Advances in Environmental Technology 10(3) 2024, 171-186.

Iranian Reasearch Organization for Science and Technology (IROST)

Advances in Environmental Technology

Journal home page: https://aet.irost.ir

Enhancing biogas production using ultrasound-assisted thermal pretreatment technology for anaerobic co-digestion of sewage sludge and microalgae substrates

Sepideh Abedi[a], Matin Khaleghi[b], Abbas Naeimi[b*]

[a]*Department of Renewable Energies Engineering, Faculty of Mechanical and Energy Engineering, Shahid Beheshti University, 1983969411, Tehran, Iran.*

[b]*Department of Energy Systems Engineering, Faculty of Mechanical and Energy Engineering, Shahid Beheshti University, 1983969411, Tehran, Iran.*

ARTICLE INFO

Document Type:
Research Paper

Article history:
Received 7 January 2024
Received in revised form 17 June 2024
Accepted 18 June 2024

Keywords:
Biogas
Anaerobic co-digestion
Pretreatment
Ultrasound
Microalgae

ABSTRACT

The study aimed to assess the effects of ultrasound-assisted thermal pretreatment on biogas production by co-digesting stress-adapted microalgae (*Synechocystis sp*.) with a combination of mixed municipal and industrial sewage sludge under anaerobic conditions. The pretreatment process involved subjecting the microalgae biomass to thermal pretreatment at temperatures of 70, 90, and 110°C while utilizing ultrasound pretreatment for the sludge (with an average solids content of 16.7 g/L) at a frequency of 25 kHz and power output of 400 W for durations of 3, 9, and 15 minutes before the main digestion process. The experiment was designed using response surface methodology (RSM) with a central composite design. The results revealed that the model exhibited statistical significance, with a probability value (Prob > F) of less than 0.05. Pre-treated samples demonstrated a substantial increase in biogas production compared to untreated samples, showing an average 1.4 to 5.6-fold enhancements. Optimization analysis indicated that the highest cumulative biogas production, amounting to 706 NmL, could be achieved after two weeks by pre-heating the microalgae cells at 110°C for one hour and subjecting the sewage sludge to ultrasound pretreatment for three minutes. These findings highlight the potential of ultrasound-assisted thermal pretreatment technology as a strategy to enhance biogas production through the co-digestion of stress-resilient microalgae and sewage sludge.

*Corresponding author Tel.: +982173932727
E-mail: a_naeimi@sbu.ac.ir
DOI: 10.22104/AET.2024.6691.1828

1. Introduction

The increasing trend in population growth and the consequent rise in demand for water and energy present a significant threat to essential fuel and drinking water resources for future generations. Freshwater withdrawals have tripled over the past 50 years, with a year-on-year demand increase of 64 billion m^3 [1]. Predictions indicate a 50% increase in global energy consumption by 2035 [2], which will undoubtedly impact water requirements. Despite this high demand for water and energy, significant amounts of wastewater (approximately 2.2 million m^3 globally) are produced annually from various sources such as agriculture, industry, and households [3]. In early 2017, approximately 80% of global sewage was being discarded without adequate treatment [4]. However, effective strategies and practices have decreased wastewater mismanagement to 48% [5], and recycling of wastewater has become a crucial aspect of water management policies in many countries, aiding in the conservation of precious water resources [6]. Nevertheless, the global demand for sewage treatment has led to an increase in sludge production with high levels of organic matter, resulting in detrimental environmental impacts such as improper disposal and groundwater contamination through leachate. Treating the sludge through anaerobic digestion (AD) can mitigate these negative consequences and produce renewable energies, turning it into a valuable resource [7]. Therefore, it is imperative to prioritize alternative energies, especially sustainable ones, and address water resources by developing technologies for wastewater treatment and recycling for safe reuse in nature. In recent years, there has been significant global development of biofuels, attributed to the utilization of economically viable non-edible sources [8], waste materials [9], and even sewage sludge [10] as renewable energy sources, contributing to a more sustainable and environmentally friendly future. Biogas generated from sewage sludge, with a methane content ranging from 40-75% by volume [11] and 25-60% of CO_2 [12], has the potential to generate significant amounts of energy. When used as fuel for a combined heat and power plant, it can produce both electrical and thermal energy, with up to 81% efficiency equating to 2 kWh and 3 kWh, respectively [13]. The biogas production process involves four essential biochemical reactions: hydrolysis, acidogenesis, acetogenesis, and methanogenesis [14]. Hydrolysis is the initial step where complex organic compounds are converted into simpler organic matter and monomers by hydrolyzing bacteria. Following hydrolysis, acidogenesis occurs, where acidic bacteria convert the monomers into acetic acid and hydrogen. Next, during the acetogenesis stage, acids are further transformed into acetate and CO_2. Finally, in the methanogenesis stage, the products from the previous stages are converted into methane [14]. Recently, there has been notable attention given to biogas upgrading technologies, including reaction kinetics [9] and current developments and applications of methane enrichment in microbial methanation processes [15]. As anaerobic processes are required for methane production from sewage sludge, various factors must be considered. These microbial processes occur in the absence of oxygen [11,16] and are influenced by a range of complex factors that impact their efficiency on an industrial scale [17,18]. These factors include temperature [19], pH [20,21], retention time [22], C.N ratio [23], nanoparticles [24], and the type and size of precursor particles, which can optimally affect biogas production efficiency (Table 1). The choice of substrate heavily influences the efficiency of biogas production, with agricultural waste [25], municipal solid waste (MSW) [26,27], industrial waste [28], and co-digestion of MWS [29] being utilized as substrates. To address this issue, a consortium substrate has been developed to combine various organic sources to provide sufficient nutrients for the microorganisms involved in the process. Microalgae represent a substrate with significant benefits for the AD process [36]. The biomass of microalgae contains essential components such as proteins, carbohydrates, and lipids. Certain species of microalgae exhibit a remarkable ability to absorb carbon [37], as well as trace amounts of metals and non-metallic minerals during their growth cycle, making them an effective biological treatment option for waste management. Furthermore, specific strains of microalgae, such as *Chlorella* [38], *Nannochloropsis* [38], and

Scynecosystis [39], have been found to enhance biogas production efficiency in AD systems. Studies have indicated that the use of *Chlorella*, for example, can significantly improve biogas production rates [40, 41]. Therefore, selecting an appropriate precursor, such as microalgae, can promote a better balance and ultimately increase the efficiency of the AD process. Moreover, microalgae show promise for coupling wastewater treatment and biofuel production [42]. They offer a sustainable approach to CO_2 bio-fixation, biomass production, nutrient removal from wastewater, and bioenergy generation [43]. Utilizing algal biomass provides economic advantages over conventional methods by allowing the recovery of various value-added bioproducts, including biopolymers, biochemicals, biofertilizers, and biofuels. This approach not only reduces production costs but also supports the development of a sustainable, algal-based green economy [44]. Furthermore, pretreatment methods for substrates play a crucial role in biogas production efficiency as they can impact methane content and reaction rates. Researchers have explored various pretreatment techniques, including mechanical [11], chemical [45], thermal [46], and biological [47] methods, with the aim of enhancing biogas production efficiency. Among these methods, ultrasound (US) pretreatment has shown particular effectiveness in inoculum preparation [48]. US waves create acoustic cavitation, which generates microscopic bubbles in the liquid medium. These bubbles collapse due to the rapid change in pressure, creating localized hotspots and intense turbulence. This mechanical shear force facilitates faster nutrient entry into the reaction and promotes the uptake of food by anaerobic bacteria [49]. However, it is important to note that the effects of ultrasonic pretreatment can vary depending on the specific application range. Outside of this range, the use of ultrasonic waves may have negative effects on the final efficiency due to certain factors [36]. Additionally, thermal pretreatment has proven to be highly efficient for algae substrates. Studies have indicated that applying thermal processing to an algae consortium can increase the reaction rate by improving the solubility of organic matter and nutrients, resulting in higher methane yields [36,38]. Several pretreatment methods have also been developed for biomass conversion into value-added products, with a focus on detoxifying inhibitors present in hydrolysate. Different strategies can be applied to mitigate the inhibitory effects of various compounds on microbial fermentation. These strategies involve the detoxification of hydrolysate using various physicochemical methods and the engineering of microbes to enhance their tolerance to inhibitors [50]. Therefore, pretreatment methods offer opportunities to optimize biogas production efficiency by manipulating reaction conditions and enhancing microbial activity. Ongoing research aims to explore further and refine these pretreatment techniques to maximize biogas yields and improve the overall sustainability of the process. While several studies have evaluated the impact of anaerobic co-digestion on methane yield [51], a few have investigated real continuous case studies on microalgal anaerobic co-digestion. Co-digestion of microalgae and sewage sludge involves combining these organic materials to undergo anaerobic digestion. In this biological process, microorganisms break down organic matter in the absence of oxygen to produce biogas, a mixture of primarily methane and carbon dioxide. The co-digestion reaction pathways and mechanisms of microalgae and sewage sludge are interconnected and leverage the unique properties of each material to enhance biogas production [52]. Microalgae, being rich in proteins, lipids, and carbohydrates, can complement the nutrient composition of sewage sludge, which typically contains a mix of organic materials derived from domestic and industrial sources. When combined, the microorganisms in the anaerobic digestion process can utilize the diverse substrate the microalgae and sewage sludge offer to enhance biogas production [53]. The co-digestion process can benefit from synergistic effects, such as improved microbial activity and metabolic pathways, leading to higher biogas yields compared to individual digestion of these materials. The novelty of this study lies in the development of a model for biogas production that takes into account different combinations of proper microalgae cells and sewage sludge pretreatment technologies during the co-digestion process. This study aimed to investigate the impact of ultrasonic-assisted thermal pretreatment on biogas production through the co-digestion of stress-adapted microalgae with municipal sewage sludge under anaerobic conditions. The combination of ultrasonic and thermal energy serves to improve the overall performance of pre-processing techniques by enhancing mass transfer, improving reaction kinetics, and facilitating the breakdown of complex structures within the material. Additionally, the potential of stress-resilient microalgae as an effective means of treating contaminated wastewater and extracting valuable refined materials in co-digestion with mixed sewage sludge was explored. The study also sought to investigate the impact of the simultaneous thermal pretreatment of microalgae cells and ultrasonic pretreatment of sewage sludge on biogas production efficiency. The optimal ranges of using mixed pretreatment parameters in microbial co-digestion were identified for the first time in this study, offering significant advantages in terms of enhanced biogas yields and overall process efficiency.

Table 1. Factors effect on biogas production using different substrates under AD process.

Parameter	Substrate	Value	Results	Ref.
Substrate	Animal manure (goat and pig)	157-500 mL.gVS^{-1}	Pig manure: 204–438.4 mL.gVS^{-1} Goat manure: 402–500 mL.gVS^{-1}	[30]
	Lignocellulose biomass	160 to 212 mL.gVS^{-1}	160.1-211.8 mL.gVS^{-1}	
	MSW	143–516 mL.gVS^{-1}	143 (Cucumber waste) 516 (Fruit and vegetable)	
	Industrial waste	25–429 mL.gVS^{-1}	25 mL.gVS^{-1} (Barley) 429 mL.gVS^{-1} (Pulp.paper mill sludge)	
	Glycerol and MWS activated sludge and MSW	1% Glycerol 50:50 (v.v)	115 % increase in biogas production 89 % increase in biogas production	[29]
	Mud primitive with returned Dough Paper	Mesophilic Conditions	131% increase in biogas production	
	MWS With Cheese Curd	90:10 (v.v)	121% increase in biogas production	
	MWS.Oil ,fat And Grace	52:48 (v.v)	198% increase in biogas production	
Temperature	MWS	30-40 °C (Mesophilic) 50-70°C (Thermophilic)	12.4% increase in biogas production (Thermophilic Condition)	[19,31]
pH	Co-digestion MSW.Activated sludge	7.1 (Mesophilic) 7.2 (Thermophilic)	35% increase in biogas production	[20]
	MWS	pH: 5-7	24% increase in methane content (pH=7)	[21]
TS	Concentrated sewage sludge	TS: 2.61-37.86%	1.7% increase in biogas production	[32,33]
	Digested sludge	TS: 2-10%	62% increase in biogas production (TS 8%)	[34]
C.N	MWS combined with fruit waste and cheese whey	C.N≈2-16.33	31% increase in biogas production (C.N:16.33)	[23]
Retention Time	Sewage Sludge	15-25 day	50 % of increase in biogas production	[22]
Size Particles	Cow manure Pig manure Chicken manure MWS	25-500 μm	59% increase in biogas production 39% increase in biogas production 53% increase in biogas production 39% increase in biogas production	[32]

2. Materials and methods

2.1. Sewage Sludge and Microalgae Preparation as Mixture Substrate

For this study, a consortium substrate composed of resilient microalgae and mixed municipal and industrial sewage sludge obtained from the Tehran South Wastewater Treatment Plant was utilized. This treatment plant is recognized as one of the most significant wastewater projects in the Middle East. The sewage sludge samples, with an average solids content of 16.7 g/L, were taken from the final wastewater pool created by condensing a mixture of domestic and industrial sewage sludge. In this pool, the total suspended solids (TSS) ranged from 20-40 kg/L, while the volatile suspended solids (VSS) content was 0.0336 kg/L. The COD.VS ratio was measured as 1.5±0.5. The *Synechocystis sp.* PCC 6803 cells as microalgae biomass, obtained from the Culture Collection of Algae and Protozoa (CCAP, Scotland), were gradually acclimated to mixed domestic and industrial wastewater before the pretreatment process. The optical density (OD) of the microalgae biomass (with a fresh weight of 1.23 mg/mL) was measured using a spectrophotometer at a wavelength of 600 nm. The pH was set to 7.1. The AD rate of the sewage sludge in TS was 8%, achieved using a weight ratio of 37:63 (v.v) for the mixed sewage sludge and microalgae, respectively.

2.2. Ultrasonic- assisted thermal pretreatment of substrates

The UP 400 S (400 W, 24 kHz) was utilized as the probing ultrasonic device to generate US waves for sludge pretreatment. Sludge samples with a volume of 63% (v.v) were exposed to a frequency of 25 kHz with amplitude of 70%, in accordance with the experiment matrix outlined in section 2.4. The ultrasonic intensity was set at 25 $W.cm^{-2}$. The pretreatment time for the sewage sludge was determined using Eq. 1, based on ultrasonic specific energy [49]:

$$E_s = \frac{P * t}{V * T_s} \quad (1)$$

where E_s represents the specific energy (kJ/kg), P is the device power (kW), t is the ultrasonic exposure time (s), V represents the sample volume (L), and T_s is the total suspended solids (kg/L). The values of E_s, P, V, and T_s were 5500 kJ/kg (based on the VS of sample and the extractable energy from the produced biogas [54,55]), 0.4 kW (based on the device specifications), 0.315 L, and 0.04 kg/L, respectively. In order to pre-treat the microalgal biomass, thermal treatment was employed on the cells (with biomass density of 1.236 kg/L) for a duration of 1 h using a digital oven set to 70°C, 90°C, and 110°C.

