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Intensification of Waste Valorization Techniques for Biogas Production on the Example of *Clarias gariepinus* Droppings

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Abstract: This study aims to evaluate the process of biogas production from the droppings of *Clarias gariepinus* under intensification of methanogenesis using electrolysis pretreatment and electro-fermentation in comparison with the addition of stimulating substances (humates and zeolites). For the realization of a series of experiments, laboratory installations of electrolysis and electro-fermentation were developed. The following parameters were monitored: biogas composition, chemical oxygen demand, redox potential, hydrogen potential, nitrates, ammonia–ammonium, and nitrites. A taxonomic classification and review of the metabolic pathways were performed using the KEGG, MetaCyc, and EzTaxon databases. The stimulation of biomethanogenesis in the utilization of catfish droppings by the introduction of additional electron donors—exogenous hydrogen (electro-fermentation)—was confirmed. The electro-fermentation process released 4.3 times more methane compared to conventional conditions and stimulant additives and released 1.7 times more with electrolysis pretreatment. The main metabolic pathways of electron acceptor recruitment using bioinformatic databases are highlighted, and models of CO_2 transformation involving exogenous hydrogen along the chain of metabolic reactions of methanogenesis are generated. The summary model of metabolic pathways of methanogenesis are also proposed. Based on the results of the present and previous studies, two technological solutions are proposed to implement the process of anaerobic treatment intensification of excreta of the clariid catfish. Additional studies should include the optimization of the operation mode of electro-fermentation and electrolysis pretreatment of the substrate during the aquacultivation process.

Keywords: droppings; *Clarias gariepinus*; biogas; electrolysis pretreatment; electro-fermentation; stimulating additives

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1. Introduction

At present, industrial aquaculture is becoming increasingly more widespread and developed in the research. The number of fish farms is increasing every year, and, accordingly, the amount of waste products released by hydration during the process of their cultivation is also increasing [1]. There is a significant amount of research being conducted in the field of technology transfer for the treatment of such liquid-phase wastes, and the implementation of closed-loop water-consumption processes utilized in industrial aquaculture practices with the extraction and reuse of nutrients [2].

A considerable amount of attention in the research has been paid to the improvement of aquaculture conditions, particularly for the clariid catfish, including the optimization of their diet [3], and the assessment of environmental risks [4]; however, there remains a significant gap in the study of the intensification of the bioremediation of their liquid-phase waste to produce biogas. The metabolic products of clariid catfish are rich in nitrogen-containing compounds, which is a problem when the issue of discharging such effluents into the environment arises. This problem stems from the fact that nitrogen substances released during the decomposition of organic waste, namely, ammonia/ammonium, nitrites, and, to a lesser extent, nitrates, are toxic to living organisms and can have severe effects on the body even at low concentrations. In addition, organic matter present in the litter places a strain on the ecosystem through a general increase in COD and BOD, negatively affecting living organisms in the water and on land.

In this regard, the question of how to obtain an effective method for purifying these effluents from both the organic matter and the most toxic classes of nitrogen compounds is relevant in the research. A potential prospect is the processing of aquaculture waste products to produce bio-based products as part of the development of a circular bioeconomy, which reinforces the global demand for the development of innovative industries based on responsible consumption and production practices [5].

Studying the effect of biologically active substances used in ultra-low concentrations, which are close to the natural growth regulators in their properties, is of particular interest to biotechnology specialists to solve problems in the field of environmental protection [6]. For example, the study [7] highlights the data that present the ability of humic preparation and melaphene to produce different effects on the growth of microorganisms of activated sludge, depending on the concentration and growth stage [8]. Zeolite is known to absorb toxic metals and filter nitrogenous wastes from aquaculture effluents [9–11]. Natural zeolites and zeolite-containing rocks are widespread and possess adsorption properties, which have resulted in their wide application in the practice of wastewater treatment under anaerobic conditions [12,13]. The ion-exchange potential makes it possible to remove ionic forms of pollutants from water, on which, zeolite has a selective effect [14,15], including heavy metals, radionuclides, and ammonium nitrogen. A previous study [16] justified the use of zeolite in the anaerobic digestion of food, sludge, and slaughterhouse waste, as these raw materials are rich in nitrogen.

A promising direction in the research is the intensification of anaerobic processes using a constant electric current [17]. The data obtained from previous studies [18–21] indicate the positive effect of electrolysis on the growth and development of various microbial communities, which suggests the feasibility of using electrolysis as a growth and cell division stimulator during the anaerobic digestion process. In addition, under the action of introducing an electric current to the solutions, various complex organic substances that have a toxic effect on the microorganisms of activated sludge are decomposed, which contributes to a more complete and rapid decomposition of the substrate [22]. The electrolytic decomposition of water releases hydrogen, which is absorbed by anaerobic microorganisms and increases the methane content in biogas [23,24].

The concept of electro-fermentation technology uses electrochemical principles to activate the metabolic activity of microorganisms during fermentation reactions, and its use for biogas production has been considered for use in agricultural waste [25,26].

However, there is a gap in its application for processing the droppings of clariid catfish, which is a considerable challenge in relation to the increasing demand for this aquaculture product. In this case, it is reasonable to study the effect of an electric current on the process of the anaerobic digestion of catfish droppings and during electro-fermentation. In addition, a comparative analysis using other methods of intensification, such as the introduction of stimulating additives, will justify the effectiveness of using an electric current for the treatment processes of such excrement in the technological process of aquaculture. This would allow for a more efficient use of aquaculture waste for bioenergy production within the framework of sustainable development goals (SDGs).

Thus, it is of interest to study the effect of an electric current on the process of anaerobic digestion in the form of electro-fermentation; a comparative analysis with other methods of intensification, such as the addition of stimulating additives, is also important. This would allow for a more efficient use of aquaculture waste for bioenergy production as part of the implementation of SDG7.

This study was performed to evaluate the process of biogas production from clariid catfish droppings, with a particular focus on intensification via electrolysis treatment, and electro-fermentation compared to the addition of stimulant additives during anaerobic digestion.

- To achieve the aim of the study, the following tasks were performed:
- An investigation of the dynamics of hydrochemical parameters in a series of experiments conducted on the intensification of the anaerobic digestion of clariid catfish droppings;
- A comparative analysis of the effect of different types of treatment on biogas yield and quality in the digestion of clariid catfish droppings;
- The modeling of the species composition of a consortium of methanogenic microorganisms;
- The formalization of the electro-fermentation system in aquaculture technology.

