

Review

# Methods for Intensifying Biogas Production from Waste: A Scientometric Review of Cavitation and Electrolysis Treatments

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**Abstract:** This article presents future trends in research using microbiological methods to intensify bioprocesses for biogas production. The pretreatment by combinations of physical and chemical methods, such as cavitation and electrolysis, is considered. The approach of the article involved reviewing the residual area on the intensification technologies of anaerobic digestion with current methods to improve the quality and quantity of biogas. The most valuable reported positive results of the pretreatment of biological raw materials in the cavitation process were reviewed and are presented here. A model of the effect of electrolysis on the species diversity of bacteria in anaerobic digestion was developed, and changes in the dominance of the ecological and trophic systems were revealed on the basis of previous studies. The stimulating effect on biogas yield, reduction in the stabilization period of the reactor, and inactivation of microorganisms at lower temperatures is associated with different pretreatment methods that intensify anaerobic digestion. More research is recommended to focus on the electrolysis treatment of different types of waste and their ratios with optimization of regime parameters, as well as in combination with other pretreatments to produce biomethane and biohydrogen in larger quantities and in better qualities.

**Keywords:** bioprocesses; biogas production; cavitation; electrolysis; microbiological methods



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## 1. Introduction

Determining ways to reduce the technogenic impact on the environment of organic and mineral waste and their use as valuable secondary material resources is an important issue today. General trends in waste minimization stimulate the transition from linear to circular waste management systems. Therefore, the development of environmentally sound biotechnologies for waste recycling and further utilization in the form of various biocomposite products and the production of biofuels is considered promising. Furthermore, supporting the production of advanced forms of biofuels from residues and waste (domestic resources) can potentially contribute to the economic recovery after COVID-19, as projected by the European Commission [1] and substitute natural gas. Innovative biotechnology and eco-engineering are certainly integrated into a systematic approach to sustainable management of natural resources [2].

Bioprocesses are usually slower than standard chemical processes. Therefore, a promising area of research is the application of microbiological methods for bioprocess intensification, such as biofilm immobilization in mineral carriers and the formation of stable, permanent conditions for bioprocesses, taking into account the load on the organic matter

and the presence of toxicants. Therefore, a combination of physical–chemical methods, such as cavitation, electrolysis, and electromagnetic treatment, is beneficial. Furthermore, the combination of physico-chemical pretreatment methods such as cavitation, electrolysis, and magnetic treatment increase the biodegradability of organics, as with the addition of mineral additives/chemical by-products [3].

The intensity of the digestion process and, as a result, the formation of biogas are affected by the following factors: temperature [4], humidity of the environment, pH level, C:N:P ratio, the surface area of the raw material particles, the substrate feeding density [5], inhibitory substances [6], and stimulating additives [7]. Optimization of certain factors causes an increase in energy costs that makes the cost of biogas equal to the price of natural gas, which may not be economically feasible and will not provide the required effect. Therefore, the current task is to develop new methods and tools to intensify the metagenesis process.

It is possible to stimulate biogas production using cavitation to degrade biomass. Cavitation is the formation of cavities in a liquid filled with gas, steam, or their mixtures, in which the cavitation bubbles burst and form shock waves, moving with the flow to a higher-pressure area or during a half-period of compression. Under the influence of directed and controlled cavitation in biological raw materials, complex fiber bonds of organic substances at the molecular level are disintegrated. Due to this process, the dispersion of biological raw materials increases significantly, and its particles shrink in size to 0.1–8 microns. Therefore, it is easier for all bacterial species that participate in the biogas formation process to decompose biogenic materials at all stages because their homogeneous structure is destroyed and therefore the area covered with bacteria of biological raw materials increases [8,9]. Biomass degradation of cellular and subcellular materials releases more intensively natural enzymes that are biological catalysts for the biomass digestion process. This also increases the amount of biogas produced. The high degree of grinding and homogenization of the substrate, before entering the bioreactor, as a consequence, increases the number of particles on the surface allows the production of biogas to increase and intensify by 30–50% [10,11].

Anaerobic digestion is a type of indirect interspecies process with electron transfer between a consortium of microorganisms [12]; therefore, the influence of electromagnetic fields on the anaerobic digestion process has an effect and stimulates intergroup interactions between bacteria and archaea. The application of electromagnetic fields for the intensification of the biogas yield demonstrated that a magnetic field with induction of 0.38 Tesla has a significant effect on the fermentation process of methane [13]. The biogas yield increased by 14% compared with the untreated substrate. More significant for the anaerobic process is the electrokinetic decomposition, one of the high-voltage electrical methods. The electric field deforms the cell walls, so their content is easily accessible to bacteria [13]. Furthermore, treatment with high voltage electric pulses in a liquid medium makes it possible to achieve inactivation of microorganisms at lower temperatures and in a shorter exposure time than traditional thermal pasteurization methods, contributing to better preservation of the products of thermolabile components. Therefore, the impact of high voltage electrical pulses on liquid medium can be positioned as a promising method to inactivate the microflora of liquid medium with minimal thermal destruction of the products [14–16].

