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Optimisation of Ultrasound Pretreatment of Microalgal Biomass for Effective Biogas Production through Anaerobic Digestion Process

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Abstract: The anaerobic digestion, AD, process presents a solution for sustainable waste management, greenhouse gas mitigation and energy production for growing population needs and requirements. Adopting a biorefinery approach that utilises different feedstock may enhance energy production and support optimisation of the anaerobic digestion process. Algae is a promising feedstock that could be used for energy production via the anaerobic digestion process. Microalgal biomass is rich in carbohydrates and lipids; however, many species of algae exhibit tough cell walls that could also be difficult to digest and may influence or inhibit the efficiency of the AD process. This study concentrated on the comparison of AD remediation of two marine algal biomass species, *Tetraselmis suecica* and *Nannochloropsis oceanica*. The two species were pre-treated with an ultrasound technique and compared for their methane production using biochemical methane potential tests. For *Tetraselmis*, a specific methane production of 0.165 LCH4/KgVS was observed; however, for *Nannochloropsis*, a value of 0.101 LCH_4/KgVS was observed for the samples treated with ultrasound. The BMP results from this study show that among the two micro-algae species tested, *Tetraselmis suecica* is found to be a better substrate for methane production potential. Contrary to increasing the specific methane production, ultrasound cavitation caused a slight decrease in the specific methane production values for both *Nannochloropsis oceanica* and *Tetraselmis suecica* biomass residues. The pre-treatment of the biomass using ultrasound techniques provided comparable results and can be recommended for effective bioenergy production. However, further research is required for the optimisation of the pre-treatment of microalgae and for the integration of microalgal biorefineries for circular economy.

Keywords: *Tetraselmis suecica*; *Nannochloropsis oceanica*; bioenergy production; algal biomass valorisation; ultrasound pretreatment; algal biotechnology; anaerobic digestion

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1. Introduction

The environmental pressure of human-generated pollution is one of the main challenges of modern society. Anaerobic digestion (AD) is an effective process widely used globally for the reduction of organic wastes and for the generation of energy from waste resources worldwide, particularly in the UK. AD has been evidenced to reduce pollution from methane (by 90%) and nitrogen oxide (by 50%) produced from food, manure and agricultural waste processes and has a high capability of producing biogas, including for electricity generation. The limiting factors for the high-end usage of AD are the economics, which include high capital costs, lack of funding via insufficient tariff systems and the current legislation related to the application of the process [1]. However, recent

studies have shown that on a European level, adoption of AD technology on a smaller scale (small-scale anaerobic digestion, SSAD) has potential in utilising various feedstocks for bioenergy utilisation applications [2]. With the nations' COP-26 commitments, AD can also help achieve long-term reduction targets such as EU landfill reduction by 35% compared to 1995 levels [1]. Despite scientific research, the interest in farm-scale AD or AD processes in general in the UK is still underutilised and can therefore offer a significant opportunity to contribute to UK government's net zero strategy for 2050 for renewable energy generation and waste management with supportive AD policies, incentives and regulations in place [3]. The life cycle analysis (LCA) study on the AD process is also promising from a life cycle analysis perspective for a complex feedstock such as food waste, where the results agreed that AD scenarios were preferable to incineration with their added environmental benefits [4].

Algal biomass is a viable feedstock for the AD process. Algae are facultative photosynthetic organisms, with high growth rates compared to terrestrial plants and rapid nutrient assimilation potential. Algal biomass does not contain recalcitrant lignocellulosic compounds and hence the process of biochemical disintegration through anaerobic digestion is comparatively easier than with other feedstocks and provides a higher energy production rate [5]. Additionally, algae can be used to remediate the waste nutrients coming after the AD process [6].

The research into combining microalgae and anaerobic digestion is still current with the recent focus valorising microalgal products including the growth and cultivation of microalgae on AD wastewater to maximise energy recovery from the AD process [7,8]. Microalgae, due to their biochemical properties, have been identified as a suitable biomass for bioremediation of liquid effluents; hence, integration of AD and microalgae cultivation allows nutrients and CO_2 recovery, thus enhancing the sustainability of the process [7].

