JOURNAL OF ENGINEERING SCIENCES

Volume 9, Issue 2 (2022)

Chernysh, Y., Bataltsev, Y., Shen, X.-J., Bohdanovych, O., Yakhnenko, O. (2022). Electro-fermentation for biopolymers production: trends determination with bioinformatics data analysis. Journal of Engineering Sciences, Vol. 9(2), pp. H1-H8, doi: 10.21272/jes.2022.9(2).h1

Electro-Fermentation for Biopolymers Production: Trends Determination with Bioinformatics Data Analysis

Chernysh Y.[1*][0000-0003-4103-4306], Bataltsev Y.[1][0000-0003-2035-2014], Shen X.-J.[2][0000-0003-1951-0927], Bohdanovych O.[1][0000-0002-5812-1169], Yakhnenko O.[1][0000-0001-8455-1536]

[1] Sumy State University, 2, Rymskogo-Korsakova St., 40007, Sumy, Ukraine;

[2] Key Laboratory of Yarn Materials Forming and Composite Processing Technology of Zhejiang Province, Jiaxing University, 314001, Jiaxing, China

Article info:
Submitted: June 17, 2022
Accepted for publication: September 9, 2022
Available online: September 12, 2022

***Corresponding email:**
e.chernish@ssu.edu.ua

Abstract. The paper is devoted to the study of directions of application of combined systems for obtaining biofuels and biopolymers using electro-oxidative processes, namely electro-fermentation. In the course of the work, a step-by-step methodology of research is shown, and the relationship between different bioinformatic databases in their combined use is described, which made it possible to identify trends in electro-fermentation systems with the production of bio-based products. A review of possible electro-fermentation systems with major bio-product production was performed. The possibility of including anaerobic producers of organic acids, namely lactic acid, for the needs of biopolymerization, with bioinformatic databases was substantiated. The model of the process of anaerobic fermentation with the production of organic acids for biopolymerization has been formed. The analysis of bioinformatic databases showed that the strains Anaerotignum propionicum X2, isolated from silty bottom sediments, and Anaerotignum propionicum 19acry 3, isolated from an operating anaerobic reactor, have the most significant indicators of lactate productivity. The conditions for their cultivation with an indication of nutrient media and modification of their composition are considered.

Keywords: biofuels, biopolymers, electro-fermentation, bioinformatic databases, nutrient medium.

1 Introduction

Modern advances in the efficient use of waste to create value-added products can ensure the sustainability of food systems and become a tool for solving the global problem of conserving fossil resources and minimizing environmental risks for the biosphere.

Synthetic polymeric materials are derived from the processing of non-renewable natural carbon sources - oil or products of its processing. After use, such products do not decompose for a long time in the environment due to the lack of enzymatic systems capable of destroying chemical bonds in the synthetic polymer molecule and become the cause of environmental pollution, both micro- and macroplastics, and the release into the environment of dioxin and other highly toxic substances, the release of large amounts of CO_2, increasing the greenhouse effect, destructive effect on living organisms of different levels of organization in the ecosystems.

There is increasing interest in obtaining biopolymers as substitutes for synthetic polymeric materials from recycling secondary raw materials [1-4].

The advantages of this direction are the reduction or in the future, even rejection of non-renewable raw material sources, the extraction of which causes environmental pollution, and obtaining bioplastics from renewable raw material sources (biomass of plant origin, agricultures, organic waste in the form of low-grade sewage and lignocellulose biomass), reduction of greenhouse gas emissions and harmful effects on the climate change in the production of bioplastics compared to the production of synthetic plastics.

Considering that the obtained bioplastic is also compost-resistant like synthetic bioplastic and is not automatically biodegradable, recycling it after use is possible. As a result, we can conclude that the production and use of bioplastic will have less impact on the environment than the production of traditional synthetic plastic and can replace it in a wide range of human economic activities.

Thus, biopolymers are vital substitutes for petroleum-based plastics due to environmentally safe production methods, biocompatibility, and biodegradability [5]. The use of biopolymers for 3D printing technologies and their promising applications in various areas of the aerospace, textile, food, biomedical and bioindustrial industries has a significant academic, environmental and social interest. Additive manufacturing includes a subset of processes that convert a computer-aided design into a metal, polymer, ceramic, or composite structure layer by layer. For example, materials such as starch can be extruded, while some inherently non-printable products such as meat, vegetables, and rice must be processed into a powder/paste before being 3D printed. The biomedical field requires a material to have consistent performance in terms of printability, biocompatibility, degradability, and mechanical properties [6]. At the same time, bioelectrochemical systems (BES) can be used to generate electricity directly from the treatment of waste or wastewater, together with the production of valuable chemicals such as biopolymer, various alcohols, acetate, butyrate, as well as for the production of biogas, such as CH_4 and H_2.

The work aims to study the directions of application of combined systems for producing biofuels and biopolymers using electro-oxidative processes, namely electro-fermentation. In connection with this aim, two tasks were solved:

– overview of possible systems for electro-fermentation with the production of bio-basic products;

– analysis of anaerobic processes of organic acids using various bioinformatic databases.

2 Research Methodology

Bioinformation technologies implement methods that allow the administration of data on biological objects. A biological object is an open system consisting of highly organized systems of perception, sorting, and distribution of information from the environment. This ensures a biological object's organizational, structural, and functional development, which can be considered a complex structure of a multi-stage hierarchical sequence of interacting systems and subsystems in close contact with the environment.

This study used the following bioinformatics databases: GenBank, KEGG (Kyoto Encyclopedia of Genes and Genomes), EzBioCloud Database, and BacDive (The Bacterial Diversity) Metadatabase.

Figure 1 shows the dynamics in time for databases of sequences and three-dimensional structures, which indicates a significant potential for the development of information about the genome of various ecological and trophic groups of microorganisms. At the same time, GenBank and KEGG databases have free access and are most convenient for the tasks set in this study in combination with other bioinformatics platforms.

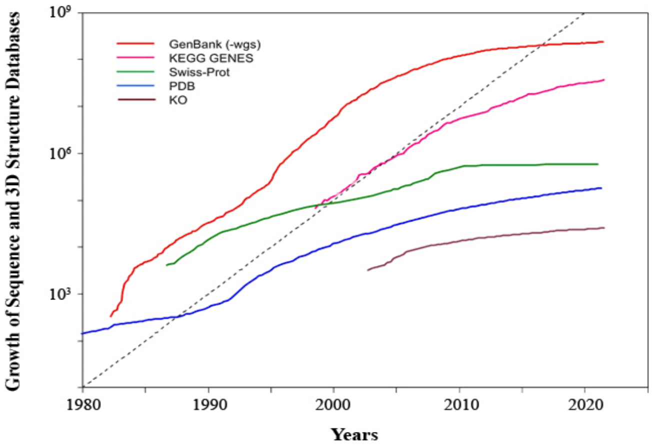

Figure 1 – Growth of Sequence and 3D Structure Databases

The step-by-step methodology of the study is shown in Fig. 2 and describes the relationship between different bioinformatic databases when they are used together.

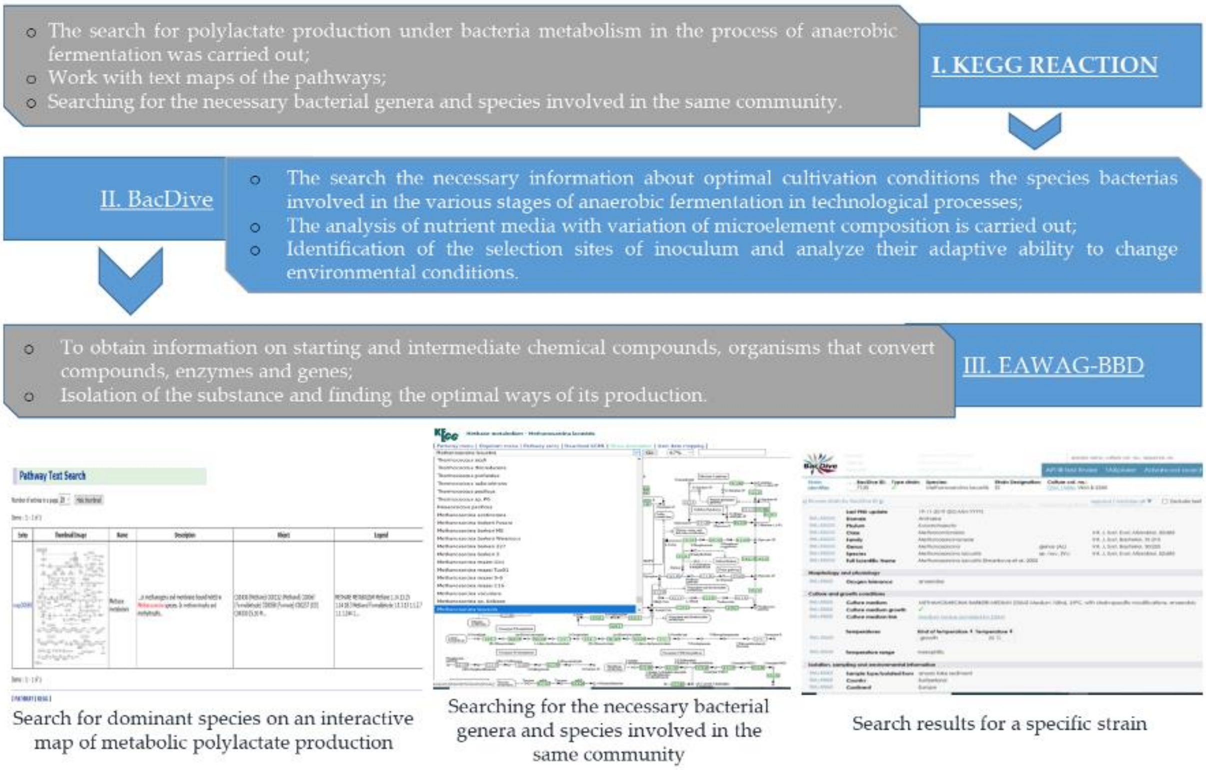

Figure 2 – Search in bioinformatic databases

3 Results and Discussion

3.1 Determination of trends in electro-fermentation systems with obtaining bio-basic products

The rapid uptake of glucose enhances lower Parallel fermentations in nature's food chains to produce numerous products, including molecular hydrogen (H_2), carbon dioxide (CO_2), formate, acetate, ethanol, lactate, succinate, and propionate. Facultative aerobes associated with *Aeromonadaceae* and obligate anaerobes associated with *Lachnospiraceae, Veillonellaceae, and Ruminococcaceae* have been related to various fermentations.

Methanogenesis continued during incubation, and 13C CH_4 labeling confirmed that the extra [13C]glucose carbon had been dissimilated to CH_4. Hydrogenotrophic methanogens belonging to the *Methanobacteriaceae* and *Methanoregulaceae* have been associated with methanogenesis, and acetogens belonging to the *Peptostreptoccocaceae* are participants in the methanogenic food web. Electrochemical processes based on renewable electricity sources at ambient temperature are an attractive alternative [7].

Dark fermentation produces hydrogen with a relatively low yield (maximum $4H_2$ per glucose) with an accumulation of metabolites such as volatile fatty acids (VFAs). On the other hand, the dark fermentation process is still attractive because of its high productivity and simple reactor design. In this regard, hybrid systems that add a second process for treating dark fermentation effluents are the subject of much research interest. By adding a second process, hybrid systems can have a potentially attractive high hydrogen yield while solving the dark fermentation wastewater treatment problem.

There are several possible combinations of a hybrid system [8]:

1) dark fermentation + photofermentation. VFAs, which are formed during dark fermentation, are ideal substrates for photofermentation;

2) dark fermentation + cell for microbial electrolysis;

3) dark fermentation + cell-free enzyme system.

4) dark fermentation + anaerobic fermentation in a fermenter. VFAs in dark fermentation effluents can be substrates for methanogens. In this process, a mixture of hydrogen and methane can be formed.

The first three combinations have the potential to achieve the maximum yield of $12H_2$ on glucose.

Many bacteria can release hydrogen as a result of dark fermentation using organic compounds: $[CH_2O]_n \rightarrow CO_2 + H_2$. It is microorganisms that can be used to dispose of various organic wastes.

A possible attractive application of the electrochemical oxidation reaction is its use to intensify the metabolism of the necessary eco-trophic groups of bacteria in the process of dark fermentation [9]. Considering the possibility of increasing the yield of organic acids in dark fermentation, interest in the electrolyzer increases.

Thus, electro-fermentation (EF) is based on electrochemical processes affecting microbial metabolism. Electron transfer during anodic or cathodic electrolysis can regulate ORP and the NAD+/NADH ratio, affecting intracellular metabolism. Recently, anodic electro fermentation using Corynebacterium glutamicum was performed to obtain L-lysine. The results showed that using anodic electro fermentation can balance the oxidation-reduction and energy states of C. glutamicum and thus improve the anaerobic production of L-lysine. Cathodic electro fermentation was also carried out to simultaneously increase biogas and biochemical production, while the highest biogas content [96 % (v/v)] and acetate production (358 mg/l) were achieved [11].

Regulation of the kinetics of the EF processes due to the action of electrodes in the microbial environment is a mechanism of electrocatalysis (Fig. 3) aimed at the regulated formation of biobased products.

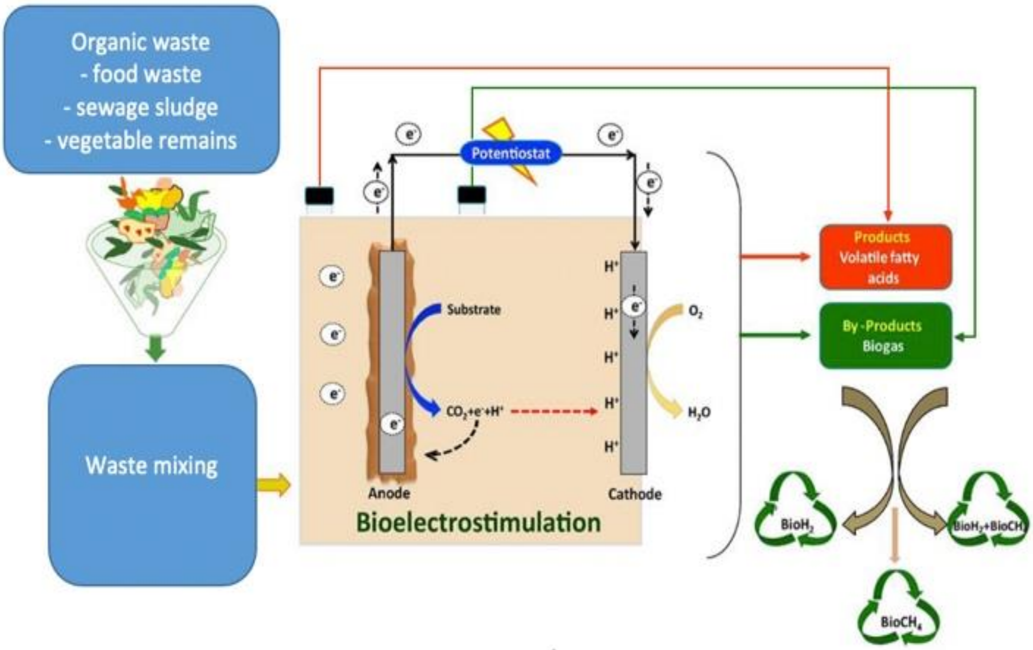

Figure 3 – Electro-fermentation under the anaerobic condition with the production of biobased products

Such organic waste, including low-grade wastewater and lignocellulosic biomass, was converted into electricity with the help of microbial fuel cells (MFC). Thus, electrical energy was used to produce hydrogen in microbial electrolysis cells (MEC) or other products, including caustic and peroxide and several organic acids. The variety of microbial and enzymatic catalysts that nature offers provides many potential applications. Compared to conventional fuel cells, bioelectrochemical systems (BES) operate under relatively mild conditions and do not use expensive precious metals as catalysts. Recently discovered microbial electrosynthesis (MES) of high-value chemicals has significantly expanded the horizons of BES application.

New application concepts, developing alternative materials for electrodes, separators, and catalysts, and innovative design have made BES a promising technology [10].

The co-production of various biofuels and organic compounds using waste and CO_2 as direct substrates points to a promising future for microbial fuel cell technologies. Likewise, an electrodialysis system can be used to extract organic acids from the dark fermentation of glucose for H_2 production by E. coli. This process was later successfully demonstrated on food waste hydrolysates. This extractive fermentation is expected to be maintained indefinitely. However, membrane fouling, the requirement for constant ionic composition, pH imbalance, etc., are major obstacles. Microbial electrolysis cells were also integrated with reverse electrodialysis, which showed a natural generation of H_2 by combining the momentum from the oxidation of organics at the anode and the energy of the salinity gradient; in addition, saline solutions can be continuously regenerated with waste heat (~38–40 °C) [12].

The work [13] performed a series of experiments using a range of commercial electron donors with varying degrees of lactate (polylactate) polymerization. These experiments were carried out using sediments from the Hanford Formation (coarse sand and gravel) submerged in Hanford groundwater, to which Cr(VI) and several types of lactate-based electron donors (Hydrogen Release Compound, HRC; primer-HRC, pHRC; extended-release HRC) and polylactate-cysteine form (Metal Remediation Compound, MRC) were added. The results showed that polylactate compounds increased bacterial biomass and activity more than sodium lactate when applied at equivalent carbon concentrations. At the same time, the concentration of hydrogen and methane in the headspace increased and correlated with changes in the microbial community [13].

Figure 4 provides an integrated flowchart of the anaerobic fermentation process, showing the bio-basic products produced at different stages of fermentation. The emphasis is on the production of organic acids that can be used to obtain biodegradable polymers, in particular, in the process of using a combination of dark fermentation (acidogenic phase of anaerobic digestion) and electrochemical processes (Fig. 3).

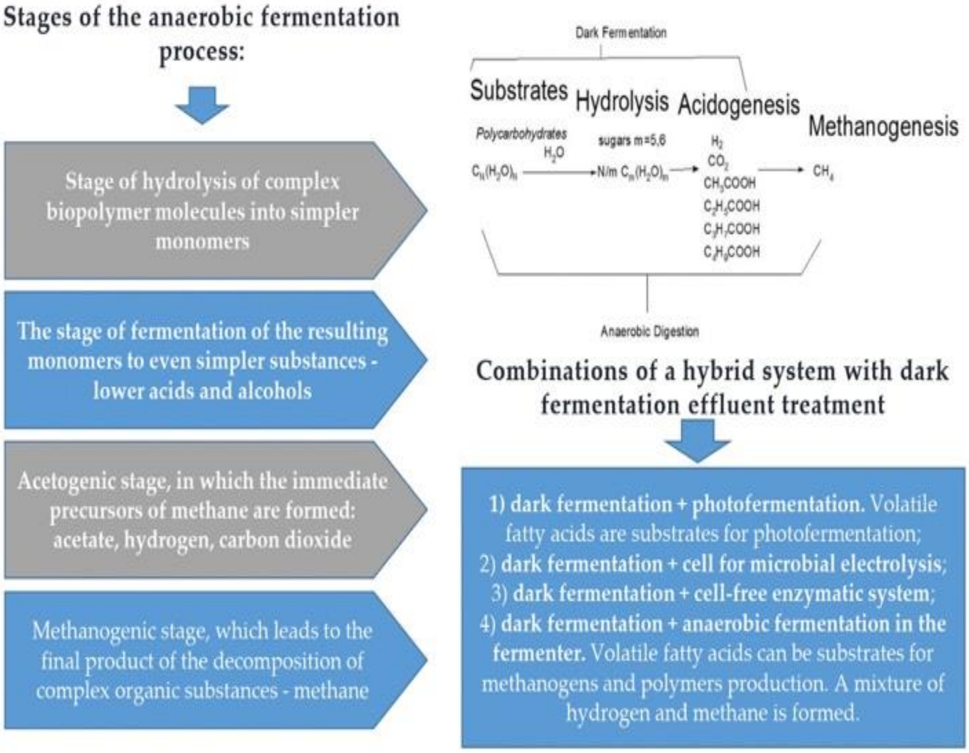

Figure 4 – Model of the anaerobic fermentation process with the production of organic acids for biopolymerization

Modern additive manufacturing (AM) of biopolymers, with a particular focus on cellulose, lignin, alginate, chitosan, starch, polylactic acid (PLA), and polycaprolactone (PCL), is increasing of interest to manufacturers of environmentally friendly polymers [6].

3.2 Analysis of anaerobic processes of organic acids using various bioinformatic databases

This paper considers the bioproduction of lactic acid for the needs of biopolymerization. Initially, GenomNet was used to identify the metabolic activity of anaerobic microorganisms for their production during dark fermentation (Fig. 5).

Polylactate (PLA) is a representative biodegradable polymer derived from lactate and is a polymer having high applicability as a general-purpose polymer or a medical polymer.

Currently, PLA is produced by the polymerization of lactate obtained from microbial fermentation. To synthesize PLA over 100,000 daltons, there is a polymerization method from low molecular weight PLA obtained by lactate direct polymerization into higher molecular weight PLA using a chain joining agent but using an organic solvent or a pair of chains. The addition of ring agents complicates the process and has the disadvantage that they are not easily removed.

Currently, a commercially available high molecular weight PLA manufacturing process is used to convert lactate to lactide and then synthesize PLA via a lactide ring condensation reaction [14].

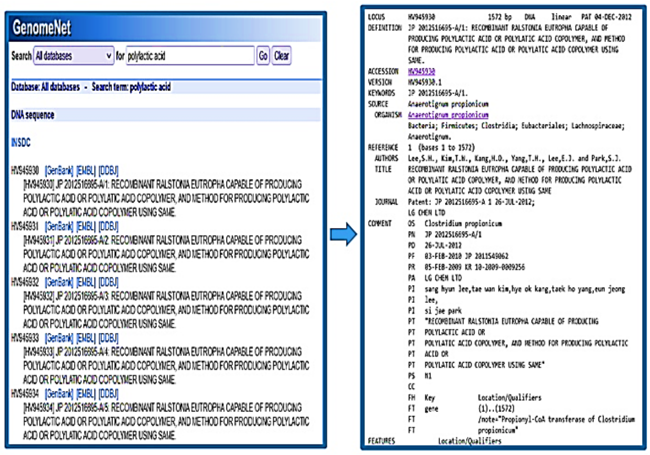

Figure 5 – Search for producers of lactic and polylactic acids

It has been determined that anaerobic strains of bacteria isolated from the methanogenic reactor have significant productivity indicators for lactate (Fig. 6).

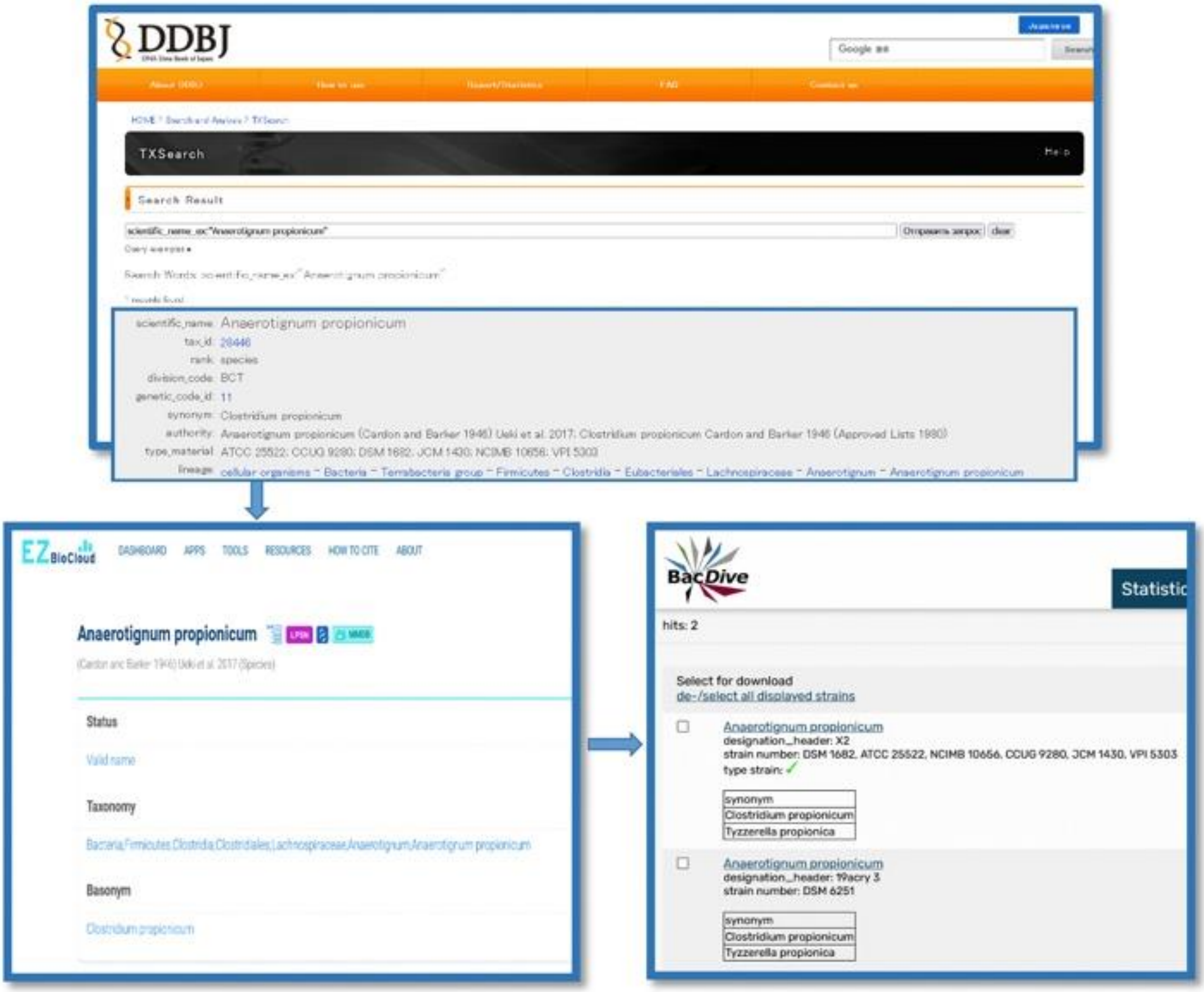

Figure 6 – Search in bioinformatic databases for the required types of anaerobic bacteria

Anaerotignum propionicum X2 is an anaerobic, chemoorganotrophic, spore-forming bacterium that was isolated from silty bottom sediments. *Anaerotignum propionicum* 19acry 3 is an anaerobe, a mesophilic bacterium, which has been isolated from an active anaerobic reactor. The conditions for their cultivation with an indication of nutrient media are shown in Figure 7.

Both strains can be cultivated on an identical nutrient medium. Dissolve the ingredients (except bicarbonate and cysteine), adjust the pH to 7.0, then saturate the medium with 100 % N_2 gas for 30–45 minutes to make it anoxic. Add and dissolve the bicarbonate and cysteine, then dispense the medium under 100 % N_2 gas into anoxic Hungat or sulfur tubes and autoclave. If necessary, adjust the pH of the whole medium to 7.0–7.2.

Based on previous studies [15], it was proposed to modify the nutrient medium for cultivating the desired anaerobic species by introducing phosphogypsum instead of calcium sulfate (Table 1).

Phosphogypsum is a multi-ton industrial waste from phosphate fertilizer production and when accumulated, can cause the alienation of vast areas of functioning ecosystems and contamination of the air, soil, and groundwater with acidic compounds containing fluorine, sulfur, phosphorus, and heavy metals.

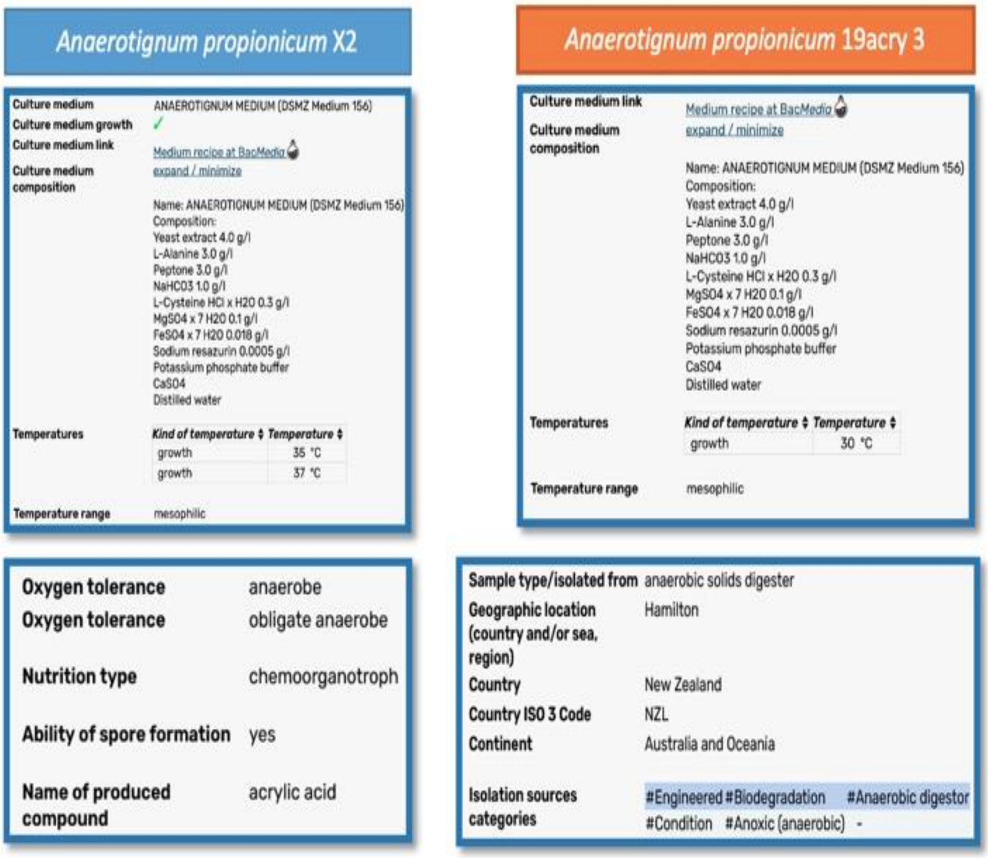

Figure 7 – Culture conditions

But it is the presence in phosphogypsum of calcium, phosphorus, residual traces of sulfur, and rare-earth elements, which are essential biogenic components for many species of living organisms, including microorganisms, makes it a source of additional macro- and microelements for bacteria used in biotechnology processes [15].

Table 1 – Culture medium for Anaerotignum propionicum

Compound	Amount	Unit	Conc. [g/L]	Conc. [mM]
L-Alanine	3.0	g	3	33.674
Peptone	3.0	g	3	–
Yeast extract	4.0	g	4	–
$MgSO_4$ x 7 H_2O	0.1	g	0.1	0.406
$FeSO_4$ x 7 H_2O	18.0	mg	0.018	0.065
Potassium phosphate buffer (1 M, pH 7.1)	5.0	ml	–	5
Phosphogypsum $CaSO_4 \times 2H_2O$ (saturated aq. solution)	2.5	ml	–	–
Sodium resazurin (0.1 % w/v)	0.5	ml	$5 \cdot 10^{-4}$	0.002
$NaHCO_3$	1.0	g	1	11.904
L-Cysteine HCl x H_2O	0.3	g	0.3	1.708
Distilled water	1000.0	ml	–	–

An important direction is the selection of the necessary types of anaerobes from local systems of anaerobic digestion of waste and effluents for their effective introduction into bioelectrochemical systems for the production of biobased products.

4 Conclusions

The article provides an overview of possible directions for using electro fermentation to intensify the processes of obtaining biobased products, such as biohydrogen, biomethane, and organic acids for producing biopolymers.

The analysis of anaerobic lactic acid processes for biopolymerization was carried out using various bioinformatic databases, namely the GenBank, KEGG, EzBioCloud, and BacDive Metadatabase. The analysis of bioinformatic databases showed that the strains *Anaerotignum propionicum* X2, isolated from silty bottom sediments, and *Anaerotignum propionicum* 19acry 3, isolated from an operating anaerobic reactor, have the most significant indicators of lactate productivity. The conditions for their cultivation with an indication of nutrient media and modification of their composition are considered.

5 Acknowledgments

We are thankful for the support of International Innovation and Applied Center "Aquatic Artery" (Sumy, Ukraine), which allowed this scientific cooperation to start. This research project was carried out as a planned research project of the Department of Ecology and Environmental Protection Technologies of Sumy State University, related to the topic "Assessment of the technogenic load of the region with changes in industrial infrastructure" according to the scientific and technical program of the Ministry of Education and Science of Ukraine (state registration No 0121U114478).

References

1. Armistead, S. J., Smith, C. C., Staniland, S. S. (2022). Sustainable biopolymer soil stabilization in saline rich, arid conditions: a 'micro to macro' approach. *Scientific Reports*, Vol. 12(1), 2880. https://doi.org/10.1038/s41598-022-06374-6.
2. Saleh, A. K., El-Gendi, H., Soliman, N. A., El-Zawawy, W. K., Abdel-Fattah, Y. R. (2022). Bioprocess development for bacterial cellulose biosynthesis by novel Lactiplantibacillus plantarum isolate along with characterization and antimicrobial assessment of fabricated membrane. *Scientific Reports*, Vol. 12(1), 2181. https://doi.org/10.1038/s41598-022-06117-7.
3. Wang, Q., Chen, W., Zhu, W., McClements, D. J., Liu, X., Liu, F. (2022). A review of multilayer and composite films and coatings for active biodegradable packaging. *Npj Science of Food*, Vol. 6(1), 18. https://doi.org/10.1038/s41538-022-00132-8.
4. Yamada, M., Kawamura, M., Yamada, T. (2022). Preparation of bioplastic consisting of salmon milt DNA. Scientific Reports, Vol. 12(1), 7423. https://doi.org/10.1038/s41598-022-11482-4.
5. Banu, J. R., Kumar, M. D., Gunasekaran, M., Kumar, G. (2019). Biopolymer production in bio electrochemical system: Literature survey. *Bioresource Technology Reports*, Vol. 7, 100283. https://doi.org/10.1016/j.biteb.2019.100283.
6. Pradhan, R. A., Rahman, S. S., Qureshi, A., Ullah, A. (2021). Biopolymers. *In Biopolymers and their Industrial Applications*, pp. 281-303. Elsevier. https://doi.org/10.1016/B978-0-12-819240-5.00012-2.
7. Schulz, K., Hunger, S., Brown, G. G., Tsai, S. M., Cerri, C. C., Conrad, R., Drake, H. L. (2015). Methanogenic food web in the gut contents of methane-emitting earthworm Eudrilus eugeniae from Brazil. *The ISME Journal*, Vol. 9(8), pp. 1778-1792. https://doi.org/10.1038/ismej.2014.262.
8. Ding, C., Yang, K.-L., He, J. (2016). Biological and fermentative production of hydrogen. *In Handbook of Biofuels Production*, pp. 303-333. Elsevier. https://doi.org/10.1016/B978-0-08-100455-5.00011-4.
9. Hauke, P., Klingenhof, M., Wang, X., De Araújo, J. F., Strasser, P. (2021). Efficient electrolysis of 5-hydroxymethylfurfural to the biopolymer-precursor furandicarboxylic acid in a zero-gap MEA-type electrolyzer. *Cell Reports Physical Science*, Vol. 2(12), 100650. https://doi.org/10.1016/j.xcrp.2021.100650.
10. Bajracharya, S., Sharma, M., Mohanakrishna, G., Dominguez Benneton, X., Strik, D. P. B. T. B., Sarma, P. M., Pant, D. (2016). An overview on emerging bioelectrochemical systems (BESs): Technology for sustainable electricity, waste remediation, resource recovery, chemical production and beyond. *Renewable Energy*, Vol. 98, pp. 153-170. https://doi.org/10.1016/j.renene.2016.03.002.
11. Zheng, T., Li, J., Ji, Y., Zhang, W., Fang, Y., Xin, F., Dong, W., Wei, P., Ma, J., Jiang, M. (2020). Progress and Prospects of Bioelectrochemical Systems: Electron Transfer and Its Applications in the Microbial Metabolism. *Frontiers in Bioengineering and Biotechnology*, Vol. 8, 10. https://doi.org/10.3389/fbioe.2020.00010.
12. Kumar, P., Chandrasekhar, K., Kumari, A., Sathiyamoorthi, E., Kim, B. (2018). Electro-Fermentation in Aid of Bioenergy and Biopolymers. *Energies*, Vol. 11(2), 343. https://doi.org/10.3390/en11020343.
13. Brodie, E. L., Joyner, D. C., Faybishenko, B., Conrad, M. E., Rios-Velazquez, C., Malave, J., Martinez, R., Mork, B., Willett, A., Koenigsberg, S., Herman, D. J., Firestone, M. K., Hazen, T. C. (2011). Microbial community response to addition of polylactate compounds to stimulate hexavalent chromium reduction in groundwater. *Chemosphere*, Vol. 85(4), pp. 660-665. https://doi.org/10.1016/j.chemosphere.2011.07.021.
14. Yang, T. H., Park, S. J., Lee, E. J., Kang, H. O., Kim, T. W., Lee, S. H. (2010). Recombinant ralstonia eutropha capable of producing polylactic acid or polylatic acid copolymer, and method for producing polylactic acid or polylatic acid copolymer using same. Patent No. WO2010090436A2.
15. Chernysh, Y., Yakhnenko, O., Chubur, V., Roubík, H. (2021). Phosphogypsum Recycling: A Review of Environmental Issues, Current Trends, and Prospects. *Applied Sciences*, Vol. 11(4), 1575. https://doi.org/10.3390/app11041575.