2. Materials and Methods

2.1. Characteristics of the Substrate Used—Excreta of the Clariid Catfish

The substrate for the experiment was the droppings of the clariid catfish (Clarias gariepinus) obtained from the experimental setup (Figure 1). Nitrogen is mainly excreted in the form of urine through the gills; only a small part of it is excreted in the form of feces through the anus. Phosphorus is excreted only through feces. Thus, most of the nitrogen is completely dissolved in the water and cannot be removed by a mechanical filter. The removal of the feces by a mechanical filter retains less nitrogen and more phosphorus. The moisture content of the substrate was 93%. The chemical oxygen demand (COD) of the solution was 123 mg/L.

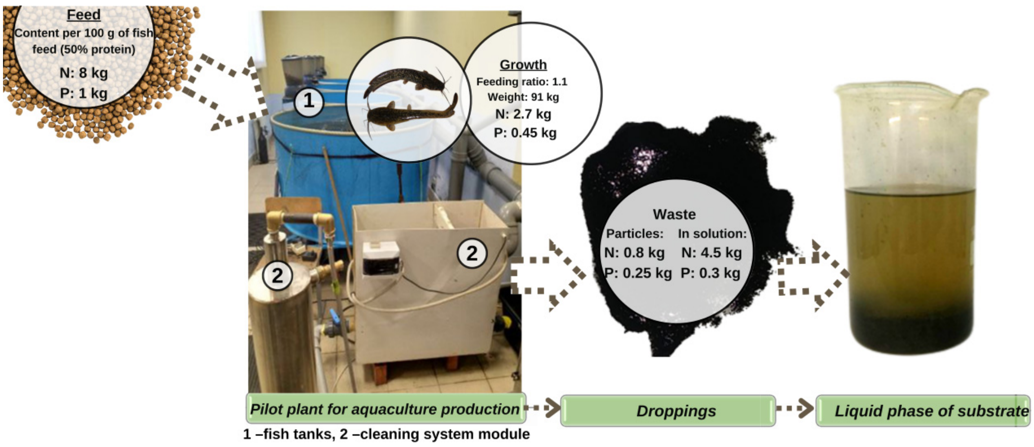

Figure 1. Aquaculture excreta as a substrate.

2.2. Characteristics of Stimulant Supplements

Zeolites and humates were used as additives in the anaerobic digestion process.

Zeolites have a fraction size ranging from 3 mm to 10 mm, and a porosity of 44%. The composition was mainly represented by silicon oxides (SiO_2), 71.5%; aluminum (Al_2O), 13.1%; and sodium and potassium oxides (Na_2O + K_2O), 5%. The manufacturer was Zeolit-Bio, Kyiv, Ukraine.

Humic fertilizers: properties—pH: 7.1–7.4, dry matter: 35–40%; composition—organic matter (per dry matter): 70–71%, humic acids (per dry matter): at least 1.44%, fulvic acids (per dry matter): at least 2.18%, total nitrogen (on dry matter): at least 1.52%, total phosphorus (on dry matter): at least 3.28%, total potassium (on dry matter): at least 1.68%, origin: producer: LLC "AVIK-Agro", Vasylkiv, Kyiv, Ukraine.

2.3. Lab Benches Used for Biogas Production Stimulation

Four series of experiments were performed (Figure 2):

1. Substrate digestion with stimulating additives: zeolite and humic fertilizer;
2. Pretreatment of the substrate by electrolysis;
3. Treatment of the substrate by electric current during anaerobic digestion every day (electro-fermentation);
4. Anaerobic digestion of substrate without treatment and stimulating additives (control).

Figure 2 presents a set of experimental facilities used in the present study. A series of experiments, with three repetitions, were conducted under 23 °C.

The typical bioreactor (Figure 2) was a tank of 20 L containing two taps, one of which was in the closed position, and was intended for sampling in the analysis stage, and the second was always in the open position, and connected the tank to the gas holder (volume: 60 L).

The parameters of the bioreactor–electrolyzer and electrolyzer were: height—30 cm, base length—15.2 cm, and base width—10.3 cm. Graphite rods used in the experiment acted as a cathode and anode; they were separated from each other by a membrane in proportions of two-thirds of the bioreactor–electrolyzer volume for the cathode and one-third for the anode.

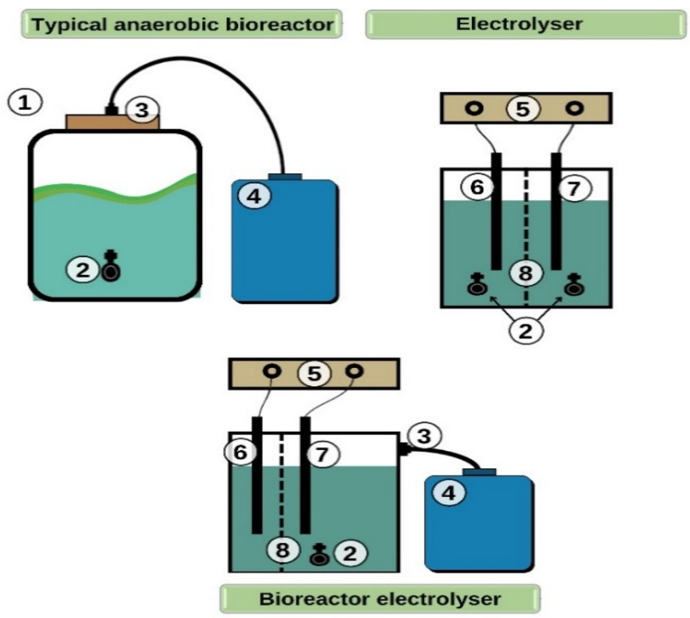

Figure 2. Lab-benches used for biogas production stimulation: 1—anaerobic bioreactor, 2—sampling tap, 3—biogas outlet tap, 4—gas holder, 5—power source, 6—anode, 7—cathode, 8—membrane.

In the experiment conducted to assess substrate digestion activity with the addition of zeolite and humic fertilizer to the substrate, the following concentrations were selected in previous studies [7,27]: zeolites in the amount of 3 g/L, and humic fertilizer in the amount of 0.1 mL/L. Zeolites were used as a means of immobilizing microorganisms to increase their effectiveness. Humates were applied as growth stimulators of the microorganisms, and the positive effect of both factors is indicated in [7,27].

In the present experiment, when pretreating the substrate in the electrolyzer (Figure 2), the substrate was treated with a direct electric current for 15 min before being introduced into a typical anaerobic bioreactor.

To implement electro-fermentation in the anaerobic digestion process, a microbial electrolysis cell (MEC) and anaerobic digestion (AD) in a two-chamber reactor composed of heat-resistant polymeric material (bioreactor–electrolyzer) were combined. In the bioreactor–electrolyzer (Figure 2), electrolysis was conducted using a welding inverter, which provided a direct electric current of 2.5 A and a voltage of 12 V. During anaerobic digestion, the substrate was treated with a constant electric current for 2 min every day. The mode of treatment was determined by the hydrogen capacity of the system; this allowed us to achieve a stable hydrogen yield of at least 18.9–21.5% in the biogas, and had no negative effect on the biocoenosis of activated sludge during the microcopying process.

2.4. Methods

2.4.1. Microbiological Studies

The microcopying of preparations was performed using phase-contrast microscopy on a Binocular biological XS-5520 microscope (LLC Trading House "MICROMED", Poltava, Ukraine) with a video camera.

Gram and Ziehl–Neelsen staining methods were used according to the standard methods [28].

A taxonomic classification and review of the metabolic pathways of the transformation of organic compounds at different stages of methanogenesis were performed using the electronic bioinformation databases KEGG, MetaCyc, and EzTaxon.

2.4.2. Control of Parameters during the Anaerobic Digestion Process

During the experiment, the following hydrochemical parameters were monitored:

- Nitrate level (NO_3^-);
- Ammonia–ammonium level (NH_3/NH_4^+);
- Nitrite level (NO_2^-);
- Solution salinity (TDS);
- Redox potential (ORP);
- Hydrogen potential (pH).

These parameters were monitored in all reactors during the experiments to judge the degree of purification obtained from organics, and the efficiency of the anaerobic digestion stages.

To determine the level of nitrogen compounds, we used express "NILPA" tests by methods developed by the Research Laboratory of Professional Aquarium [6].

During the study, hydrogen pH and ORP were measured using HI5221 (P-Lab, Praha, Czech Republic) with the following measurement errors: ±0.01 pH; ±0.2 mV; and 0.1 °C/±0.2 °C.

A TDS-3 portable total dissolved-solids detector (HM Digital, Guangzhou Juanjuan Electronic Technology Co., Ltd., Guangzhou, China) was used to measure TDS with the following characteristics: hardness measurement range of liquids: 0–9990 ppm (mg/L); and accuracy: +/−2%.

COD values prior to and following anaerobic digestion were also measured in all series of experiments. COD was measured by the photometric method, according to the standard technique (methods for measurement: "Return, surface, underground waters. Methods for measurement of chemical oxygen demand (COD) by the spectrophotometric method" MSP No 081/12-0647-09).

The volume of separated biogas was also measured using the method of the displaced liquid column [29].

Biogas composition determination: A Teflon® inert plastic sampling bag (1 dm^3 volume) was purged three times with analyzed gas. The sample was analyzed on the day of sampling. The gradient mixture was also sampled in the sampling bags using a three-way valve connected to a cylinder with a calibration gas mixture (CGM).

Studies of the gas phase were conducted on a laboratory gas chromatograph SELMICHROM 1 (JSC SELMI, Sumy, Ukraine). The primary processing of chromatograms (determination of position, peak height, and peak area), calibration of chromatograph, identification and concentration determination of components of mixtures to be separated, and storage of chromatographic information were performed using a computer system, software version 1.52x of Multichrome.

3. Results and Discussions

3.1. Comparison of Changes Occurring in Control Parameters in a Series of Experiments Conducted on the Intensification of the Anaerobic Digestion of Clarionic Catfish Excreta

The pH values present in bioreactors whose contents were subjected to electrolysis tended to be more alkaline, which suggests the positive effect on the decomposition of organic acids. In addition, it is worth noting that such pH values are more favorable for microorganisms and promote their growth and reproduction [30]. In addition, during the daily two-minute treatment in the bioreactor–electrolyzer, the pH levels behaved less stably, presenting more fluctuations than after receiving a single treatment for a period of 15 min. The index shifted to a more acidic value in the reactors. The dynamic change occurring in the pH level is presented in a graph (Figure 3). Additionally, the change occurring in the acid–acid balance during the electro-fermentation process was noted in [31].

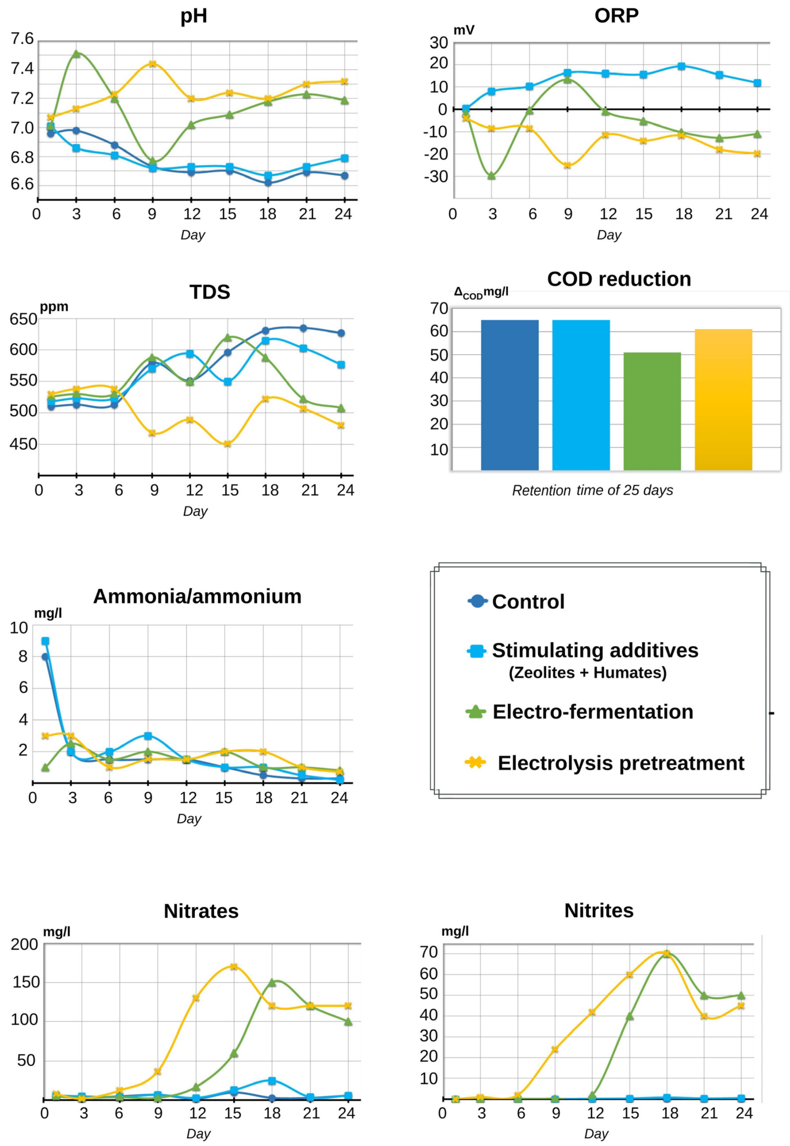

Figure 3. Dynamics of changes in the process of anaerobic digestion of clariid catfish droppings under different types of methanogenesis stimulations.

ORP was also measured, which, similar to pH level, is an important factor determining the vital activity of microorganisms. Therefore, in the study [32], it was shown that negative values of ORPs are necessary for the vital activity of the organism.

As a result of the study, it is possible to observe the situation of ORP divergence occurring in different experiments. Experiments conducted without the use of electric-current treatment presented positive ORP values, which indicates the development of facultative anaerobic microorganisms, and prolongs the stage of methanogenesis, where obligate anaerobes are activated. It should be noted that, in the bioreactor in which additives were introduced, ORP values were lower by 30 mV than those in the control, indicating a positive effect of introducing zeolites and humates. The bioreactor–electrolyzer presented negative ORP values reaching −29.6 mV (Figure 3). Such dynamics of ORP confirm the results obtained in previous studies [31,33].

TDS indicates the number of charge carriers in the solution. These are, in most cases, salt anions, metal cations, etc. To clarify this further, a value of 1000 ppm corresponds to a sodium chloride concentration of 1 g/L in solution. By relating the TDS value to the concentrations of nitrogen compounds present in the solution, it is possible to judge the degree of purification of the solution from other compounds, such as chlorides, phosphates, etc. [34]. Therefore, from Figure 4, we can observe that total mineralization value is the lowest in the reactor whose substrate was treated once by electrolysis for 15 min, while the values of nitrates placed in the tank for a long period of time were the highest and exhibited the most obvious changes. This indicates that the ions of other salts were successfully removed from the solution, while the significant amount of nitrogen compounds present allowed us to assess the decomposition of organic matter. At the same time, the highest TDS values were observed in the control tank; however, at the same time, the level of nitrogenous compounds did not increase, which suggests the presence of other salts in the solution. This agrees with the data for the study of other types of substrates presented in [21,31,35].

Nitrate ions are an extreme form of the decomposition of organic fish waste through the formation of ammonia/ammonium and nitrite. The control of this indicator allows us to assess the degree of removal of organic substances, namely, proteins and peptides, from the solution [36].

Thus, in the bioreactor–electrolyser exposed to current treatment for 2 min daily, and the bioreactor containing the substrate after a single electrolysis pretreatment for 15 min, the nitrate content by the end of the experiment was 17 and 20 times higher, respectively, than in the control and bioreactor containing simulant additives. The nitrate content increased more dynamically after one pretreatment of the substrate; however, over time, the values of substrates exposed to continuous daily treatment in the bioreactor–electrolyzer also increased and sometimes exceeded the values of a single pretreatment process. The results are presented in the graph in Figure 3. The processes of the nitrification–denitrification cycle were also studied in several works [27,37,38] for other types of substrates, namely, poultry manure and the manure of large farm animals.

The amount of nitrite ions present indicates the degree to which the solution has been purified of organic matter. The process of nitrite formation is an intermediate stage between the decomposition of organic matter into ammonia/ammonium and the formation of nitrates [39].

The dynamics of nitrite concentrations are presented in the graph (Figure 3). It is worth noting that it resembles the dynamics of changes occurring in the amount of nitrates. However, it differs in that the amount of nitrite in the substrate treated in the bioreactor–electrolyzer (on day 18) is equal to the amount of nitrite in the substrate exposed to anaerobic digestion after pretreatment with electrolysis, and exceeds it by 20 mg/L by the end of the experiment. In the control, and in the experiment with additives, the amount of nitrite did not exhibit such an increase.

Thus, the abovementioned results indicate that an electric current intensifies the anaerobic processes occurring in bioreactors.

Ammonium ions are products of the decomposition of protein molecules during the anaerobic digestion process. High ammonia/ammonium levels in a solution are detrimental to microorganisms [27].

In a series of experiments conducted in all the bioreactors, the process of ammonium decomposition into nitrite was effective, and the values of the amount of this ion present in the substrate solution ranged from 0.1 mg/L to 3 mg/L. Thus, the values of nitrite in the control experiment and in the experiment with additives did not increase, and the values of ammonia/ammonium in the solution were within the normal range. This indicates that the decomposition of organic matter is less efficient than in the experiments utilizing an electric current (electro-fermentation). Simultaneously, the ammonia/ammonium decomposition process was the same in all experiments. The results are presented in Figure 3. Over time, the amount of ammonia/ammonium present in each bioreactor decreased. However, the initial values of the quantitative content of this substance in the bioreactors containing the substrate not treated with electrolysis were 8–9 times higher than those in the bioreactors whose substrate was exposed to an electric current, which indicates the effectiveness of using electrolysis as a method for cleaning the substrate from ammonium ions during the initial stage of digestion.

In this respect, the addition of zeolite particles as an auxiliary material to reactors containing suspended biomass seemed very effective in helping to retain anaerobic biomass. Additionally, as indicated in [12], zeolite particles improve the operation and performance of the Anammox process. The addition of zeolite also, to some extent, counteracted the inhibitory effect of ammonia on the batch's anaerobic digestion process.

The results show that the COD level decreases in all series of experiments (Figure 4), which demonstrates a decrease in the amount of total organic matter present in the substrate solution; however, the values of the control reactor and the reactor containing zeolite and humates were 19% lower than those for the bioreactor–electrolyzer and the pretreatment of the substrate in the electrolyzer. At the same time, the amount of nitrogenous compounds in the substrate treated by electrolysis was much higher than in the control and bioreactor containing humates and zeolites. This suggests that the electric-current treatment used in the bioreactor–electrolyzer helps to destroy complex oxidized compounds, which are not considered in the COD analysis, and to transfer them into an easily accessible form for decomposition. In general, the indicated dynamics are consistent with the results of the previous studies conducted on the processes of the anaerobic digestion of liquid-phase substrates and anaerobic treatment of various wastewaters [40,41].

3.2. Comparative Analysis of the Effect of Different Types of Treatment on Biogas Yield and Its Quality

The dynamics of biogas production are presented in Figure 4. Thus, we can observe that in a single pretreatment phase of the substrate by electrolysis, the process of gas formation was most active during the initial stage when it was saturated with hydrogen, which agrees with the studies [31,33]. However, in the end, the bioreactor–electrolyzer presented a higher biogas yield per 1 l of substrate, compared to the pretreatment of the substrate in the electrolyzer. There was a 1.56-times difference in favor of periodic treatment in the bioreactor–electrolyzer, i.e., the process of electro-fermentation.

During electrolysis treatment, the lysis of accumulated microbial biomass and insoluble organic compounds occurs, which we assume could help maintain the productivity of the system producing biogas yields during the anaerobic digestion period, and which is consistent with the studies using an MEC [42] и в MEC–AD coupled system [43]. As can be observed from Figure 4, the electro-fermentation process produces a significant biogas yield on days 20–24, compared to the control and the application of stimulant additives.

The proportion of methane present in the biogas on the eighth day is higher when using electro-fermentation (15.30%) and electrolysis pretreatment (18.30%) processes, compared to other treatments (control: 10.24% and stimulant additives: 14.27%). This indicates a decrease in the lag period of the anaerobic digestion phase (Figure 4).

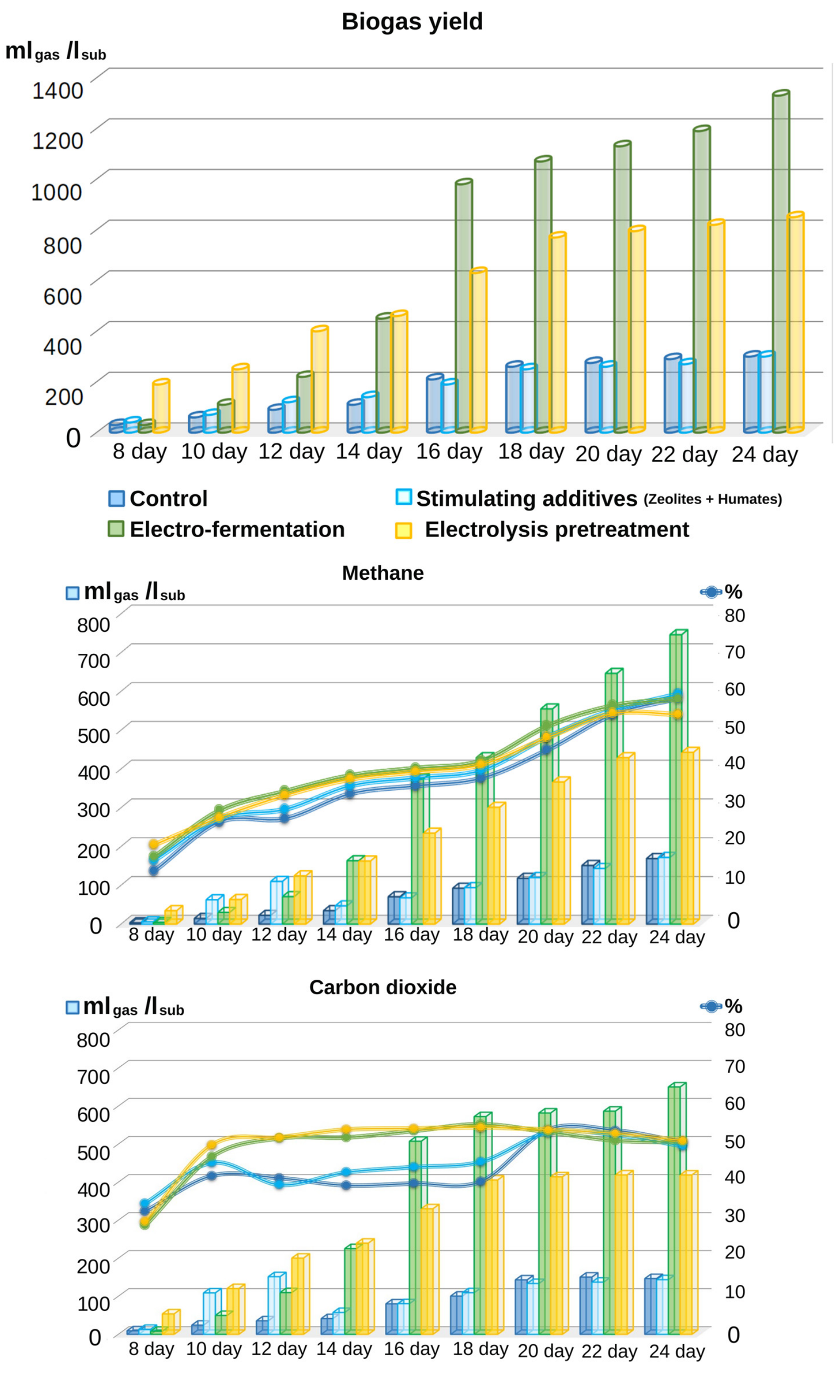

Figure 4. Biogas and methane yields in a series of experiments conducted on the intensification of anaerobic digestion of clariid catfish droppings.

The electro-fermentation process released 4.3 times more methane (746.13 mL on day 24) compared to the control conditions (168.90 mL) and stimulant additives (171.90 mL), and released 1.7 times more following electrolysis pretreatment (442.85 mL) (Figure 4), which is consistent with the results of the studies conducted on other types of substrates [26,40]. Autotrophic methanogens utilize CO_2 as their electron acceptor and H_2 as their donor, a process which was studied in [44]. When exogenous hydrogen is introduced, i.e., not produced by microorganisms themselves during the anaerobic digestion process (acidogenic and acetatogenic stages), but by means of electric-current treatment, autotrophic methanogenesis is stimulated. Electrical discharge, affecting the growth of methane-forming bacteria, makes it possible to obtain energy through the reaction of the reduction in CO_2 to methane, which was also confirmed in our previous study [31] and in studies conducted by other authors on the digestion of other types of substrates and inoculants occurring during the electro-methanogenic process [24].

Thus, it is proposed to stimulate biomethanogenesis by introducing additional electron donors—exogenous hydrogen. At the same time, the use of electrolysis treatment in the process of anaerobic digestion in a combined bioreactor–electrolyzer also promotes cell lysis and the hydrolysis of complex organic compounds, which also stimulates the enzymatic process stepwise from hydrolytic to terminal stages—proper biological methane production—which requires the experimental study of different regimes of electrolysis treatment. An additional direction of the research that can also be studied is the stimulation caused by the induction of exogenous hydrogen via dark fermentation to obtain biohydrogen.

3.3. Microscopy and Modeling of the Species Composition of the Methanogenic Microorganisms Consortium

The microscopy results of the Gram and Ziehl–Neelsen stained preparations obtained from the anaerobic reactor reveal the presence of bacilliform (bacilli) and globular (cocci) Gram-negative microorganisms that are resistant to acids. This is consistent with the results of a previous study [45], where, as the C/N ratio decreased and as time progressed to the terminal stage of methanogenesis, the anaerobic sludge surface became rougher, numbers of streptococci and filamentous bacteria decreased, and the number of cocci and bacilli increased. In addition, streptococci and filamentous bacteria were predominantly present on the surface of the sediment particles, and they were rarely observed inside the particles.

In addition to the process of methanation, the above mentioned data suggest that the genera *Methanococcus* and *Methanobacillus* were present in the solution by the end of the study. They are mesophiles and are capable of living in room-temperature conditions. Biogenic hydrogen is in high demand in the field as an electron donor, not only for the development of methanogenic Archaea, but also for sulfate-reducing bacteria. Accordingly, additional exogenous hydrogen is introduced via electrolysis, which activates the growth of lithotrophs, and the biosystem catalyzes the formation of methane. Bioinformatic databases, namely, KEGG and EAWAG-BBD, were used to examine these mechanisms at greater depth. Figure 5 presents a consortium of methanogenic microorganisms based on the data on metabolism, diversity, the presence of certain enzymes, major transformation pathways, and experimental confirmation of the dominance of hydrogenotrophic methanogens.

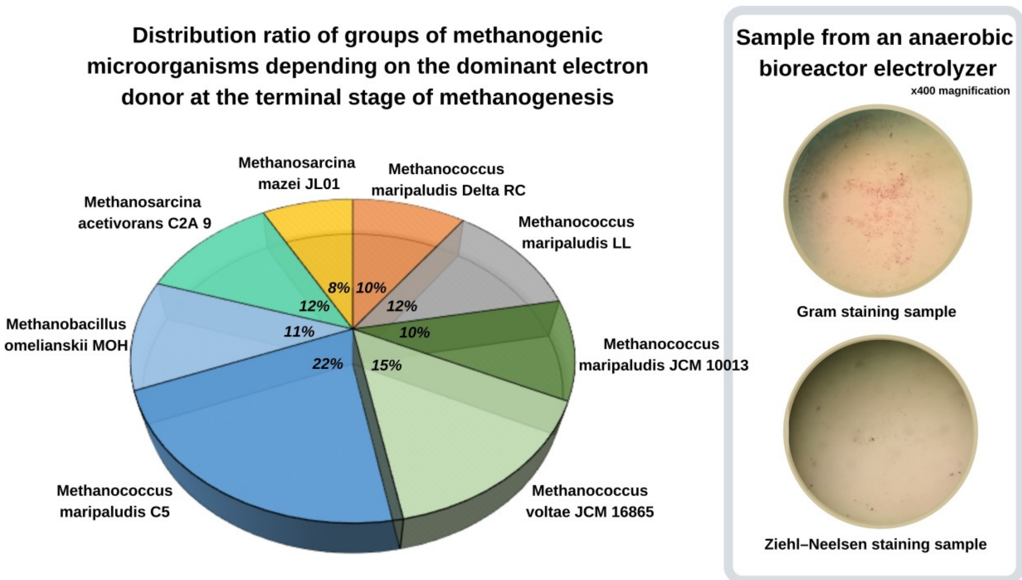

Figure 5. Modeling the composition of methanogens under the influence of an electric current during anaerobic digestion.

A complete set of enzyme systems involved in the anaerobic fermentation of organic matter, in particular, aquaculture products, taking into account the experimental identification of mesophilic methanogenic archaebacteria species present in the consortium, was modeled using the following bioinformatics databases: *Methanococcus maripaludis* Delta RC (10%), *Methanococcus maripaludis* LL (12%), *Methanococcus maripaludis* JCM 10013 (10%), *Methanococcus voltae* JCM 16865 (15%), *Methanococcus maripaludis* C5 (22%), *Methanobacillus omelianskii* MOH (11%) (hydrogen utilization methanogenesis), and slightly represented by *Methanosarcina acetivorans* C2A 9 (12%), and *Methanosarcina mazei* JL01 (8%) (acetatoclastic methanogenesis).

Thus, in our study, an environment with an additional exogenous source of hydrogen was formed in the bioreactor–electrolyzer; therefore, *Methanobacillus* and *Methanococcus* sp. are rather widely represented in the consortium.

A study [46] showed *Thioclava* sp. and *Sulfurovum* sp. to be the most abundant at lower temperatures, followed by *Proteocatella* (6.3–14.1%), *Sulfuricurvum* (1.8–12.1%), the noran families *Anaerolineaceae* (3.2–11.0%), *Proteiniclasticum* (5.7–9.1%), *Sedimentibacter* (2.9–4.2%), and *Longilinea* (1.8–3.5%). Multivariate sensitivity analysis showed that temperature ($p < 0.01$) significantly affected the microbial consortium structure in hydrogenotrophic methanogenic mixed cultures. The pH level ($0.01 < p < 0.05$) also influenced the relative abundance of dominant archaea. Clostridia accounted for the majority of the matches, including species of *Ruminococcus* and *Lachnoclostridium* known to ferment H2, as indicated in [5,47].

Consequently, electro-fermentation processes with biogas production are dominated by hydrogenotrophic methanogens (80%) among all methanogens, rather than acatatoclastic, which also agrees with the results of [44]. This is a distinctive feature of electrobiochemical activation processes occurring under anaerobic substrate processing conditions, in contrast to the standard processes of industrial biogas production, where acetoclastic methanogens dominate. For example, *Methanosaetaceae* is one of the most predominant acetatoclastic methanogens in most anaerobic methanogenic consortia, accounting for 35–75% of the total Archaea [46].

Highlighting the main metabolic pathways of electron-acceptor recruitment using the MetaCyc bioinformatic database, Figure 6 exhibits the pathways of CO_2 transformation occurring along the chain of metabolic reactions of methanogenesis and represents fragments of the chain. In general, the complete cycle of methane metabolism has a significant dependence on coenzymes, which are involved by Archaea as natural gas pedals of biochemical reactions.

The modeled consortium includes Archean strains at the terminal stage of methanogenesis in varying ratios, assuming that the group is 100%. The difference in the mass ratio value between the strains used for this model is due to the differences in the required enzymes taking into account decay intermediates that are substrates for other microorganisms in the consortium in the ecological–trophic relationships noted in Figure 6. Understanding the trophic relationships of microbial consortia in hydrogenotrophic methanogenic mixed cultures during electrolysis treatment related to the consideration and interpretation of several environmental variables (temperature, pH, ORP) requires further research to reveal the microbial ecology in the process of biogas production from aquaculture waste.

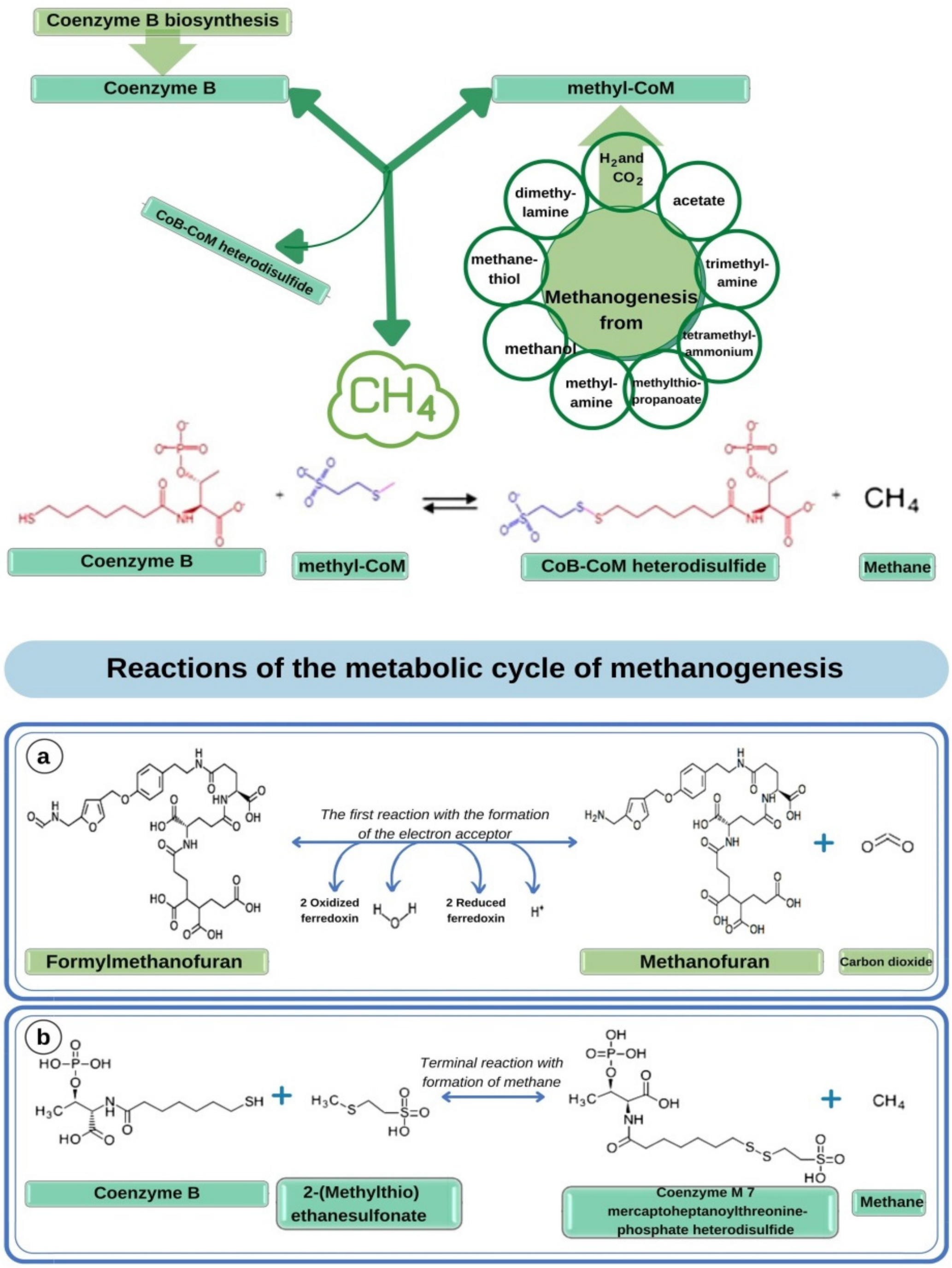

Figure 6. Summary model of metabolic pathways of methanogenesis: (**a**) the first reaction with the formation of the electron acceptor CO_2; (**b**) terminal reaction with formation of methane.

3.4. Formalization of the Electro-Fermentation System in Aquaculture Technology

Several reports [48–50] present different scenarios for converting carbon to biogas, reducing mineral nitrogen to microbial protein, and the implementation of hydroponic systems for realization in combination with existing or new anaerobic digestion plants in aquaculture.

In the study conducted by Yogev et al. (2016), nitrogen recovery was accomplished by (a) the aeration of the plant root medium and, hence, minimizing nitrogen loss through denitrification; and (b) by reducing organic nitrogen obtained from solid fish waste after its biodegradation to total ammonia nitrogen in an anaerobic digester. During anaerobic digestion, organic carbon is converted to biogas. There is a direct correlation between the amount of feed applied and the amount of waste produced, and therefore the amount of energy created in biogas production. By increasing the size of the system, the energy demand per kilogram of produced fish is expected to decrease. It is worth noting that rearing fish species, such as catfish (e.g., *Clarius* sp. and *Pangasius* sp.), which are aerialists, reduces the energy requirement of the system; therefore, perhaps smaller plants can maintain an offline operation while using plants, such as lettuce, which have a smaller proportion of non-edible parts, will negatively impact the model results and may require an additional external energy source, as defined in [51].

Thus, biotechnology solutions present advantages over standard aquaculture treatments: (1) optimal resource and waste management, (2) a closed system with a low carbon footprint, (3) production of a high-quality sustainable fish product for the end user, and (4) waste valorization using bioprocesses to produce bio-based products. In terms of resource management, a clariid catfish recirculation aquaculture system recycles 93% of the water required to grow the fish, and 7% are fed with fresh water. However, this all depends on the fish species and feed; the ratio (percentage) can vary between water treated for reuse and fresh water. The management of organic waste obtained from aquaculture is sustainable because fish excrement and any uneaten food are concentrated in containers and used on-site for biogas production.

Two solutions for the implementation of the intensification process of the anaerobic treatment of clariid catfish waste were proposed (Figure 7).

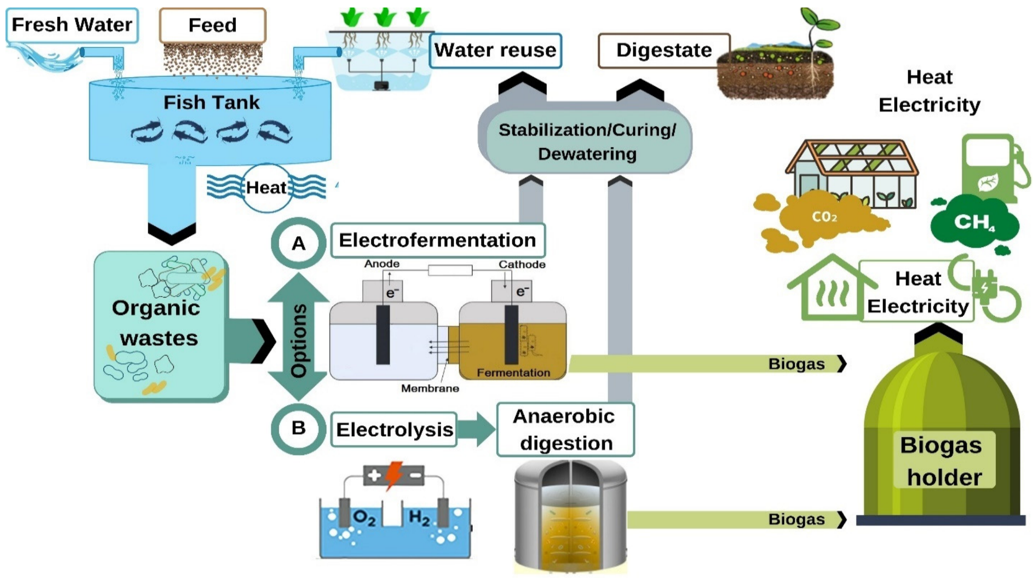

Figure 7. Technological solutions for the complex processing of waste from the life cycles of the clariid catfish with the production of useful bio-based products.

The technological scheme presented in Figure 7 (Option A) functions in the following manner:

- In a closed water supply system designed for the cultivation of clariid catfish, the following materials are supplied: prepared water (feed), combined feed, and heat to maintain the required temperature of the water solution;
- The catfish consume and digest food, and release metabolic waste products into the water. These include proteins, peptides, and nitrogen compounds harmful to the fish, for which protein molecules from the feces can be broken down;
- Aquaculture waste is removed from the fish tank using filtering elements and extracted for further processing;
- The separated droppings are sent to an anaerobic bioreactor, integrating a substrate electrolysis process into its structure. Furthermore, during the digestion cycle, the waste is treated daily with a constant electric current (electro-fermentation process);

- During the process of anaerobic conversion, biogas is released as a by-product of methanogenic microorganisms. The gas is drained from the reactor into a gas holder. Methane contained in this gas mixture is an energy carrier and can be used to generate heat and electricity;
- Carbon dioxide (CO_2) can be accumulated in liquefied form, and used to feed plants in greenhouses as one of the possible applications;
- Water obtained after dewatering the sludge can be sent back to the fish farm by passing through a hydroponic system, and the dry residue rich in mineral salts and available forms of nitrogen can be used as fertilizer.

The scheme of electro-technological utilization of aquaculture waste, presented in Figure 7 (option B), functions as follows:

- Until the organic substrate is obtained from the fish farm, the algorithm of action is similar to that described for the scheme presented in Figure 7 (option A);
- After the droppings are removed from the filter, they are sent to the electrolyzer, where they are treated with a direct electric current;
- Following the electrolysis treatment, the substrate is sent to the classical anaerobic bioreactor;
- The subsequent algorithm obtained from the end of the digestion period is the same as in option A.

Thus, the first option, (A), offers primary treatment by performing the electrolysis of fish excreta effluent prior to the stage of anaerobic digestion, and the second option, (B), offers a constructive solution of a biogas plant in the form of a two-chamber bioreactor with the placement of electrodes for conducting electro-fermentation. The obtained digestate has no toxic components in its composition, and can be used in hydroponic systems as a fertilizer (liquid phase) and in agriculture (solid phase).

Further research is required to optimize the mode of operation of electro-fermentation and electrolysis pretreatment of the substrate, and the feasibility study for the implementation of such solutions in the cultivation of *Clarium catfish.*

4. Conclusions

The process of biogas production from *Clarium catfish* droppings was evaluated under the intensification process conducted by electrolysis and electro-fermentation compared to the addition of stimulating additives during anaerobic digestion. For the realization of a series of experiments, laboratory installations of electrolysis and electro-fermentation were developed in the present study. The COD value decreased in all series of experiments, which demonstrates a decrease in the amount of total organic matter produced. The amount of nitrogenous compounds in the substrate treated by electrolysis was much higher than those in the control and in the bioreactor containing humates and zeolites. The electro-fermentation released 4.3 times more methane compared to the control conditions and stimulant additives, and released 1.7 times more during electrolysis pretreatment. Thus, the stimulation of biomethanogenesis in the utilization of catfish droppings by the introduction of additional electron donors–exogenous hydrogen was confirmed. The modeled consortium included Archean strains at the terminal stage of methanogenesis. The main metabolic pathways of the electron-acceptor recruitment using bioinformatic databases were highlighted and models of CO_2 transformation involving exogenous hydrogen (electro-fermentation) along the chain of metabolic reactions of methanogenesis were generated. Furthermore, two solutions for the implementation of the process of intensification of the anaerobic treatment of clariid catfish waste were proposed. These biotechnological solutions have advantages over the standard treatments used in the field: optimal resources, a closed system with a low carbon footprint, and waste valorization under bioenergy production. At the same time, the use of electrolysis in the process of anaerobic digestion in a combined bioreactor–electrolyzer also promotes cell lysis and the hydrolysis of complex organic compounds, which also requires further experimental studies of different modes of electrolysis treatment. An additional research direction, which will also be investigated in the future, is the stimulation caused by the induction of exogenous hydrogen via dark fermentation to obtain biohydrogen.

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