Ultrasound was first used and shown as successful to harvest microalgae in a lab or at the pilot scale, as it has the advantage of being operated continuously with no shear stress and less costs [9]. Further studies into utilising ultrasound explored the extraction of lipids from microalgal cells in comparison to traditional methods and found that all ultrasound-assisted methods recovered more lipids than the conventional methods [10]. The research into ultrasound was further extended to its effect on the growth and viability of microalgae cells (*Dunaliella salina, Chlamydomonas concordia and Nannocholoropsis oculata*) and found that the cell walls of *D. salina* were completely disrupted after four minutes of sonication; however, for *C. concordia* the same process took sixteen minutes for total cell wall disruption. The same process was less effective for *N.oculata*, (a species with a thick cell wall), which did not show complete cell disruption; however, a decrease in the level of intracellular chlorophyll was found [11]. These studies indicate that the effectiveness of ultrasound is dependent on the efficiency of the ultrasound equipment (energy applied), time used for cavitation and mainly on the choice of microalgal strain.

Comparing different microalgal pre-treatment methods such as thermal, hydrothermal, microwave, and especially ultrasound technologies has been researched well since the 2010s to study microalgae characteristics, structure and applications as biofuels [12–15]. Nevertheless, studies have shown both negative [10] and positive results [14] for utilising ultrasound for improving methane yield and solubilisation of the microalgal biomass. These studies have had their focus on the physical pre-treatment spectrum, which has been shown to be not the only way of pre-treating microalgal cells.

This also indicates the need for further exploration in the microalgal pre-treatment research sector to be more cost- and energy-effective, which still continues to be the case with new methods such as pressurised liquid extraction, supercritical fluid extraction and chemical-based methods [16]. Furthermore, recent reviews have stressed that microalgae biomass such as *Botryococcus braunii and Chlorella vulgaris* harvested from different technologies (microwave) are unique in their properties, as the former provided better lipid extraction results with microwave pretreatment than the latter species and that still needs further understanding prior to valorisation or bioenergy routes [17,18]. In addition, cost

and energy requirements are the main limitations for pre-treatment [19]. Even though microalgae have been vastly studied for their pre-treatment, structural and biochemical properties and biotechnology applications, there is still a gap in the literature that requires results from pretreatment experiments conducted on microalgal biomass that are then analysed for their anaerobic digestion potential.

Ultrasound was also studied as a pre-treatment for microalgal anaerobic digestion to increase the biodegradability of cells. When ultrasound was compared with thermal and mechanical pretreatments for AD, methane production from the microalgae species (*Monoraphidium* sp. and *Stigeoclonium* sp.) was not significantly improved; however, proteins and carbohydrates were solubilised better, which are the main composition of biomass and the cell wall [14]. In another study, when *Arthrospira maxima*, *Scenedesmus Obliquus* and *Chlorella sorokiniana* were subjected to ultrasound as a pretreatment to AD, increasing the amount of ultrasound energy demonstrated a linear correlation between the energy input and soluble chemical oxygen demand (sCOD) released for *S.Obliquus* and *C. sorokiniana*. However, ultrasound produced more structural damage to the cells of *A. maxima* as more intracellular material was discharged [15]. In another study, when *Chlorella vulgaris* was subjected to ultrasound pre-treatment prior to anaerobic digestion, it was found that specific biogas production and biodegradation were increased due to increased cell disintegration from the cavitation [12]. Therefore, from the literature, it can be seen that the efficiency and application of the ultrasound pre-treatment method depend upon the selected microalgal strain, cell wall characteristics and overall cost and energy requirements of the AD process. The studies reviewed in this research has shown that ultrasound has the potential to maximize biogas yields and is cost-effective for large-scale applications.

Biogas production from microalgae has been focused on green species, especially from the genera Chlorella and Scenedesmus, and has reported low biomethane yields due to their rigid cell wall characteristics that hinder biodegradation during digestion. This has resulted in the exploration of other microalga species and genera and even macroalgae. Among the pre-treatments studied, ultrasound is the most-studied mechanical method used for microalgae digestion. It has been studied for its effectiveness on AD, more for species of Chlorella and less for *Nannochloropsis* and *Tetraselmis*. The theoretical methane production for *Tetraselmis suecica* has been given to be 0.41 LCH_4/KgVS for the whole cell without any pretreatment on the species; however, with any extraction pretreatments, the estimated methane production is reported to be between 0.34–0.20 LCH_4/KgVS when either the protein and lipid fraction is utilised for methane production or only the protein fraction is utilised for methane production [20]. For *Nannochloropsis* sp., the theoretical methane potentials of biomass based on chemical composition were 0.73, 0.67, 0.67 and 0.78 m3 CH4 VS /kg. Despite a stable macromolecular composition and high methane potential due to its high lipid content, the *Nannochloropsis* species is less exploited due to its recalcitrant cell wall [21]. In a recent comparative research where the effects of ultrasound treatment and hot water were studied on *Nannochloropsis sp.*, ultrasound pre-treatment alone produced higher methane production and suggested that pre-treatments significantly affected the overall structure of the biomass; however, the relative abundance of the species remained mostly unchanged [22]. Thus it can be seen that further research is required into ultrasound studies utilising novel species such as *N. oceanica* and *T.suecica.* This is also valuable to increase the potential applications of marine microalgae for high-value compounds and their role in biorefinery. *Nannochloropsis oceanica* is also a unicellular, free floating green micro-algae with subspherical cells 2–4 µm in diameter. This strain, a marine species, is also halotolerant (tolerating salinities) and a preferred species known for its high biomass productivity and high lipid content [23]. Recently, the species has also been studied for its capacity for nutrient removal capacity from anaerobically digested municipal wastewater effluent [24]. *Nannochloropsis*, being a marine microalga, has fast growth rate, high lipid content and high efficiency in CO_2 fixation and is also a noted species for mass production of commercial products, such as high-value fish oil [21].