To control the physico-chemical parameters for intensification of the metagenesis process, we consider cavitation, electrolysis, electromagnetic treatment, and their combinations as relevant methods for intensification increasing substrate degradation and microbial activation.

Therefore, this article focused on the search for future trends in research using microbiological methods to intensify bioprocesses in biogas production in the form of pretreatment by combinations of physical and chemical methods, such as cavitation and electrolysis.

This article is structured into three sections, which cover the analysis of publications on anaerobic digestion intensification technologies. The following section describes the

methodological approach to analyzing publications on the intensification of anaerobic digestion from the Scopus and Web of Science databases. The main part analyzes publication activity by years and trends that have changed in the research of the selected magnetic and electrolysis treatment and cavitation methods. A more detailed review of the influence of cavitation processes on the enhancement of biogas yield is provided, as well as an overview of the simulation result depending on the type of substrate, as well as the electrolysis treatment to stimulate ecological and trophic groups of microorganisms in bioprocesses to increase biogas production.

## 2. Methodological Approach for the Analytical Review of Intensification Anaerobic Digestion

The methodological approach for the integration methods of intensification anaerobic digestion involved:

- Reviewing the subject area of research on anaerobic digestion intensification technologies;
- Keywords search;
- Most current methods to improve the quality and quantity of biogas for their combination, which are most beneficial.

Two databases, Scopus and Web of Science, were used for the analytical review of research studies, shown in Figure 1. These databases are considered the largest and most important international scientific platforms for publications with specific features.



Figure 1. Methodological approach for the analytical review from Scopus and Web of Science databases.

These platforms provide an opportunity to publish personal scientific work and search for and analyze existing material in a specific area, the comparison between Scopus and Web of Science. The databases provide a quick and simple overview of the citations of scholarly journal articles.

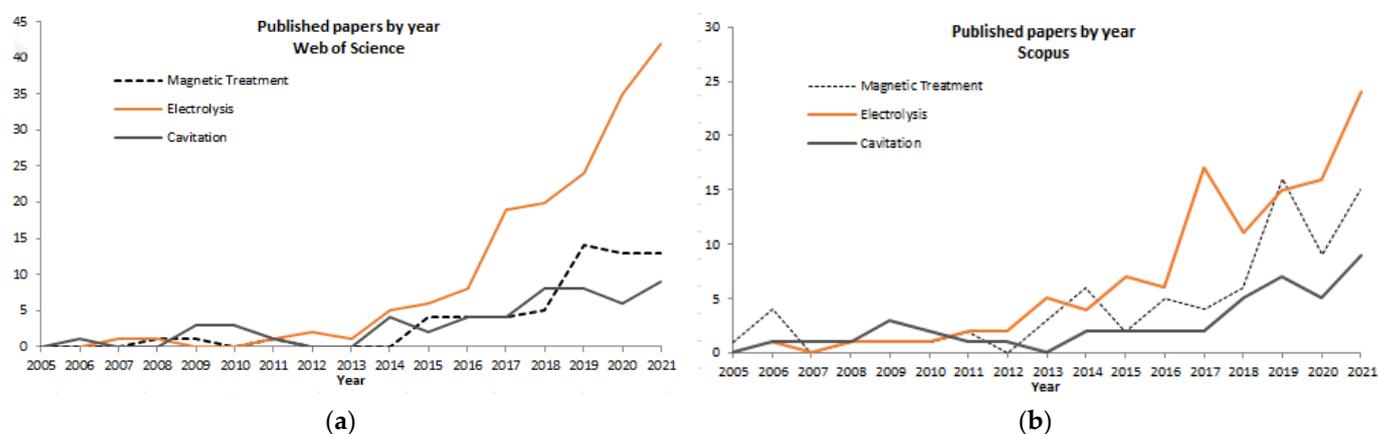
The Web of Science database selects only high-quality articles for publication and provides a thorough peer review and professional expertise [17]. The publication must correspond to the list of criteria [18]. The Scopus database is considered a more modern database [19], which allows for a more prospective and predictable data search. Scopus collects diverse works mainly uploaded to the resource digitally, including papers in different languages.

The databases also provide an additional tool to simplify and speed up searches on the resources. Advanced search and additional tools make data search more detailed and used for statistical evaluation of the data. Additionally, another important feature is the citation report, which presents the dynamics of the citation and publication activity, as well as the citation by year, along with the creation of citation maps, allowing us to trace the relationship between the studies.

Analyzing the publication activity over the years is important to identify keywords in the search that are specific to the chosen topics. In our case, we use words such as “bioprocesses, anaerobic digestion, biogas, cavitation, electrolysis, and pretreatment” and similar words in variations according to the desired query. All articles that met the query were analyzed. A detailed analysis was carried out to find the reasons for the growth and decline of publishing activity and the beginning of the discussion of the subject matter in the articles.

### 3. Results and Discussion

Through scientific periodicals and the Web of Science and Scopus databases, we can consider the interest in the use of various methods of intensification of bioprocesses, as shown in Figure 2. According to the Scopus database, fluctuations in publication activity by year are more visible.



**Figure 2.** Research articles in the field of various methods of intensification of bioprocesses using the (a) Web of Science and (b) Scopus databases (2005–2021).

Since 1997, the use of cavitation in bioprocesses has been considered, and since 2017, the topic has received more attention. In the published literature, the most common applications of cavitation processes are the ultrasonic and hydrocavitation technologies as effective pretreatment methods to increase the biological decomposition of various substrates. The impact of different effects and technical characteristics of constructions on cavitation processes and the further intensification of the processes are also discussed. In the investigation [20], the physical methods of cavitation of lignocellulosic biomass before ultrasonic and hydrothermal treatment were compared. The solubilization effects of biomass were achieved at approximately the same values (about 30% chemical oxygen demand (COD)), but biomass pretreated with hydrothermal cavitation pretreatment achieved a higher biogas production rate than ultrasonic pretreatment at the same consumption, but the biogas production yield was higher with ultrasonic pretreatment. Cavitation offers an effective way to make the lignocellulosic biomass more suitable for subsequent microbial degradation. The study showed that the biogas yield of the pretreated biomass was high in the anaerobic digestion process. This review article [21] summarizes the benefits of ultrasonic sludge treatment, the effect of ultrasonic treatment parameters, and its effect on increasing biogas production in an anaerobic digester.

According to database data, the initial interest in the subject of electrolysis in bioprocesses was in the years 2006–2007 [22,23], and later after 2013, this topic became increasingly popular, with a growing trend. The study by Tartakovsky et al. [24] showed improved methane production from wastewater in laboratory anaerobic reactors equipped with electrodes for water electrolysis. Hydrogen obtained by electrolysis is converted to biogas, improving its combustible properties, and converted to methane by hydrogenotrophic

methanogens, improving the production of methane by 10–25% and stabilizing the reactor at the required pH value without the addition of NaOH.

Liu et al. [25] found that the microbial electrolysis cell considered for the purpose of extracting bioenergy and using waste sludge can provide higher energy efficiencies and increase biogas production. Hassanein et al. [26] determined the effects of energy production and waste treatment of dairy manure use in a combined anaerobic digestion and microbial electrolysis cell system. The addition of an electrolysis cell increased overall energy production and removed organic matter from the dairy manure. In Hua et al. [27] microbial electrolysis cells were studied in a wide range of potential applications such as the removal of recalcitrant pollutants, the synthesis of chemicals, the recovery of resources, and biosensors.

The use of magnetic processing in bioprocesses developed slowly until 2014 and after it began to increase in interest, while the peak values are 2019 and 2021. The effect of the magnetic field on the qualitative composition of biogas is different. Debowski et al. [28] showed the dependence of the methane yield time on the time of exposure to a constant magnetic field. The increase in time leads to a decrease in the methane content in biogas. An increase in methane content and yield is observed in the anaerobic digestion process of animal waste [29] under the influence of a magnetic field with an induction of 10–50 mT and a decrease in productivity under conditions of more than 90 mT. The biological effect of an external magnetic field must be associated with the effect of external fields on water that is in a state different from the equilibrium metastable state, which is transmitted further to the biological level through the participation of water in various metabolic reactions [30].

This topic is widely discussed in agricultural countries and countries with a large amount of organic waste. Therefore, the study of bioprocess intensification methods can help increase qualitative and quantitative indicators of biogas yield by using a combination of intensification methods.

According to the analysis of scientometric databases, a significant number of publications [31–34] focus on improving the ecological situation in regions and in the energy sector of the economy, the production of biofuels, and the chemical engineering with the production of different useful chemical additives (e.g., magnesium salt [31]).

### 3.1. Cavitation Processes for Intensification of Biogas Yield

Cavitation can occur as a result of a decrease in liquid pressure as its velocity increases or high-intensity sound waves pass through during a half-period. The industrial application of cavitation is for the homogenization, mixing, and precipitation of suspended particles in colloidal liquid compositions. Many industrial mixers are based on this established principle. This is usually achieved by designing a turbine or by passing the mixture through an annular orifice with a narrow inlet and a large outlet: a forced reduction in pressure leads to cavitation as the liquid runs towards the larger volume side. This method can be controlled by hydraulic devices that control the size of the inlet orifice; therefore, the process can be controlled in different mediums [35,36].

Ultrasound is elastic vibrations and waves with frequencies from 15,000 to 20,000 Hz (15 to 20 kHz) and up to 1,000,000,000 Hz (1 GHz), the frequency range of ultrasound between a 1109 and 11,012 Hz is commonly called hypersonic. The ultrasound frequency range can be divided into three subareas: ultrasound at low frequencies (15,000–100,000 Hz)-LFU, ultrasound at medium frequencies (100,000–10,000,000 Hz)-MFU, and ultrasound at high frequencies (10,000,000–1,000,000,000 Hz)-HFU [33]. Thus, the different frequency ranges of ultrasound were shown with the possibility of extending its application to substrate preparation in biogas technology.

As a result of the application of the controlled cavitation process in biomass destructors, they are widely used in biogas production, as shown in Table 1. The percentage of methane in biogas increases to 70–75% [9]. Nykyforov et al. [37] studied anaerobic digestion of blue-green algae that causes blooms in water bodies, it was experimentally established that mechanical cavitation pre-treatment of blue-green algae biomass increases the biogas yield

by 21.5%. Furthermore, the biogas produced contains up to 72% methane and hydrogen. Hydrogen is also an important component of biogas, and in the context of the development of hydrogen energy [38] as the most environmentally friendly field, its production and production biologically are relevant.

In Polettini et al. [39], the effect of ultrasonic treatment on methane production was evaluated in anaerobic digestion of lignocellulosic waste. It was determined that with a mixture ratio of sonicated and untreated substrate 75/25, methane production improved by 20% and kinetic parameters increased by 64–82%. As can be seen, there is a trend towards an increase in biogas methane production, which has a certain range with a maximum value of 82%, which can be achieved in the study [39], which is consistent with several other studies [8,9].

Joshi et al. [40] showed that the use of ultrasound promotes the solubilization of organic matter present in food waste, and the continued application of ultrasound in the anaerobic digestion process resulted in increased biogas production (almost doubled) of pretreated food waste [41]. The pretreatment of different types of waste is important, as food waste has a significant inclusion of cellulose-containing components, so its treatment with ultrasound can have a positive effect in terms of improving the biodegradability of these compounds and homogenization.

As a result of the high dispersibility and intensification of the anaerobic fermentation processes, the fermentation period of biomass is considerably reduced. The use of ultrasound in secondary sludge at the Molina de Segura wastewater treatment plant [42] significantly reduces the minimum time required for the anaerobic digestion process. By hydrolysis of the existing cell walls in secondary sludge, biodegradable substances increase immediately, accelerating the anaerobic digestion processes and moving to the stages of acidogenesis, acetogenesis, and methanogenesis. The use of an ultrasonic sludge treatment system increased digester capacity by 8%, causing an 18% increase in average biogas production and a 10% decrease in the production of dewatered sludge on average. The cogeneration process at the Molina de Segura wastewater treatment plant reduced the required electricity consumption since cogeneration increased by 20%.

**Table 1.** Process characteristics with the additional pretreatment.

Substrate	Increased Production	Pretreatment Conditions	Limiting Factors	Benefits	Reference	
Wastewater treatment plant	Sewage sludge	Increase the <b>methane yield coefficient up to 95%</b>	USPP: power 150 W; exposure time 15, 30, 45, and 60 min	<ul style="list-style-type: none"> <li>Economically feasible at the specific energy input</li> </ul>	<ul style="list-style-type: none"> <li>Increase in the methane yield coefficient balance the energy requirements;</li> <li>Higher biodegradability and enhanced hydrolysis phase would entail smaller reactor sizes along with a reduction in high capital requirements;</li> <li>Methane yield coefficient 172 mL/g VS</li> </ul>	[43]
	Sewage sludge	Increase the <b>methane content</b> in biogas to <b>68.3 ± 2.5%</b>	USPP: power 125 W; field intensity 1.9–4.3 W cm <sup>-2</sup>	<ul style="list-style-type: none"> <li>The volume and composition of biogas directly dependent on the ultrasound power</li> </ul>	<ul style="list-style-type: none"> <li>The efficiency of the unit depends on the acoustic and electronic construction set</li> </ul>	[44]
	High organic content wastewater	Increase the <b>methane yield up to 60%</b>	USPP: power 400 W; frequency 24 kHz with different amplitude ratios; exposure time 1, 2, 3 h	<ul style="list-style-type: none"> <li>Higher COD and BOD removals were in combination with ultrasonic with alkaline pre-treatment</li> </ul>	<ul style="list-style-type: none"> <li>Ultrasonic pre-treatment with the following alkaline pre-treatment: CH<sub>4</sub> yield of 211 mL/g VS. within the first two days</li> </ul>	[45]
	Waste-activated sludge	Increases <b>biogas production by 25%</b>	USPP: power 225 W, frequency 20 kHz	<ul style="list-style-type: none"> <li>Ultrasound led to increased sludge biodegradability, but they were not fully biodegradable</li> </ul>	<ul style="list-style-type: none"> <li>Biogas volume higher with supplied energies of 7000 and 15,000 kJ/kg TS;</li> <li>Biogas production limit at 7000 kJ/kg TS</li> </ul>	[46]
Food waste	Fruit and vegetable wastes	Increase the <b>methane production by 29–80%</b>	USPP: frequency 20 kHz; the amplitude of 80 μm; exposure time 9 min, 18 min, 27 min	<ul style="list-style-type: none"> <li>Longer ultrasonic time not only upsurges the energy input of the digester, but also decreases the net energy yield;</li> <li>Longer treatment time may reduce the microbial activity</li> </ul>	<ul style="list-style-type: none"> <li>Power of the ultrasound has a stronger influence than the treatment time;</li> <li>The energy output of the bioprocess compared with the energy input of ultrasonic treatment is two times higher;</li> <li>Shorter batch time means more economic profitability</li> </ul>	[47]
	Food waste and cardboard	Increase the <b>biogas yield up to 26%</b>	USPP: power 750 kW; frequency of 20 kHz; exposure time 30 min, 45 min, 60 min	<ul style="list-style-type: none"> <li>Inverse relationship exists between the specific energy required and the TS concentration of the substrate;</li> <li>Proportional relationship exists between the energy required and the time of ultrasound pre-treatment</li> </ul>	<ul style="list-style-type: none"> <li>Indicates that ultrasound pre-treatment would be beneficial for large quantities of substrate pre-treatment</li> </ul>	[41]
Organic fraction of municipal solid waste		Increase the <b>biogas production up to 24%</b>	USPP: density 0.1–0.4 W/mL, exposure time 30 min, 69 min	<ul style="list-style-type: none"> <li>Operating conditions and potential costs and benefits</li> </ul>	<ul style="list-style-type: none"> <li>Mass and energy balance on full-scale studies showed that 1 kW of ultrasonic energy used to pretreat sludge generates about 7 kW of electrical power after losses;</li> <li>Higher capital and operating cost savings can be reached by reducing digesters' size</li> </ul>	[48]
		Increase the <b>biogas production up to 16%</b>	USPP: frequency 20 kHz	<ul style="list-style-type: none"> <li>Dependable scale for mass and energy balances is needed to evaluate the process upgrade's technical and economic feasibility</li> </ul>	<ul style="list-style-type: none"> <li>Application of specific energy values higher than 15,000 kJ/kgTS does not provide significant solubilization effects</li> </ul>	[49]
		Maximum <b>biogas yield</b> produced after 72 h of digestion <b>increase</b>	USPP: frequency 20 kHz; density 0.2, 0.4, 0.6 W/mL; exposure time 10 min, 20 min, and 30 min.	<ul style="list-style-type: none"> <li>Ultrasonic power densities and exposure times had little influence on samples with 10% total solid content. The time required for the hydrolysis phase decreased</li> </ul>	<ul style="list-style-type: none"> <li>High amount of energy input during sonication pretreatment caused significant TVFA generation, effective for low TS content</li> </ul>	[50]

Table 1. Cont.

Substrate	Increased Production	Pretreatment Conditions	Limiting Factors	Benefits	Reference	
Agricultural waste	Maize silage	Increase in <b>biogas and methane production up to 29.5%</b>	USPP: field intensity 40–50 W·cm <sup>-2</sup> ; frequency 20 kHz; production ceases after 300 h	<ul style="list-style-type: none"> <li>Consuming energy for disintegration</li> </ul>	<ul style="list-style-type: none"> <li>Suppose the production of electricity with increased CH<sub>4</sub> on 30% leads to electric energy balance value of approximately 467 kJ/kgVS, might be expected that the electricity production of the biogas plant would rise by about 20.7%</li> </ul>	[51]
	Cattle manure mixed with straw wheat (2:1)	Increase in <b>methane production by 1.6–4.1%</b> Increase in <b>biogas yield production by 8.7–64.2%</b>	USPP: power 400 W; frequency 24 kHz; treatment time 4.41–54.14 s	<ul style="list-style-type: none"> <li>With specific energy input, hydrothermal cavitation pretreatment and ultrasonic pretreatment provided release of CODsol and increased solubilization;</li> <li>Crucial aspects of the economics of the conversion of lignocellulosic biomass for commercialization is the energy requirement;</li> <li>Industrial-scale application</li> </ul>	<ul style="list-style-type: none"> <li>Highest provided energy inputs were 4034 kJ/kg TS;</li> <li>Hydrothermal cavitation equipment is easier to scale up for industrial-scale applications and consumes less energy than ultrasonic</li> </ul>	[20]
		Increase in <b>methane production by 2.0–5.4%</b> Increase in <b>biogas yield production by 5.7–39.4%</b>	HCPP: hydrosonic pump 1.2 kW; treatment time 4.41–54.14 s			
	Silage with bovine liquid manure	Increase in <b>biogas yield by 23.5%</b>	USPP: power 400 W; frequency 24 kHz; treatment time 60 s, 120 s, 180 s	<ul style="list-style-type: none"> <li>Volumes of biogas produced at the sonification times of 120 s and 180 s did not change significantly</li> </ul>	<ul style="list-style-type: none"> <li>Production of biogas was not significantly increased by extending the ultrasonic field’s activity time</li> </ul>	[52]
	Mixture of organic wastes	Increase in <b>methane production by 20%</b>	USPP: intensity 2.3, 7.7, 13.4 W·cm <sup>-2</sup>	<ul style="list-style-type: none"> <li>Treatment conditions have a high energy consumption, creating a challenge to full-scale implementation</li> </ul>	<ul style="list-style-type: none"> <li>Efforts should be made to reduce the US energy applied in order to improve the energetic profile of the combined process corresponding to overall environmental sustainability</li> </ul>	[39]
Algae	residues	Increases the methane yield by 21.5%	HCPP: 4000 rpm for 15 min	<ul style="list-style-type: none"> <li>Actual effectiveness of methanogenesis</li> </ul>	<ul style="list-style-type: none"> <li>Biogas produced calorific value Q = 5100–5200 kJ/m<sup>3</sup> are close to the propane-butane mixture</li> </ul>	[37]

USPP, ultrasound pretreatment parameters; HCPP, hydrothermal cavitation pretreatment parameters; COD, chemical oxygen demand; BOD, biochemical oxygen demand; TS, total solids; VS, volatile solids.

The biological processes are substantially stabilized, leading to the absence of foaming and floating crusts in the upper part of the bioreactor. Thus, the entire productive volume of the reactor is used efficiently. Machnicka et al. [53] showed how the addition of cavitation-disintegrated foam to mesophilic anaerobic digestion can improve the process and the production of biogas. The thermophilic digestion mode is more efficient in terms of biogas yield per unit time, but its use can be limited because of economic indicators of the cost of heating the substrate in the bioreactor, and it depends on external temperatures and thus on the climatic zone. Therefore, in temperate latitudes, including Ukraine, it is relevant to work in the mesophilic mode.

### 3.2. Electrolysis Treatment in Processes of Stimulation of the Ecological and Trophic Groups of Microorganisms Required in Biogas Production

The microbial electrolysis cell provides a higher energy efficiency and biogas production, which have been studied and confirmed by several studies. A systematic understanding of microbial interactions and the production of biomethane and hydrogen in the microbial electrolysis cell is limited [54]. Liu et al. [25] showed that biohydrogen can be produced directly in microbial electrolysis cells by biocathodes using sludge. The predominant population at the alkali-pretreated sludge-fed microbial electrolysis cell anode was represented by exoelectrogenic *Geobacter*, while fermentative *Clostridium* dominated at the biocathode. Most of the methanogenic archaea in the cathodes belonged to the hydrogenotrophic *Methanobacterium* and *Methanocorpusculum*. The microbial electrolysis cell enhances the production of biomethane and hydrogen from waste sludge through syntrophic interactions between fermentative bacteria, exoelectrogenes, and methanogenic archaea. Furthermore, multiple gas production pathways are observed in the microbial electrolysis cell reactor: fermentation and electrolytic H<sub>2</sub> production, as well as hydrogenotrophic methanogenesis and electromethanogenesis. The possibility of additional electrolytic hydrogen production in the electrolysis system also provides perspective on the application in innovative bioprocesses such as photo-fermentation [55] and dark fermentation [56]. Higher efficiency biohydrogen production by two-stage dark fermentation combined with microbial electrolysis was proven on palm oil mill effluent [57] and cassava starch processing wastewater [58]. Furthermore, the integration of microbial electrolysis cells with photo-fermentation positively changed the microbial community with the predominance of electrogenic microorganisms, which increased hydrogen production [59].

In electromethanogenic bioelectrochemical systems, there are three known pathways: CO<sub>2</sub> reduction, methylotrophic, and acetoclastic pathways. The CO<sub>2</sub> reduction pathway, which stimulates methane production, is considered the main determinant of overall system performance, but other pathways are also important [60]. Therefore, acetoclastic methanogenesis very often prevails in industrial condition [61] biogas production because it is based on the possibility of bioconversion of acetates with the obtaining of methane ( $\text{CH}_3\text{COOH} \rightarrow \text{CH}_4 + \text{CO}_2$ ).

Some methane production reactions carried out by biocathode communities have been described in the literature, but further research is needed to elucidate the molecular pathways. In a review by Blasco-Gómez et al. [62], some molecular research findings are collected in the field of electromethanogenesis (Table 2).

Therefore, each of these pathways can be used to produce methane, and it is important to find an integrated opportunity to stimulate their dominant relationships in a consortium of methanogens to improve the quality of biogas.

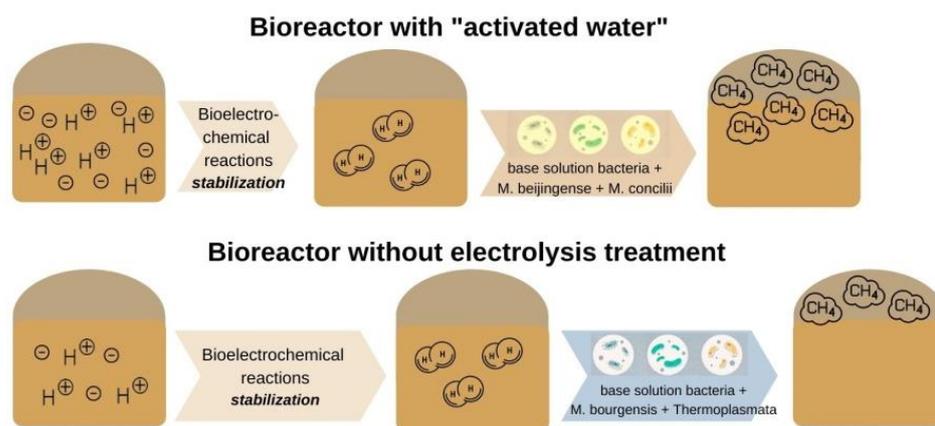
**Table 2.** Production of the components required in biocathode processes [62].

Element to Catalyze Processes	Type of Reaction	Description	Bacterial Groups
Electrons from electrodes	$\text{HCO}_3^- + \text{H}_2 + \text{H}^+ \rightarrow \text{CH}_4 + 3\text{H}_2\text{O}$	<ul style="list-style-type: none"> <li>Can catalyze the production of hydrogen and methane through electrons</li> <li>Reduction in <math>\text{CO}_2</math> into <math>\text{CH}_4</math></li> </ul>	<i>Hydrogenotrophic methanogens</i> (i.e., <i>Methanobacterium</i> or <i>Methanobrevibacter</i> )
Electron or hydrogen transfer	$2\text{H}^+ + 2\text{e}^- \xrightarrow{\text{(electricity)}} \text{H}_2$	<ul style="list-style-type: none"> <li>Functional correlation between species ensures</li> <li>Involvement in energy transduction (direct electron or hydrogen transfer) between the electrode surface and the methanogenic populations</li> </ul>	Between <i>Desulfovibrionaceae</i> family and the phylum <i>Euryarcheota</i>
Reduction in oxygen	$\text{CH}_4 + 2\text{O}_2 \rightarrow \text{CO}_2 + 2\text{H}_2\text{O}$	<ul style="list-style-type: none"> <li>Species of bacteria can enhance methane production by consuming oxygen, a factor that is toxic to methanogenic archaea</li> </ul>	<i>Hydrogenophaga caeni</i> , <i>Methylocystis</i> sp. and <i>Acidovorax caeni</i>
Reduced compounds (hydrogen or formate) as available substrate for other	$\text{HCO}_3^- + \text{H}_2 + \text{H}^+ \rightarrow \text{CH}_4 + 3\text{H}_2\text{O}$ $\text{HCOO}^- + 3\text{H}_2 + \text{H}^+ \rightarrow \text{CH}_4 + 2\text{H}_2\text{O}$	<ul style="list-style-type: none"> <li>Use cathodic electrons to produce reduced compounds faster than their metabolic capacity to use them</li> </ul>	<i>Methanothermobacter</i> , <i>Methanomicrobiales</i> , <i>Methanococcales</i> , <i>Methanocellales</i>

According to Cerrillo et al. [63], the combination of anaerobic digestion and a microbial electrolysis cell with a methanogenic biocathode also proved to be a promising waste treatment strategy for pig manure. Microbiological analysis showed that the methylotrophic family of *Methanossiliococcaceae* (genus *Methanomassiliococcus*) was the most abundant among active archaea in anaerobic digestion during the inhibited state. The *Methanobacteriaceae* family (genera *Methanobrevibacter* and *Methanobacterium*), generally considered the most abundant in methanogenic biocathodes, shared dominance with the *Methanomassiliococcaceae* families (genus *Methanomassiliococcus*) and *Methanotrichaceae* (genus *Methanotrix*) in the cathode.

The results of the Liu et al. study [64] showed that stimulation of direct electron transfer in the microbial electrolysis cell improves the processing of biogas. The methane content of biogas increased from 71% to >90% and 8.2%  $\text{CO}_2$  converted to methane, due to the fact that the acetoclastic methanogen *Methanotrix* in the cathode used the  $\text{CO}_2$  reduction pathway, while in the bulk sludge it used the acetate decarboxylation pathway to produce methane. Therefore, methane production was stimulated by hydrogen generation, which was used by autotrophic methanogens as electron donors through the  $\text{CO}_2$  reduction pathway.

One of the most recent studies by Park et al. [65] focused on microbial communities and the performance of a conventional anaerobic reactor combined with microbial electrolysis cells. According to the study bioelectrochemical reactions can reduce the stabilization period of the reactor and increase the amount of methane produced. Furthermore, an increase in the yield of methane was assumed because  $\text{e}^-$  and  $\text{H}^+$  produced during this process were converted to  $\text{H}_2$  by electrochemical reactions and then combined with  $\text{CO}_2$  to produce  $\text{CH}_4$ . Therefore,  $\text{H}_2$  and  $\text{CO}_2$  react with the formation of additional methane. However, the hydrogen content of the system with ‘activated water’ (AW) was higher than that of the experiment without electrolysis treatment during the start-up period. On the contrary, the gas compositions in the two reactors were the same during the stationary period. On this basis, it was assumed that the increase in methane yield and production was associated with changes in microbial communities under bioelectrochemical reactions, as shown in Figure 3.



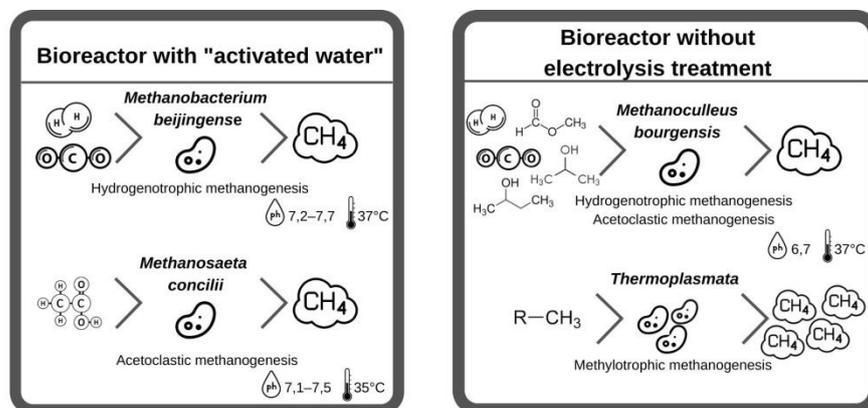
**Figure 3.** Bioelectrochemical reactions in the system with ‘activated water’ and the experiment without electrolysis treatment during the initial period.

There are definite differences between the microorganisms of the archaea communities in the basic solution, the dominant species in the unclassified solution with AW was *Methanobacterium beijingense* (63% of the total population), followed by *Methanosaeta concilii* (20% of the total population). In the unclassified solution without electrolysis treatment, *Methanoculleus bourgensis* was the dominant species (69% of the total population and unclassified microorganisms, 25%). The *Methanoculleus bourgensis* and *Thermoplasmata* classes, which dominated without electrolysis treatment, inhabit the anode surface of bioelectrochemical systems. *Methanobacterium beijingense*, which represents most of the microbial community with AW, is a hydrogenotrophic methanogen that generates methane from  $H_2$ ,  $CO_2$ , and formate and does not use substrates from methanol, ethanol, trimethylamine, isobutanol, or isopropanol. *Methanosaeta concilii*, which is the next largest fraction, is an acetoclastic methanogen that generates methane from acetic acid. Methane formation stops if methanol, trimethylamine, formic acid, propionic acid, butyric acid, and pyruvic acid come into contact with this microorganism. The main microorganisms with *Methanobacterium beijingense* and *Methanosaeta concilii* of AW use their respective substrates (acetic acid and formate) to produce methane, and the presence of other compounds negatively affects the formation of methane.

*Methanoculleus bourgensis* was the dominant species in the reactor without electrolysis treatment. The introduction of additional electron acceptors in the form of hydrogen, as well as the disintegration of complex organic compounds under the influence of redox reactions during electrolysis, was the main contributor to this, which is consistent with [65]. This species uses  $CO_2$ ,  $H_2$ , formate, 2-propanol, 2-butanol, and other secondary alcohols to generate methane with simultaneous acetoclastic and hydrogenotrophic methanogenesis, with a Gibbs free energy for the production of  $CO_2$  methanol lower than for reactions of acetoclastic and hydrogenotrophic methanogenesis. The non-electrolysis approach bioelectrochemically accelerates the methanol pathway and the production rate, and then the methanol produced in the cathode is converted directly to methane by advanced methylotrophic methanogens (Figure 4).

To summarize the changes in the archaea community in bioreactors with and without electrolysis, microbial communities that generate methane from certain substrates (formate and acetate) were found with AW, while *Methanoculleus bourgensis*, which generates methane using different substrates (formate, 2-propanol, 2-butanol, etc.), was found with AW, the *Thermoplasmata* community, which is a methylotrophic methanogen that generates more methane than other methanogens (such as acetoclastic or hydrogenotrophic methanogens), dominated without electrolysis treatment. Combined with the results on species diversity, the diversity and population of archaea communities were lower without electrolysis treatment than with AW. This change in the microbial community resulted in a difference in methane production. Furthermore, when analyzing the microbial community

without electrolysis treatment using food waste filtrate, the population of *Methanosarcina thermophila*, which grows with acetate, methanol, methylated amines, etc., and is part of the *Methanosarcinaceae* family, increased significantly. These results demonstrate that this medium promotes the growth of methylotrophic methanogens in the reactor without electrolysis treatment [65].



**Figure 4.** Comparison of bacterial bioprocesses in reactors with ‘activated water’ and without electrolysis treatment.

Overall, certain aspects ensure higher biogas production with electrolysis treatment for the stimulation bioprocess:

- The microbial electrolysis cell can increase the methane content;
- Electrokinetic decomposition deforms the cell walls of substrates, making their contents easily accessible to bacteria for anaerobic digestion [13];
- Treatment of liquid media with high-voltage electric pulses leads to inactivation of microorganisms at lower temperatures and in a shorter soaking time [15];
- The same increase in the yield and production of methane is associated with changes in the microbial community resulting in a difference in methane production [50].

#### 4. Conclusions

Prospective implementation methods, such as cavitation and electrolysis were considered to stimulate biofuel production processes. The cavitation method has a stimulating effect on the biogas yield and percentage of methane due to better biomass destruction and homogenization, release of natural enzymes and reduction in the digestion period. Bioelectrochemical reactions can shorten the stabilization period of the reactor and increase the amount of released methane due to the deformation of cell walls of substrate, inactivation of microorganisms, and changes in the microbial community under the influence of electrolysis treatment. It should be noted that it is important to find the best option for the use of intensification methods with a feasibility study of the effectiveness of use on an industrial scale.

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