2.3. Biogas production using AD reactor

The water displacement method was used to measure the biogas production volume [56-58]. To this end, nine glass digester tanks with a volume of 50 mL and nine glass measurement containers containing a dilute acid solution with a volume concentration of 10% HCl were applied. A dilute acid solution was introduced into the measurement container via glass tubes. The volume of the produced biogas in each reactor was measured daily for 15 days. A water bath heated by a 400 W element and controlled via a temperature sensor, thermocouple, and thermostat were employed to control the temperature balance within the range of 38±1°C. Argon gas was injected into the headspace of the digester tanks before starting the AD. The reactors were spun by a mechanical mixer for one min every 6 h to prevent two-phase conditions. Each digester was filled with 37 wt% cultivated *Synechocystis sp.*, which was then mixed with water in a relative ratio of 1:3 (v.v).

2.4. Experiment design through response surface methodology

The experiment was designed via response surface methodology (RSM) [59] with central composite design (CCD) in Design Expert x13 to analyze the interaction of factors and predict an optimum pretreatment model for the highest biogas production. Figure 1 demonstrates a scheme of the designed experiment to investigate the interaction effect of the US-assisted thermal pretreatment of mixed substrate on biogas production. The independent variables for this study were sludge US pretreatment time (A) and microalgae biomass thermal pretreatment temperature (B), each at three levels of 3, 9, and 15 min and 70, 90, and 110°C, respectively (Table 2).

Table 2. RSM experimental design for optimizing biogas production using pretreatment.

Pretreatment type	Factors	Name	Unit	-1	0	+1
Ultrasound sludge pretreatment	A	Time	min	3	9	15
Thermal microalgae pretreatment	B	Temperature	°C	70	90	110

Also, control samples were prepared without a pretreatment process. Total biogas was measured every day as response data. Based on the designed matrix, a quadratic model was used to model the biogas production; A and B were defined as duration time of US treatment for sewage sludge and temperature of thermal treatment for microalgae cells, respectively. The Design Expert software offers a range of statistical tools for evaluating the reliability of a statistical model. In this study, the validity of the statistical model was evaluated using analysis of variance (ANOVA), residual analysis, and prediction error assessment. ANOVA serves as a statistical technique employed to determine the significance of the model terms and ascertain their contribution to the variability in the response variable. Residuals denote the disparities between the predicted and actual values of the response variable. The residuals of the model were examined through plots to assess whether they adhered to a normal distribution. Prediction error quantified the effectiveness of the model in forecasting the response variable for new observations. Cross-validation was employed to estimate the prediction error of the model and determine its accuracy.

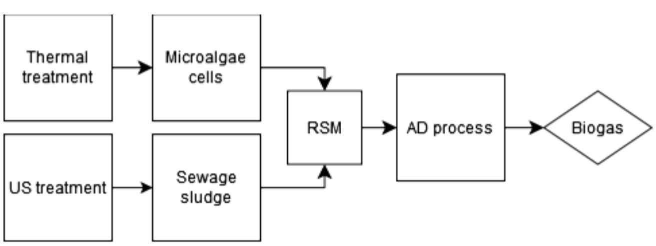

Fig. 1. A scheme of the experiment for evaluating the effect of the US-assisted thermal pretreatment of substrate on biogas production.

3. Results and discussion

3.1. Biogas production enhanced using combined pretreatment technology

Biogas production in the reactors was evaluated for 15 days based on the designed experiment (Table 3). The results demonstrated that the application of the mixed pretreatment process resulted in an average 1.4-5.6-fold increase in the accumulated production of biogas, ranging from a total amount of 167-704 mL compared to the control sample (120 mL). The cumulative biogas production results from co-digestion of treated sewage sludge and microalgae cells are presented in Figure 2.

Table 3. Accumulative biogas production amount as responses for the designed experiment.

Run	A: Time [min]	B: Temperature [°C]	Biogas [NmL]
1	0.51	90	373
2	9	90	556
3	9	61.72	210
4	17.49	90	360
5	3	70	469
6	15	70	386
7	3	110	704
8	9	118.28	302
9	15	110	167

Optimizing the temperature during thermal biomass pretreatment significantly impacts the production of biogas. The study revealed that increasing the temperature led to a corresponding increase in biogas production until it peaked at 110°C. Therefore, the most suitable temperature for the thermal pretreatment of this resilient microalgae, promoting AD, was observed to be 110°C for a duration of one hour. The chemical bonds between cellulose, hemicellulose, and pectin in the microalgae's cell wall were effectively broken down through thermal pretreatment. This process enhanced cell permeability, facilitating the release of intracellular compounds into the surrounding aqueous phase, ultimately resulting in improved biogas production [60]. On the other hand, algae are rich in volatile fatty acids (VFAs), which are also intermediate products of methanogenesis [61]. The formation and accumulation of VFAs can lead to microbial stress, resulting in acidification and a decline in the co-digesting process [62]. This factor could be investigated in future works. The results of the present study revealed a significant improvement in biogas production through US-assisted thermal pretreatment. This increment surpassed the outcomes of previous attempts involving US pretreatment before sludge AD (Table 4). US pretreatment of sewage sludge produces microbubbles that burst upon reaching a critical size, resulting in cavitations. This explosion generates high local temperature (~4726.85°C) and pressure (exceeding 500 bar), which affects the digestion process. The impact of this phenomenon on biogas production requires further investigation. Several factors contribute to enhanced hydrolysis of volatile solids, including hydro mechanical shear forces, radical formation, thermal decomposition of hydrophobic substances, and increased temperature. Although the specific mechanism by which US waves excite enzymes has yet to be extensively studied, it is known that enzyme activity depends on the active site configuration. Minor changes in medium conditions or composition can significantly alter the three-dimensional structure of the enzyme, affecting catalysis. These alterations in protein structure and enzyme activity are typically observed within the frequency range of 20 to 100 kHz [63]. This study has successfully developed stress-resilient cells of *Synechocystis sp.* through the gradual passaging of cells in highly toxic industrial and municipal wastewater effluent [6]; it is assumed that these cells may exhibit a new profile of enzymes as a result of biosynthetic changes. Identifying the enzymes and understanding how enzymes undergo changes under US waves is crucial because enzyme activity is influenced by structural alterations. It has been suggested that under appropriate US conditions, enzymes unfold, exposing the active site and facilitating faster substrate interaction, leading to increased reaction rates [64]. The effect of ultrasonic waves on enzymes is not fully understood, but it is known that enzyme activity is influenced by changes in the active site's configuration. Ultrasonic waves can cause structural alterations in the enzyme, resulting in increased enzyme activity due to an exposed active site and improved transport phenomena. However, it is unclear whether ultrasonic waves affect only the structure of the enzyme and substrate or also the reaction media. Ultrasonic intensity and duty cycle are crucial factors affecting enzymatic activity, with low-intensity and low-frequency US irradiation promoting stable cavitation bubbles that increase enzyme activity. Higher ultrasonic intensity can lead to denaturation of the polypeptide chain and decreased enzymatic activity. Enzymes can also undergo radical attack and modification of their secondary and tertiary structures under ultrasonic processing [65]. The diverse nature of enzymes requires a case-by-case study to understand their response to ultrasonic waves, with kinetic modeling providing valuable insights.

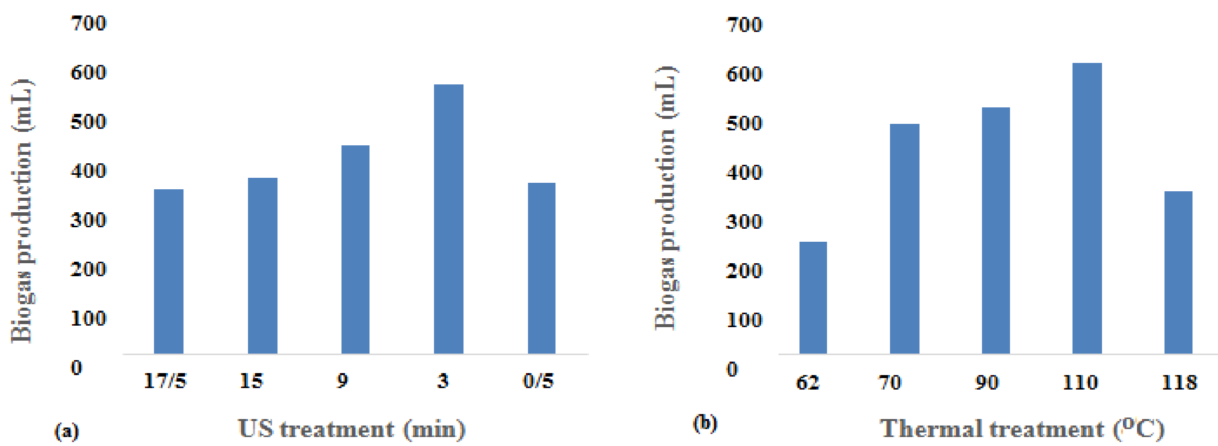

Fig. 2. Average of cumulative biogas production during co-digestion of mixed treated microalgae and sewage sludge affected by US treatment (a) and Thermal treatment (b).

Table 4. Comparison of the results of biogas production under US pretreatment with other studies.

Substrate	TS	Power	Frequency	Yield	Ref
Concentrated sludge	16.2–17.2 g/l	400 W	25 kHz	40-480 % increase in biogas	This study
Activated sludge	16.2–17.2 g/l	21KJg/TS	20 kHz	40% increase in CH_4	[66]
Activated sludge	1.78%	60 W	20 kHz	84% increase in biogas	
Activated sludge	3.5 %	100 W	20 kHz	32% increase in organic matter dissolution	[67]
Concentrated sludge	4.36%	100 W	20 kHz	27% increase in biogas	

As shown in the Table. 4, the highest increment in biogas production (40-480% increase) was observed while using different pretreatment processes particularly suitable for each substrate in co-digestion. In previous studies, it was demonstrated that thermal pretreatment of different microorganisms, such as *Arthrospira maxima, Chlorella sorokiniana, Nannochloropsis Salina, and Acutodesmus obliquus,* at various temperatures and times led to biogas production increases ranging from 12-72% [68]. Furthermore, ultrasonic pretreatment with frequencies between 20 and 40 kHz was found to enhance methane production by increasing the availability of macromolecular components for AD [69]. The highest increase in biogas production reported resulted in a 27-84% increase with an energy input between 21-35 $kJ.gTS^{-1}$ [66,67]. Moreover, a study [61] has demonstrated that US technology could enhance methane production. However, when ultrasound is combined with the thermal treatment of sludge, it hinders methane production but boosts hydrogen production [62]. Ultrasound pretreatment of sewage sludge disrupts the cell structure, facilitating the release of organic matter and making it more accessible to the microorganisms during anaerobic digestion. This can increase the rate of biogas production by enhancing the availability of substrates for microbial activity [49]. The application of ultrasound can also aid in reducing particle size and increasing the surface area of the sludge, thereby improving the efficiency of the digestion process. On the other hand, the heating pretreatment of microalgae can break down complex organic compounds, such as cell walls, and facilitate the release of intracellular components. This can lead to increased solubilization of organic matter and improved digestibility of the microalgae biomass during anaerobic digestion. As a result, the enhanced accessibility of nutrients and substrates from the pretreated microalgae can promote higher biogas production [70]. Co-digestion of microalgae and sewage sludge can lead to enhanced biogas production due to the complementary nutrient composition of the materials and synergistic effects on microbial activity. Furthermore, ultrasound and heating pretreatments can increase biogas production by improving the accessibility and digestibility of the organic matter in sewage sludge and microalgae, respectively, thereby optimizing the anaerobic digestion process.

3.3. Co-digestion improved using optimum combination of pretreatment processes

Statistical analysis of the results indicates a significant paired effect of sludge pretreatment with ultrasonic waves and algae thermal pretreatment on biogas production, as shown in the table for the given significance level (Figure 3). The ANOVA results between two effective factors on biogas production, including A: US time on sewage sludge and B: temperature on microalgae biomass, are presented in Table 5.

Table 5. ANOVA results: the impact of mixture thermal pretreatment of microalgae and US pretreatment of sludge on biogas production.

Source	Sum of Squares	df	Mean Square	F Value	p-value (Prob > F)[1]
Model	147.34	3	49.11	9.68	0.0159*
AB	46.03	1	46.03	9.07	0.0297
B^2	23.98	1	23.98	4.72	0.0818
AB^2	77.34	1	77.34	15.24	0.0114
Residual	25.38	5	5.08		
Cor Total	172.72	8			

[1] Values of "Prob > F" less than 0.05 indicate model terms are significant.

A: Sludge US pretreatment time

B: Microalgae biomass thermal pretreatment temperature

The Model F-value of 9.68 implied that the model was significant. There was only a 1.59% chance that a "Model F-Value" this large could occur due to noise. Values of "Prob > F" less than 0.050 indicated that the model terms were significant and, in this case, AB and AB^2 were significant model terms. The results of ANOVA, residual analysis, and prediction error demonstrated the validity of the statistical model in Design Expert, ensuring that it accurately represented the relationship between the independent and dependent variables. Figure 4 illustrates the relationship between pretreatment conditions and biogas production. The goal was to identify the combination that resulted in the highest desirability, which indicated the optimal solution. To minimize energy consumption during the pretreatment process, we aimed for a desirability of 83%. This was achieved by subjecting the microalgae cells to thermal pretreatment at 70°C and applying US for three minutes to the sewage sludge (Figure 4a). Under these conditions, biogas production was significantly enhanced, resulting in a total yield of 397.14 mL biogas over a span of two weeks. Furthermore, based on our second prediction, it was found that heating the microalgae cells to 110°C while maintaining the same duration of US pretreatment on the sludge could increase the desirability to 100% (Figure 4b). According to this updated model, the average biogas production throughout the entire mixed pretreatment process followed by the AD process would increase to 706 mL after two weeks. The final optimization equation is shown below:

$$\mathrm{Sqrt\ (Biogas\ production)} = 4.40\ AB^2 + 2.21\ B^2 + 3.39\ AB - 21.14 \quad (2)$$

These findings highlight the importance of optimizing the pretreatment conditions for both microalgae cells and sewage sludge to maximize biogas production. Significant improvements in biogas yields can be achieved by carefully selecting the appropriate combination of pretreatment parameters, such as temperature and US intensity. The economic aspect of pretreating sewage and microalgae is essential for improving process efficiency, reducing costs, and maximizing resource recovery. Proper pretreatment can enhance downstream processes like wastewater treatment and biofuel production, leading to reduced operation expenses and increased economic viability. By minimizing equipment damage and optimizing performance, pretreatment also helps lower maintenance costs. Investing in pretreatment offers long-term cost savings and improved economic feasibility for sewage and microalgae treatment processes. The combination of ultrasonic and thermal energy enhances pre-processing techniques by improving mass transfer, reaction kinetics, and breaking down complex material structures. Pretreatment methods with a positive energy balance are crucial for making anaerobic digestion economically feasible. The techno-economic analysis evaluates the economic viability of production processes to determine commercial feasibility [71]. Improving algal biomass productivity can reduce the cost of algae-derived biogas, achieved through strategies like recycling CO_2 for cultivation [72]. Life cycle and economic assessments explore the benefits of biogas production from microalgae biomass, highlighting factors affecting net energy ratio, economic benefit, nitrogen recovery, and greenhouse gas emissions.

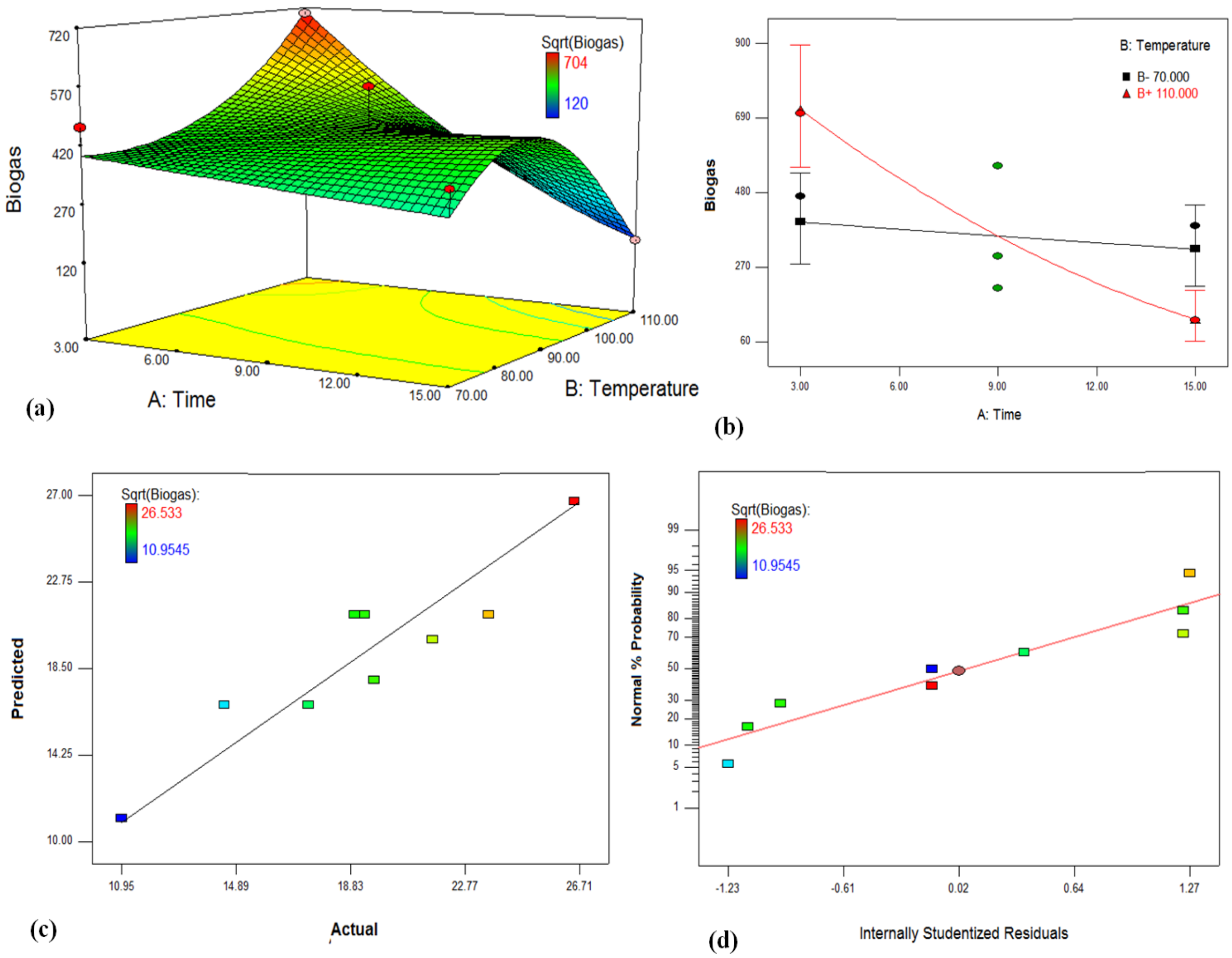

Fig. 3. Biogas production model based on the different mixture of pretreatment process on microalgae: (a) sludge, (b) interaction of factors plot, (c) normal plot of residuals and the predicted vs. actual plot, and (d) normal plot of residual.

4. Conclusions

In this study the impact of US-assisted thermal pretreatment on biogas production through the co-digestion of stress-adapted microalgae with mixed municipal and industrial sewage sludge was investigated under anaerobic conditions. The results indicated that the production of biogas could be enhanced by adjusting the temperature during thermal microalgae biomass pretreatment. Thermal pretreatment breaks down the chemical bonds present in cellulose, hemicellulose, and pectin within the cell walls of microalgae. As a result, the permeability of the cell increased, allowing for the release of intracellular compounds into the surrounding aqueous phase. This breakdown of chemical bonds and increased permeability contributed to improved biogas production during the subsequent AD process. On the other hand, ultrasonic promoted efficient mixing and enhanced mass transfer rates, leading to improved process efficiency. Ultrasonic energy could disrupt molecular and crystalline structures in sludge, making them more susceptible to subsequent processing steps during AD. This is particularly useful when dealing with complex sewage sludge, where breaking down the structure enhances extraction, digestion, and other desired transformations. The main results of this study based on the designed experiment through RSM are listed below:

i. The optimal temperature for the resilient microalgae cell pretreatment process was 110°C for duration of one hour.

ii. The optimal US time for sewage sludge pretreatment was three min at a frequency of 25 kHz, amplitude of 70%, and power output of 400 W.
iii. The prediction model and the interaction of pretreatment factors were significant based on the RSM investigation, with the highest desirability followed by an average 1.4-5.6-fold increase in biogas production.

The combination of ultrasonic and thermal energy offers several advantages in mixed pre-processing, including enhancing mass transfer, accelerating reaction kinetics, disruption of complex structures, energy efficiency, and versatility. The enhanced mass transfer resulting from ultrasonic-assisted thermal pretreatment also accelerates the reaction kinetics. By increasing the contact area and promoting the diffusion of reactants, ultrasonic waves facilitate faster reactions, reducing the required processing time. Combining ultrasonic and thermal energy allows for reduced processing temperatures and shorter processing times. This not only saves energy but also minimizes the risk of thermal degradation or unwanted side reactions, preserving the quality of the processed material.

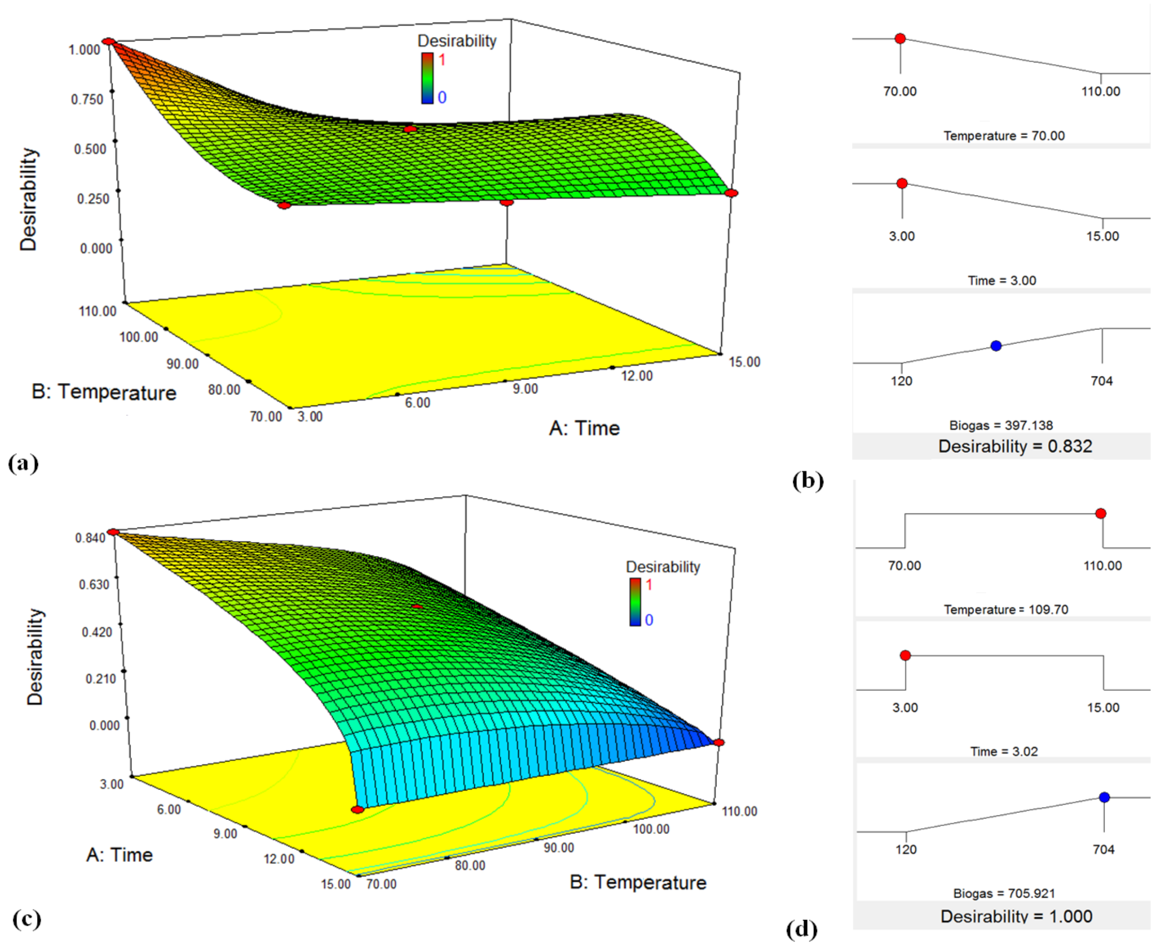

Fig. 4. Two Optimized models for the highest biogas production using combined pretreatment of microalgae and swege sludge. 3D RSM contore and optimized level of substrate mixture with desirebility of (a) 83% and (b) 100%.

Therefore, the ultrasonic-assisted thermal pretreatment is a promising technique in mixed pre-processing methods. Harnessing the synergistic effects of ultrasonic and thermal energy offers improved process efficiency, faster reaction kinetics, and enhanced product quality while being applicable to a wide range of materials and processes. Further investigation is required to comprehensively analyze the effects of the combination of ultrasound pretreatment of sludge and thermal treatment of microalgae cells on the composition of biogas. While various sludge pretreatment methods have been implemented at an industrial scale, assessing newer technologies at lab and pilot scales is necessary for a solid techno-economic analysis. Balancing operational, economic, and environmental concerns in selecting efficient pretreatment applications is challenging. Future research should focus on scaling implications and integrating energy-efficient waste management practices for sustainable solutions. Focusing on these areas aims to unlock the immense potential of stress-resilient microalgae and advance sustainable solutions for wastewater treatment while simultaneously harnessing valuable resources for energy production.

Authors contribution

AN supervising and support, SA designed the experiments and analyzed data, M.KH and SA performed experiments, SA and M. KH wrote the manuscript.

Acknowledgment

The authors wish to express their appreciation to the Tehran Water and Wastewater Company for supplying the sludge sample and its related quality attributes. Additionally, we are grateful to the Water Laboratory at the Faculty of Civil Engineering, Shahid Beheshti University, for analyzing the sewage sludge's characteristics, the Laboratory of Nano-Mineral Materials at the Faculty of Sciences, Tehran University, for their assistance with ultrasonic testing, and IRSA BioEnergy Laboratory for their support in biogas production facilitation.

References

[1] Connor, R., Faurès, J. M., Kuylenstierna, J., Margat, J., Steduto, P., Vallée, D. Hoe, W. V. D. , 2009. Chapter 7: Evolution of water use., in The United Nations World Water Development Report 3–Water in a Changing World, E.K. (WWAP), Editor. London, UNESCO: London.

[2] Matsuo, Y., A. Yanagisawa, and Y. Yamashita, (2013). A global energy outlook to 2035 with strategic considerations for Asia and Middle East energy supply and demand interdependencies. *Energy strategy reviews*, *2*(1), 79-91. https://doi.org/10.1016/j.esr.2013.04.002

[3] Connor, R., et al., (2017). The United Nations World Water Development Report 2017. Wastewater: The Untapped Resource, Facts and Figures in "WWAP (United Nations World Water Assessment Programme). : Paris, UNESCO.

[4] UNWWAP, W., (2017). The United Nations World Water Development Report 2017- Wastewater: The untapped resource, UNESCO, Paris.

[5] Jones, E.R., et al., (2021). Country-level and gridded estimates of wastewater production, collection, treatment and reuse. *Earth Syst. Sci. Data*, *13*(2), 237-254. https://doi.org/10.5194/essd-13-237-2021

[6] Abedi, S., et al., (2019). Decoupling a novel Trichormus variabilis-Synechocystis sp. interaction to boost phycoremediation. *Scientific reports*, *9*(1), 2511. https://doi.org/10.1038/s41598-019-38997-7

[7] Tyagi, V. K., Lo, S. L. (2013). Sludge: a waste or renewable source for energy and resources recovery? *Renewable and sustainable energy reviews*, *25*, 708-728. https://doi.org/10.1016/j.rser.2013.05.029

[8] Hajinezhad, A., et al., (2015). Biodiesel production from Norouzak (*Salvia lerifolia*) seeds as an indigenous source of bio fuel in Iran using ultrasound. *Energy conversion and management*, 99, 132-140. https://doi.org/10.1016/j.enconman.2015.04.034

[9] Abedi, S., A. Nozarpour, and O. Tavakoli, (2023). Evaluation of Biogas Production Rate and Leachate Treatment in Landfill Through a Water-Energy Nexus Framework for Integrated Waste Management. *Energy nexus*, *11*, 100218. https://doi.org/10.1016/j.nexus.2023.100218

[10] Abedi, S., M. Khaleghi, and A. Naemi, (2021). Bioenergy Production Potential Using Anaerobic Digestion of Wastewater Sludge (Case study: Sabzevar Wastewater Treatment Plant) in 5th International Conference on Applied Researches in Science and Engineering, University of Amsterdam.

[11] Khanh Nguyen, V., et al., (2021). Review on pretreatment techniques to improve anaerobic digestion of sewage sludge. *Fuel*, *285*, 119105. https://doi.org/10.1016/j.fuel.2020.119105

[12] Verbeeck, K., et al., (2018). Upgrading the value of anaerobic digestion via chemical production from grid injected biomethane. *Energy and environmental science*, *11*(7), 1788-1802. http://doi.org/10.1039/C8EE01059E

[13] Kaparaju, P., Rintala, J. (2013). Generation of heat and power from biogas for stationary applications: boilers, gas engines and turbines, combined heat and power (CHP) plants and fuel cells. In *The biogas handbook* (pp. 404-427). Woodhead Publishing.

[14] Uddin, M.M. and M.M. Wright, (2022). Anaerobic digestion fundamentals, challenges, and technological advances. *Physical Sciences Reviews*. https://doi.org/10.1515/psr-2021-0068

[15] Aryal, N., et al., (2018). An overview of microbial biogas enrichment. *Bioresource Technology*, 264, 359-369. https://doi.org/10.1016/j.biortech.2018.06.013

[16] Nguyen, V.K., et al., (2021). Review on pretreatment techniques to improve anaerobic digestion of sewage sludge. *Fuel*, 285, 119105. https://doi.org/10.1016/j.fuel.2020.119105

[17] Mahmudul, H.M., et al., (2017). Production, characterization and performance of biodiesel as an alternative fuel in diesel engines – A review. *Renewable and Sustainable energy reviews*, *72*, 497-509. http://doi.org/10.1016/j.rser.2017.01.001

[18] Patinvoh, R.J. and M.J. Taherzadeh, (2019). Challenges of biogas implementation in developing countries. *Current opinion in environmental science and health*, 12, 30-37. https://doi.org/10.1016/j.coesh.2019.09.006.

[19] Van, D.P., et al., (2020). A review of anaerobic digestion systems for biodegradable waste: Configurations, operating parameters, and current trends. *Environmental engineering research*, *25*(1), 1-17. https://doi.org/10.4491/eer.2018.334

[20] Liu, C.-f., et al., (2008). Prediction of methane yield at optimum pH for anaerobic digestion of organic fraction of municipal solid waste. *Bioresource technology*, *99*(4), 882-888. https://doi.org/10.1016/j.biortech.2007.01.013

[21] Latif, A., C. Mehta, and D. Batstone, (2017). Influence of low pH on continuous anaerobic digestion of waste activated sludge. *Water research*, *113*. https://doi.org/10.1016/j.watres.2017.02.002

[22] Liu, J., et al., (2020). Enhanced anaerobic digestion of sewage sludge by thermal or alkaline-thermal pretreatments: Influence of hydraulic retention time reduction. *International journal of hydrogen energy*, *45*(4), 2655-2667. https://doi.org/10.1016/j.ijhydene.2019.11.198

[23] Hallaji, S.M., M. Kuroshkarim, and S.P. Moussavi, (2019). Enhancing methane production using anaerobic co-digestion of waste activated sludge with combined fruit waste and cheese whey. *BMC Biotechnology*, *19*(1), 19. https://doi.org/10.1186/s12896-019-0513-y

[24] Hassanpourmoghadam, L., et al., (2023). Effect of Fe_3O_4 nanoparticles on anaerobic digestion of municipal wastewater sludge. *Biomass and Bioenergy*, 169, 106692. https://doi.org/10.1016/j.biombioe.2022.106692

[25] Laiq Ur Rehman, M., et al., (2019). Anaerobic digestion. *Water environment research*, *91*(10), 1253-1271. https://doi.org/10.1002/wer.1219

[26] Oladejo, J., et al. (2019). A Review of Sludge-to-Energy Recovery Methods. *Energies*, *12*. https://doi.org/10.3390/en12010060

[27] Heidari, A., Taheri, A., Rezaei, M., Jahanbakhshi, A. (2023). Biogas production and electrical power potential, challenges and barriers from municipal solid waste (MSW) for developing countries: A review study in Iran, *Journal of agriculture and food research, 13*, 100668. https://doi.org/10.1016/j.jafr.2023.100668

[28] De Mes, T. Z. D., Stams, A. J. M., Reith, J. H., Zeeman, G. (2003). Methane production by anaerobic digestion of wastewater and solid wastes. *Bio-methane and Bio-hydrogen*, *2003*, 58-102.

[29] Chow, W.L., et al., (2020). Anaerobic Co-Digestion of Wastewater Sludge: A Review of Potential Co-Substrates and Operating Factors for Improved Methane Yield. *Processes*, *8*(1), 39. https://doi.org/10.3390/pr8010039

[30] Nwokolo, N., et al., (2020). Waste to Energy: A Focus on the Impact of Substrate Type in Biogas Production. *Processes, 8*(10), 1224.

[31] Gebreeyessus, G.D. and P. Jenicek, (2016). Thermophilic versus mesophilic anaerobic digestion of sewage sludge: A comparative review. *Bioengineering (Basel)*, *3*(2). https://doi.org/10.3390/bioengineering3020015

[32] Odirile, P.T., et al. (2021). Anaerobic digestion for biogas production from municipal sewage sludge: A comparative study between fine mesh sieved primary sludge and sedimented primary sludge. *Water*, 13. https://doi.org/10.3390/w13243532

[33] Odirile, P.T., et al., (2021). Anaerobic digestion for biogas production from municipal sewage sludge: A comparative study between fine mesh sieved primary sludge and sedimented primary sludge. *Water*, *13*(24), 3532. https://doi.org/10.3390/w13243532

[34] An, D., et al., (2017). Effects of total solids content on performance of sludge mesophilic anaerobic digestion and dewaterability of digested sludge. *Waste management*, *62*, 188-193. https://doi.org/10.1016/j.wasman.2017.01.042

[35] Sebola, M., E. Muzenda, and H. Tesfagiorgis, (2015). Effect of particle size on anaerobic digestion of different feedstocks. *South African journal of chemical engineering*, *20*(3), 11-26.

[36] Thorin, E., et al., (2018). Co-digestion of sewage sludge and microalgae – Biogas production investigations. *Applied energy*, *227*, 64-72. https://doi.org/10.1016/j.apenergy.2017.08.085

[37] Lage, S., A. Toffolo, and F.G. Gentili, (2021). Microalgal growth, nitrogen uptake and storage, and dissolved oxygen production in a polyculture based-open pond fed with municipal wastewater in Northern Sweden. *Chemosphere*, 276, 130122. https://doi.org/10.1016/j.chemosphere.2021.130122

[38] Ugurlu, A. and E. Kendir, (2018). A comprehensive review on pretreatment of microalgae for biogas production. *International journal of energy research*, *42*. https://doi.org/10.1002/er.4100

[39] Yu, Y., et al., (2013). Development of Synechocystis sp. PCC 6803 as a Phototrophic Cell Factory. *Marine drugs*, *11*(8), 2894-2916. https://doi.org/10.3390/md11082894

[40] Ometto, F., et al., (2014). Impacts of microalgae pre-treatments for improved anaerobic digestion: Thermal treatment, thermal hydrolysis, ultrasound and enzymatic hydrolysis. *Water research*, *65*, 350-361. https://doi.org/10.1016/j.watres.2014.07.040

[41] Mendez, L., et al., (2013). Enhancing methane production of Chlorella vulgaris via thermochemical pretreatments. *Bioresour technol*, 149, *136*-41. https://doi.org/10.1016/j.biortech.2013.08.136.

[42] Abedi, S., et al., (2019). Bioenergy production using Trichormus variabilis–a review. *Biofuels, Bioproducts and biorefining*, *13*, 1365-1382. https://doi.org/10.1002/bbb.2023

[43] Abedi, S., et al., (2017). Evaluation of Biomass Production and Wastewater Nutrient Removal Using Microalgae: Sustainable Strategy to CO2 Bio-Fixation and Bioenergy Production Approach. *Journal of renewable energy and environment*, *4*(4), 39-48. https://doi.org/10.30501/jree.2017.88374

[44] Kannah, R.Y., et al., (2021). A Mini Review of Biochemical Conversion of Algal Biorefinery. *Energy and fuels*, *35*(21), 16995-17007. https://doi.org/10.1021/acs.energyfuels.1c02294

[45] Siami, S., et al., (2020). Process optimization and effect of thermal, alkaline, H_2O_2 oxidation and combination pretreatment of sewage sludge on solubilization and anaerobic digestion. *BMC Biotechnology*, *20*(1), 21. https://doi.org/10.1186/s12896-020-00614-1

[46] Gahlot, P., et al., (2022). Principles and potential of thermal hydrolysis of sewage sludge to enhance anaerobic digestion. *Environmental research*, *214*, 113856. https://doi.org/10.1016/j.envres.2022.113856

[47] Mitraka, G.-C., et al., (2022). A Comprehensive Review on Pretreatment Methods for Enhanced Biogas Production from Sewage Sludge. *Energies*, *15*(18), 6536.

https://doi.org/10.3390/en15186536.

[48] Karouach, F., et al., (2020). Effect of combined mechanical–ultrasonic pretreatment on mesophilic anaerobic digestion of household organic waste fraction in Morocco. *Energy reports*, *6*, 310-314. https://doi.org/10.1016/j.egyr.2019.11.081

[49] Pilli, S., et al., (2011). Ultrasonic pretreatment of sludge: A review. *Ultrasonics sonochemistry*, *18*(1), 1-18. https://doi.org/10.1016/j.ultsonch.2010.02.014

[50] Bhatia, S.K., et al., (2020). Recent developments in pretreatment technologies on lignocellulosic biomass: Effect of key parameters, technological improvements, and challenges. *Bioresource technology*, *300*, 122724. https://doi.org/10.1016/j.biortech.2019.122724

[51] Du, X., et al., (2019). Synergistic methane production from the anaerobic co-digestion of Spirulina platensis with food waste and sewage sludge at high solid concentrations. *Renewable energy*, *142*, 55-61. https://doi.org/10.1016/j.renene.2019.04.062

[52] Gonçalves, R.F., et al., (2020). Co-digestion of municipal wastewater and microalgae biomass in an upflow anaerobic sludge blanket reactor. *Algal research*, *52*, 102117. https://doi.org/10.1016/j.algal.2020.102117

[53] Wang, X., et al., (2022). Synergetic utilization of sewage sludge and microalgae: A review and proposal. *Bioresource technology reports*, *19*, 101146. https://doi.org/10.1016/j.biteb.2022.101146

[54] Safieddin Ardebili, S., (2020). Green electricity generation potential from biogas produced by anaerobic digestion of farm animal waste and agriculture residues in Iran. *Renewable energy*, *154*. https://doi.org/10.1016/j.renene.2020.02.102

[55] Staley, J.T., (2006). The bacterial species dilemma and the genomic-phylogenetic species concept. *Philos Trans R Soc Lond B Biol Sci*, *361*(1475), 1899-909. https://doi.org/10.1098/rstb.2006.1914

[56] Selvankumar, T., et al., (2017). Process optimization of biogas energy production from cow dung with alkali pre-treated coffee pulp. *3 Biotech*, *7*(4), 254. https://doi.org/10.1007/s13205-017-0884-5

[57] Zeynali, R., M. Khojastehpour, and M. Ebrahimi-Nik, (2017). Effect of ultrasonic pre-treatment on biogas yield and specific energy in anaerobic digestion of fruit and vegetable wholesale market wastes. *Sustainable environment research*, *27*. https://doi.org/10.1016/j.serj.2017.07.001

[58] Yuan, X., et al., (2014). Enhancing the anaerobic digestion of lignocellulose of municipal solid waste using a microbial pretreatment method. *Bioresour technol*, *154*, 1-9. https://doi.org/10.1016/j.biortech.2013.11.090

[59] Mohammadi, S., et al., (2022). Optimization of a three-dimensional electrochemical process with granular activated carbon for diclofenac removal using response surface methodology. *Environmental progress and sustainable energy*, *41*(1), e13715. https://doi.org/10.1002/ep.13715

[60] Wang, M., et al., (2017). Thermal pretreatment of microalgae for biomethane production: experimental studies, kinetics and energy analysis. *Journal of chemical technology and biotechnology*, *92*(2), 399-407. https://doi.org/10.1002/jctb.5018

[61] Petrovič, A., et al., (2022). The advantages of co-digestion of vegetable oil industry by-products and sewage sludge: Biogas production potential, kinetic analysis and digestate valorisation. *Journal of environmental management*, *318*, 115566. https://doi.org/10.1016/j.jenvman.2022.115566

[62] Li, L., et al., (2021). Short-chain fatty acids resource recovery potential from algal sludge via anaerobic fermentation under various pH values. *Chemosphere*, *275*, 129954. https://doi.org/10.1016/j.chemosphere.2021.129954

[63] Córdova, A., et al. (2022). Recent advances in the application of enzyme processing assisted by ultrasound in Agri-Foods: A review. *Catalysts*, *12*(1),107. https://doi.org/10.3390/catal12010107

[64] Subhedar, P.B. and P.R. Gogate, (2014). Enhancing the activity of cellulase enzyme using ultrasonic irradiations. *Journal of molecular catalysis B: Enzymatic*, *101*, 108-114.

https://doi.org/10.1016/j.molcatb.2014.01.002

[65] Mawson, R., et al., 2011. Ultrasound in enzyme activation and inactivation, in ultrasound technologies for food and bioprocessing, H. Feng, G. Barbosa-Canovas, and J. Weiss, Editors. *Springer New York*: New York, NY.369-404.

[66] Tian, X., W.J. Ng, and A.P. Trzcinski, (2018). Optimizing the synergistic effect of sodium hydroxide/ultrasound pre-treatment of sludge. *Ultrasonics sonochemistry*, *48*, 432-440. https://doi.org/10.1016/j.ultsonch.2018.07.005

[67] Lizama, A.C., et al., (2017). Effects of ultrasonic pretreatment on the solubilization and kinetic study of biogas production from anaerobic digestion of waste activated sludge. *International biodeterioration and biodegradation*, *123*, 1-9. https://doi.org/10.1016/j.ibiod.2017.05.020

[68] Kendir, E. and A. Ugurlu, (2018). A comprehensive review on pretreatment of microalgae for biogas production. *International journal of energy research*, *42*(12), 3711-3731. https://doi.org/10.1002/er.4100

[69] Volschan Junior, I., R. de Almeida, and M.C. Cammarota, (2021). A review of sludge pretreatment methods and co-digestion to boost biogas production and energy self-sufficiency in wastewater treatment plants. *Journal of water process engineering*, *40*, 101857. https://doi.org/10.1016/j.jwpe.2020.101857

[70] Passos, F., et al., (2014). Pretreatment of microalgae to improve biogas production: A review. *Bioresource technology*, *172*, 403-412. https://doi.org/10.1016/j.biortech.2014.08.114

[71] Das, T., Al-Waili, I., Balasubramanian, V., Appleby, G., Kaparaju, P., Parthasarathy, R., Eshtiaghi, N. (2024). Process modelling and techno-economic analysis of anaerobic digestion of sewage sludge integrated with wet oxidation using a gravity pressure vessel and thermal hydrolysis. *Science of the total environment*, *912*, 169024. https://doi.org/10.1016/j.scitotenv.2023.169024

[72] Wu, N., Moreira, C. M., Zhang, Y., Doan, N., Yang, S., Phlips, E. J., Pullammanappallil, P. C. (2019). Techno-economic analysis of biogas production from microalgae through anaerobic digestion. *Anaerobic digestion*, 113-117. https://doi.org/10.5772/intechopen.86090

How to site this paper:

Abedi, S., Khaleghi, M., & Naeimi, A. (2024). Enhancing Biogas Production Using Ultrasound-Assisted Thermal Pre-Treatment Technology for Anaerobic Co-Digestion of Sewage Sludge and Microalgae Substrates. Advances in Environmental Technology, 10(3), 171-186. doi: 10.22104/aet.2024.6691.1828