Similarly, *Tetraselmis suecica* is a marine, unicellular, flagellate algae with elliptical or spherical cells in shape. The cells contain a single cup-shaped chloroplast and flagella having two rows of hair shaped scales projecting from opposite sides [25]. The species is known for its ability to tolerate a wide range of salt concentrations and has been extensively studied for its physiology, as variations in salinity and nutrient concentration have a greater effect on structural and chemical properties of its biomass [26]. The species has also recently been studied for its potential in an integrated multi-trophic aquaculture system that utilises fish wastewater to grow algal biomass as a fish feed [27]. *Tetraselmis sp.* has also been found have high endurance of high CO_2 concentrations and is also shown to be cultivated in wastewaters. As a circular approach, biomass production of *Tetraselmis suecica* using biogas and wastewater as nutrients has also been reported recently [28]. This review shows that these two species are gaining importance in a variety of applications and further study is required to increase their utility in the AD sector. Additionally, it can be noted that only very few studies have reported experimental values for these two species in the literature, which inhibits further understanding of the species in detail.

Therefore, this paper investigates the impact of ultrasound on two microalgae species, i.e., *Nannochloropsis oceanica* and *Tetraselmis suecica* and their bioenergy potential through anaerobic digestion. This paper is innovative in investigating the biomethane potential of the microalgal residues after ultrasound pre-treatment, which has been less reported in the literature for these two species.

Therefore, the aims of this paper are as follows:

1. To demonstrate the feasibility of using microalgal biomass residues for anaerobic digestion under mesophilic conditions,
2. To compare the two species' methane production from two microalgal classes and their biochemical composition,
3. To determine the impact of ultrasound on micro-algal methane production.

2. Material and Methods

2.1. Species Selection, Cultivation and Growth Assessment

Nannochloropsis oceanica (Eustigmataceae) and *Tetraselmis suecica* (Chlorophyceae) were taken from CSAR culture collection, Swansea University, previously bought from CCAP culture collections, Scotland, UK. The strain references are CCAP 849/8 for *N. oceanica* and CCAP 66/22A) for *T. suecica*.

The inoculum for both species was maintained in a temperature-constant room (18 °C) with a light of 100 µmol photons m-2s-1 light: dark cycle 16:8 h. An F/2 standard media was used to maintain the master culture and inoculum of this species. In this experimental study, to assure consistent growth, the inoculum was taken in the exponential growth phase.

2.2. Cultivation System and Cultivation Condition

A horizontal tubular photo bioreactor (PBR), Biofence, was constructed and installed by Varicon Aqua Solution Ltd. with a capacity of 800 L. This PBR is housed in a greenhouse at the location of 51°36′29.1″ N 3°58′53.1″ W, Swansea UK. The light-exposed section of the PBR has a volume of 400 L and the system also includes a dark tank of 400 L. The culture was mixed with a pump and transferred from the light phase to the dark phase every 3 to 4 min. The temperature and pH of the process were monitored with probes installed within the PBR, allowing the temperature to be maintained within a range of 18–24 °C while pH was maintained at 7.5–8.5. CO^2 injection was regulated with an automated pH sensor; pH rising to 8.1 was selected as a threshold to trigger automatic CO^2 injection for pH regulation.

2.3. Sampling and Analysis

Biomass samples for biochemistry analysis and dry weight were harvested every 24 h via centrifugation (8000× *g*, 4 °C, JA-2, Beckman, Germany), suspended and washed twice

in deionised water and frozen at −80 °C overnight before freeze-drying. The freeze-dried biomass was placed in the freeze dryer (CoolSafe 110 Freeze Dryer, Scanvac, Denmark) within 24 h at −110 °C [29].

The elemental composition of the biomass was analysed via estimation of lipids. This utilised a gravimetric method proposed by [30] with modification by [31]. