BBD were used to study biochemical transformations and model anerobic fermentation of organic waste.

KEGG PATHWAY is one of the largest and best-known bases for gene networks, metabolic and signaling pathways. In 2017, the KEGG PATHWAY was one of the largest and best-known bases on genetic networks,

metabolic and signal pathways. KEGG PATHWAY contained manually created by experts graphical representations and text descriptions of 496 metabolic or signaling pathways, schemes of regulation of biological processes, diseases, classifications of medicinal substances. Each diagram presents generalized data on many types of organisms. A particular option of the base interface allows you to configure the diagram for a specific organism type, and the number of species depends on how the universal biological process is reflected in the diagram.

Information about each KEGG PATHWAY database diagram can be downloaded as a graphical and text description (including a list of gene identifiers). There is a KEGG REST API software interface, which allows you to generate and execute queries for all information contained in the KEGG database.

KEGG REST API is a base of chemical reactions, mainly enzymatic, containing all reactions that appear in KEGG metabolism maps and additional reactions that appear only in the enzyme nomenclature. Each reaction is identified by its number R, e.g. R01143 for oxygen oxidoreductase (hydroxylation) (Figure 2).

Figure 2 – Reaction search results by keyword "methane"

Following the help of the diagrams is possible. Accordingly, the system was searched and methane metabolism pathways identified in the anaerobic fermentation process, as shown in Figure 3.

Figure 3 – Search for dominant methanogen species on an interactive map of metabolic methane production

Methane is generated by biologically methanogenic bacteria, which are the main link in the Archean Kingdom, mainly through the consumption of acetates and the reduction of carbon dioxide content. The latter pathway contains new coenzymes and biochemical reactions that have not previously been observed in other organisms.

Methanogenesis is part of the C1 metabolic cycle.

The text map of methanogenesis pathways is presented below (Figure 4). An organism that can initiate this pathway is given, but other organisms can also perform later stages. For more information on compounds or reactions, please follow the appropriate link in the interactive map. This map is also available in a graphical (40k) format.
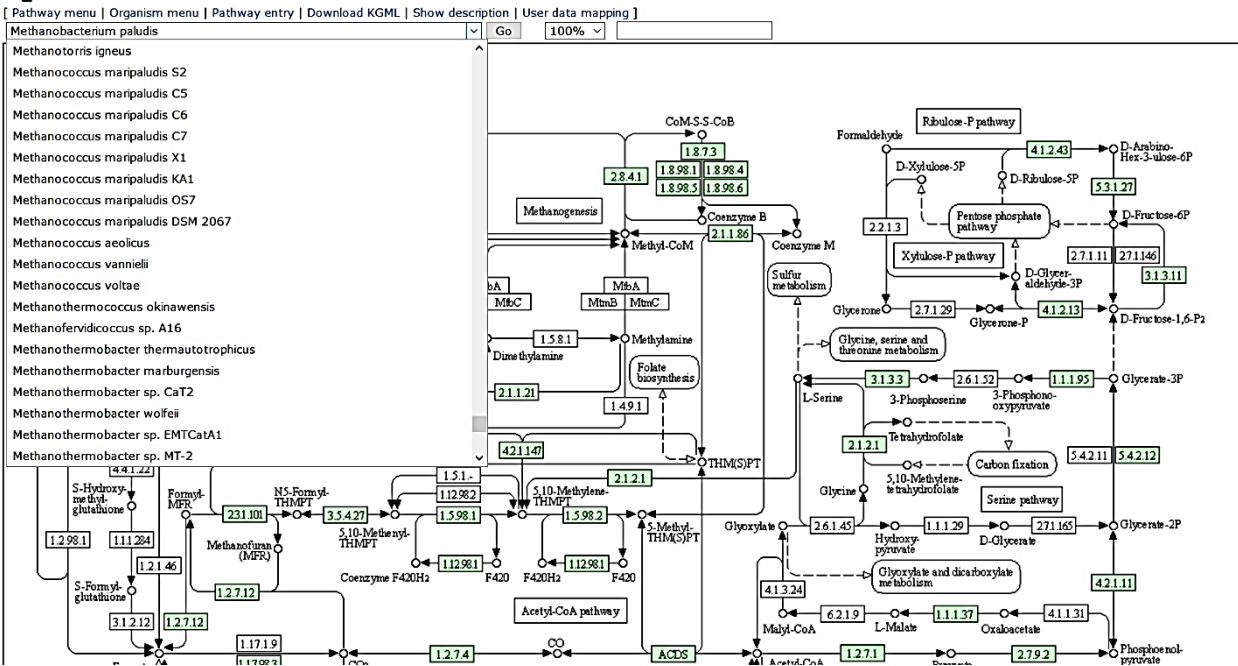
Figure 4. Display of required genera and bacterial species that are involved in the process of methanogenesis and carbon cycle in a single community

Methane, as a biogenic gas, is produced biologically from carbon dioxide by a series of 2-electron reduction in a process known as methanogenesis. It is estimated that bacteria, or methanogens, each year around the world produce 10^{15} grams of methane. In some environments, such as in lakes, it has been observed that 90 % of biogenic methane is oxidized before it enters the atmosphere. In aerobic environments, for example, in the upper lakes, methane is biologically oxidized as a result of a series of 2-electron oxidation reactions, commonly known as "methanotrophic metabolism" or "methanotrophs". The ecological link between methanogenesis and methanotrophy leads to a large turnover of C_1 compounds in the global cycle (C_1 Metabolic Cycle). C_1 fragments from many sources are metabolized by disparate bacteria and eventually enter the global C_1 cycle.

3.2   The use of the BacDive bioinformatics database

This database is a collection of body-related information covering various aspects of bacterial and archaeal biodiversity. According to species involved in different stages of anaerobic digestion, one can find the necessary information regarding their optimal cultivation conditions in technological processes (Figure 5).
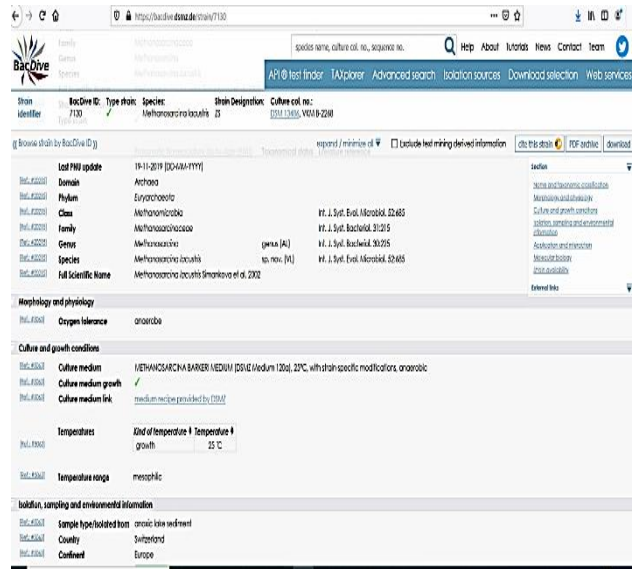

Figure 5. Search results for "Methanosarcina lacustris"

Nutrient media with a variation of the trace element composition were also analyzed. All this can be done in the BacDive database (Figure 6).

Microorganisms   **DSMZ**

120a. METHANOSARCINA BARKERI MEDIUM

K_2HPO_4	0.35	g
KH_2PO_4	0.23	g
NH_4Cl	0.50	g
$MgSO_4 \times 7\ H_2O$	0.50	g
$CaCl_2 \times 2\ H_2O$	0.25	g
NaCl	2.25	g
$FeSO_4 \times 7\ H_2O$ solution (0.1% w/v in 0.1 N H_2SO_4)	2.00	ml
Trace element solution SL-10 (see medium 320)	1.00	ml
Yeast extract (OXOID)	2.00	g
Casitone (BD BBL)	2.00	g
Na-resazurin solution (0.1% w/v)	0.50	ml
$NaHCO_3$	0.85	g
Vitamin solution (see medium 141)	10.00	ml
Methanol	10.00	ml
L-Cysteine-HCl x H_2O	0.30	g
$Na_2S \times 9\ H_2O$	0.30	g
Distilled water	1000.00	ml

Figure 6 – An example of a culture medium
for methanogen cultivation

Through the Web Services Portal, you can access not only BacDive, but also a list of taxa directories. Also determine inoculum selection sites and analyze their adaptive ability to change environmental conditions (Figure 7).

Figure 7 – Detail of information on the origin of the strain

3.3 The use of the EAWAG-BBD database

The reactions under consideration are studied with a view to a basic understanding of nature, biocatalysis leading to chemical production, and biological degradation of environmental pollutants. Individual reactions and metabolic pathways are represented by information on the source and intermediate chemical compounds, organisms that convert compounds, enzymes and genes [7]. Using maps can help with maps. Figure 8 presents a map of methanogenesis.
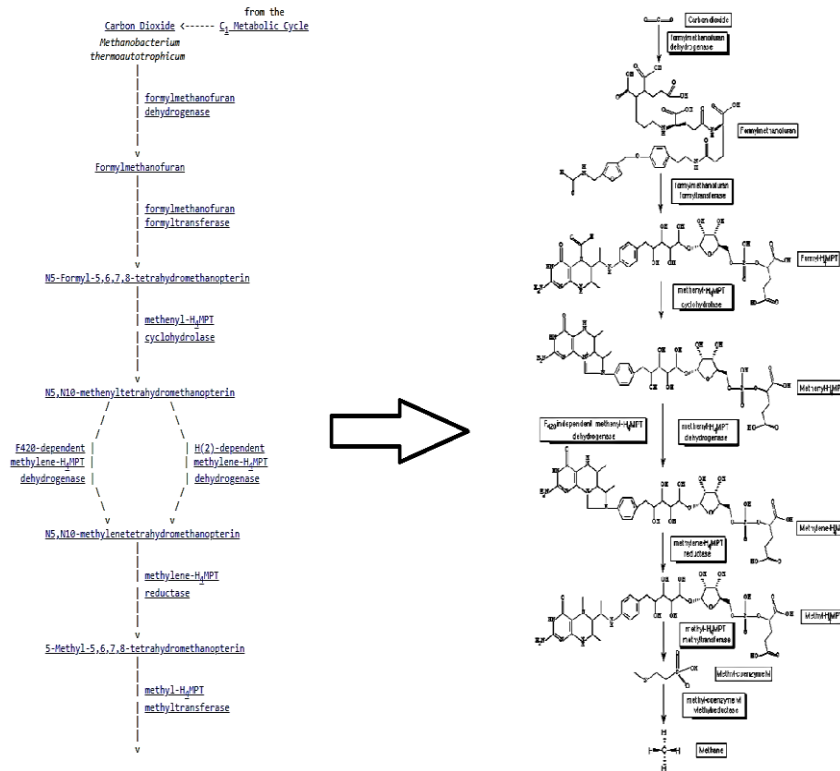

Figure 8 – Part of the methanogenesis path map

All maps are interactive, and each substance involved in the metabolism of bacteria can be considered individually to find optimal ways of its production, etc.

4 Results

In our view, it is essential to analyze the interactions in the anaerobic association of microorganisms, which will further optimize the technological process of bioconversion of organic waste with the production of useful bio-based products. Accordingly, based on the analytical data from the bioinformatics databases discussed in Section 2 and previous research, we can determine the interactions of the required ecological-trophic groups of microorganisms.

It is essential to understand the effects of mineral compounds, in particular, the ferrum contained in many industrial organic wastes, including sewage sludge and sludge in municipal wastewater treatment plants (Figure 9).
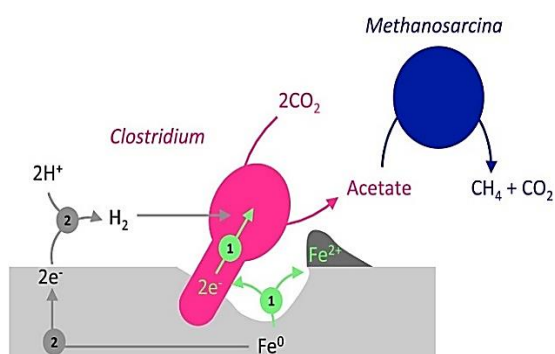

Figure 9. Estimated commensual interaction between Fe^0-chordinator *Clostridium*-acetogen and acetate-utilized *Methanosarcina*-methanogen: 1 – a direct mechanism of electron absorption; 2 – an absorption mechanism based on abiotic H_2 [7]

Thus, in the study [8] direct absorption of electron from Fe^0 for *Methanobacterium* strain IM1, because:

1. It generates more methane from Fe^0 than H_2 using *Methanococcus maripaludis* with a low H_2 uptake threshold.

2. It produces methane using only a cathode with a voltage of 400 mW in conditions.

It is speculated that *Methanosarcina* could receive electrons directly from Fe^0. The electron acceptor can be extracellular (such as electrodes or cells), after which *Geobacter* uses its extracellular electron transfer mechanisms. Only recently, through a comparative transcriptomic approach, probable scenarios for direct electron uptake in *Methanosarcina* have been substantiated [9]. In this study, *Methanosarcina*, grown by direct electron uptake rather than H_2, individually regulated several redox-active cell surface proteins. However, the exact role of these cell surface proteins in the direct absorption of electrons by methanosarcin has not yet been characterized.

The second strategy for methanogens on electron regeneration from Fe^0 is described - the use of extracellular enzymes for efficient electron capture. For efficient electron recovery enzymes such as hydrogenase, forming a dehydrogenase or supercomplex heterodisulfide reductase use enzymes derived from Fe^0, to obtain H_2 or its formation. If the extracellular enzyme-dependent strategy is to be useful in eco-corrosion communities, it remains to be explored further. This is especially true for dark fermentation with the production of biohydrogen and organic acids.

Dark fermentation produces relatively low-yield hydrogen (maximum $4H_2$ per glucose) with the accumulation of metabolites such as VFA. On the other hand, the dark fermentation process is still attractive because of the very high performance and simplicity of the reactor structure. In this regard, hybrid systems that add a second process of purification of dark fermentation effluents are of interest to many studies. With the addition of the second process, hybrid systems can have a potentially attractive high hydrogen yield. There are several possible combinations of hybrid systems [10]: (1) dark fermentation + photo fermentation. VFAs derived from dark fermentation are ideal substrates for photo-fermentation; (2) dark fermentation + microbial cell electrolysis; (3) dark fermentation plus a cell-free enzyme system. All three of these combinations have the potential to achieve maximum yield of $12H_2$ for glucose; and (4) dark fermentation + biogas plant. VFA in dark fermentation effluents can be substrates for methanogens. As a result of this process, a mixture of hydrogen and methane can be formed.

Many bacteria can produce hydrogen as a result of dark fermentation using organic compounds:

$$[CH_2O]_n \rightarrow CO_2 + H_2.$$
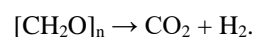
Thus, in general, the stages of the dark fermentation process can be formed, as shown in Figure 10.

It should be noted that the hydrolytic and acidogenic stages of fermentation are highly interrelated, and changes in dominant species also depend on the properties of the degradable substrate and on the concentration of nutrients, micro- and macronutrients.

Bacteria that release hydrogen during dark fermentation have been identified among the representatives of 25 % of bacterial genera presented in the latest edition of the Bergen Bacteria Identifier. Among these bacteria are known species such as Escherichia coli. Hydrogenase enzyme catalyzes this process under anaerobic conditions. The rate of formation of hydrogen by fermentation bacteria (400–650 ml per hour per 1 g of dry cell biomass) is much higher than that of other microorganisms, such as photosynthetic [11].

Therefore, the most appropriate approach is based on the integration of bio-hydrogen and bio-methane production from organic waste.

Hydrolysis of complex multi-carbon substances

Heterogeneous group of anaerobic bacteria, so-called "primary" anaerobic bacteria	Clostridium, Bacteroides, Ruminococcus, Butyrivibrio, also available Escherichia coli and Bacillus sp.

Acidogenic phase: fermentation of H_2 and CO_2 and other monocarbon compounds as well as some more complex substances into low-molecular organic acids by intermediate formation of acetyl - Coa.

Acidogenic ta acetatogenic microorganisms	Syntrophobacter, Syntrophomonas, Desulfovibrio

Reactions are based on such equations:

1) oxidation of organic acids

i. lactic acid
$$CH_3CHOHCOOH + H_2O \rightarrow CH_3COOH + CO_2 + 2H_2$$
ii. propionic acid
$$CH_3CH_2COOH + 2H_2O \rightarrow CH_3COOH + CO_2 + 3H_2$$

iii. butyric acid
$$CH_3CH_2CH_2COOH + 2H_2O \rightarrow 2CH_3COOH + 2H_2$$
2) alcohol oxidation
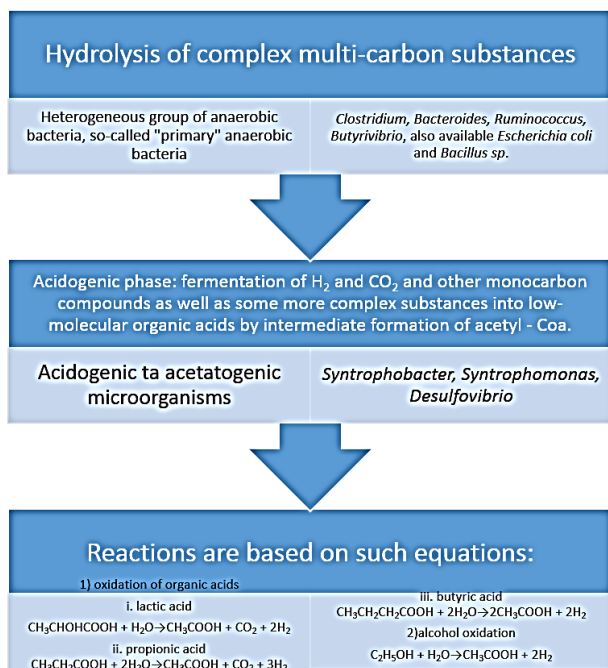$$C_2H_5OH + H_2O \rightarrow CH_3COOH + 2H_2$$

Figure 10 – Biochemical reaction of dark fermentation

In the first stage (I) of the process, hydrogen and organic acids (acetic, propionic, petroleum) are formed, which through anaerobic fermentation, is converted into a mixture of methane and carbon dioxide, well mastered by industry. The hydrogen and methane obtained can be used separately, for example, in fuel cells or in mixtures, for example, for internal combustion engines of vehicles. The second-degree liquid wastewater, low in organic matter but rich in nitrogen and phosphorus, is a fertilizer, for example, for energy plantations (agricultural biofuels or raw materials for it) that are increasingly used.

In the first reactor, due to the activation of the activity of bacteria that carry out biochemical oxidation at the stage of hydrolysis, it is possible to achieve almost complete decomposition of the original complex organic substances passing into this phase, as well as the formation of acids with almost no oxidation to methane. In the second rector, where in general the conditions are adapted to the methanogenic group of bacteria (primarily pH and Eh), it is possible to achieve the maximum degree of conversion of volatile fatty acids to methane in the practical absence of their new formation. Regulation of such indicators as residence time, the acidity of the environment, and temperature in each of the reactors allow achieving high activity of each of the required ecological-trophic groups of bacteria. The active phase of development of methanogens in such conditions can be achieved in the period from 4 to 15 days, instead of the usual 30 days, the speed of conversion of acetic acid anions to methane increases. The flow of the first phase of the process is ensured by a high load on the organic matter, which gives advantages in the development of the first groups of acid-forming bacteria. Their excessive growth is constrained by the hydrolysis phase of the solid organic matter contained in the substrate.

The second stage (II) – methane formation – should provide the growth conditions for methanogenic and acetogenic bacteria that decompose volatile fatty acids into simple carboxylic acids. Compliance with this two-phase separation principle greatly improves the performance of the anaerobic digestion system. Thus, two-phase fermentation increases the efficiency of the process in terms of biogas output, the rate of formation and the percentage of methane in biogas.

Mesophilic reaction conditions are suitable for this process, as they provide equilibrium conditions for both phases of the process, being optimal for methanogen metabolism and acetogenesis processes in which oxidation to acetate and capron salts, as well as volatile fatty acids with branched structure, occurs [12].

In addition to the stage of methanogenesis, the speed of the process may be limited by the stage of hydrolysis preceding the acidogenesis (can be combined with it or the separation of a separate technological stage), this nature of the course is due to the origin of organic matter. For this reason, pre-treatment of the waste, which may contain pre-heating, is used to accelerate the process. This not only increases the amount of biogas produced but also improves its quality by increasing the methane content. In addition to thermal pre-treatment, wastes containing large amounts of cellulose and lignin, which are poorly biochemically oxidized, can also be subjected to alkaline hydrolysis. Depth of hydrolysis can also be achieved by recirculating the precipitate, which has already been anaerobically digested.

In the process of hydrogen production, both optional and obligate anaerobic bacteria are involved by fermentation. The various metabolic pathways that are thus established may be either stimulated or inhibited, depending on the operating conditions that govern the production of specific volatile fatty acids and alcohols, including acetate, propionate, butyrate, lactate and ethanol. Fermentation of carbohydrates by conversion of acetate and butyrate results in the production of, respectively, 4 and 2 molecules of molecular hydrogen per molecule of degraded glucose. But propionate, ethanol, and lactic acid can also be produced in mixed bacterial cultures, adversely affecting H_2 production: propionate is a metabolite of the H_2 consumer pathway, while ethanol and lactic acid are associated with zero H_2 pathway. To increase H_2 production, crop conditions such as C/N and C/P ratios, carbon sources, pH, and temperature have been widely studied. Metal ions can significantly affect the activity of enzymes related to hydrogen production [13].

In the study [14] was estimated a part of anaerobic metabolic pathways of association of methanogenic and sulfate reduction bacteria under municipal sewage sludge digested. Comparison of kinetics data by Fukui et al. between freshwater species of *Desulfotomaculum acetoxidance,* including other acetate utilizing sulfidogens and acetoclastic methanogens, is one of the keys to resolving dominance of acetoclastic methanogen over acetate-utilizing SRB in anaerobic digesters.

A large number of organic substrates can be used for biogas production. In agricultural biogas plants, liquid manure of cattle and pigs, as well as energy crops, plants are grown for energy production, is a widely used substrate. Energy crops help to generate new biomass for electricity, heat, and fuel production each year, but it is necessary to assess the environmental security of their large-scale use comprehensively, their impact on soil fertility and biodiversity in the ecosystem. Other organic wastes from manufacturing and municipalities, as well as organic residues from agriculture and industry, can also be fermented to produce bio-hydrogen and/or biomethane, which contributes to climate protection, reducing greenhouse gas emissions.

5 Conclusions

One approach to systematizing the available information, in particular concerning anaerobic fermentation, is ontologizing them. In current conditions, when working with patent information, in addition to the intellectual property databases of individual countries, it is advisable to use the possibilities of search systems WEB-systems, which conveniently provide information about patents, taken into account in the examination of the patent office. Thus, the development of modern communications has greatly expanded the opportunities for a researcher to enter the world level of his developments. Significant scientific support for biochemical research is provided by bioinformation electronic databases such as KEGG, BacDive and EAWAG-BBD, etc., which provide access to a collection of graphical representations and text descriptions of metabolic or signal pathways, regulatory schemes of biological processes, information on the body. Covering various aspects of bacterial and archaic biodiversity; information on microbial biocatalytic reactions and biodegradation pathways, mainly xenobiotic chemical compounds. The use of such information ontological tools significantly expands the possibilities of developing anaerobic fermentation technologies. With the help of ontological online platforms, biochemical simulation of anaerobic fermentation processes was carried out, a scheme of anaerobic fermentation reactions was formed, technological combinations of hydrolysis, acidogenesis, and methanogeneration processes at individual fermentation stages were analyzed. The directions for optimization of the technological process of obtaining biohydrogen and biomethane with account for changes in the association of microorganisms at different stages of the bioprocess have been determined.

References

1. Shapovalov, E. B. (2019). *Improving the Biotechnology of Anaerobic Fermentation of Chicken Manure with Reduced Runoff.* D.Sc. Thesis. Kyiv, Ukraine.
2. Strizhak, O. E., Gorborkov, V. V., Franchuk, O. V. (2014). Ontology of the problem of choice and its application in the analysis of limnological systems. *Ecological Safety and in the Use of Labor*, Vol. 15, pp. 172–183.
3. Velychko, V. Y., Popova, M. A., Prykhodniuk, V. V., Strizhak, O. E. (2017). Todos is an IT platform for creating transdisciplinary information environments. *Weapons Systems and Military Equipment*, Vol. 1(49), pp. 10–19.
4. Golub, N. B., Drapoy, D. I. (2016). Hydrogen production during anaerobic fermentation of corn and sunflower waste. *Scientific News of NTUU "KPI"*, Vol. 3, pp. 13–19.
5. Kireyeva V. (2010). Microbial transformation of vegetative plant mass residues with obtaining feedstuff for ruminants. *Journal of Don State Technical University*, Vol. 10(4), pp. 520–525.
6. Prikhodko, V. Y., Safranov, T. A., Shanina, T. P. (2019). Evaluation of the involvement of biogenic elements in the natural cycle when composting the organic component of municipal solid waste. *International Scientific Ecological Conference "Waste, Causes of their Formation and Prospects for Use"*, pp. 566–568.
7. About the EAWAG Biocatalysis / Biodegradation Database: Biocatalysis / Biodegradation Database: Website, URL: http://eawag-bbd.ethz.ch/aboutBBD.html.
8. Palacios Jaramillo, P. A., Snoeyenbos-West, O., Loscher, C., Thamdrup, B., Rotaru, A.-E. (2019). Baltic Methanosarcina and Clostridium compete for electrons from metallic iron. *BioRxiv*, 530386, doi: 10.1101/530386.
9. Rotaru A.-E., Malla Shrestha P., Liu F., Markovaite B., Chen Sh., Nevin K. P., Lovley D. R. (2014). Direct Interspecies Electron Transfer between Geobacter metallireducens and Methanosarcina barkers. *Applied and Environmental Microbiology*, Vol. 80(15), pp. 4599–4605, doi: 10.1128/AEM.00895-14.
10. Ding C., He J. (2016). *Handbook of Biofuels Production. Processes and Technologies* (2nd ed.). Boston, Woodhead Publishing.
11. Markov, S. A., Protasov, E. S., Bybin, V. A., Stom, D. I. (2013). Hydrogen production by microorganisms and microbial fuel cells using wastewater and waste products. *International Scientific Journal for Alternative Energy and Ecology*, Vol. 01/2, pp.108−116.
12. Kalyuzhny S. V. (2006). Biotechnological hydrogen production: fundamental principles and limiting factors. *Catalysis in Industry*, Vol. 6, pp. 33−41.
13. Cardoso V., Betania, B. R., Felipe, T. M. Silva, Santos, J. G., Batista, F. R. X., Juliana S. (2014). Hydrogen production by dark fermentation. *Ferreira Schematic Engineeringtransactions*, Vol. 38, pp. 481-486.
14. Fukui M., Jung-In S., Yoshikuni U. (2000). In situ substrates for sulfidogens and methanogens in municipal anaerobic sewage digesters with different levels of sulfate. *Wat. Res.*, Vol. 34(5), pp. 1515–1524.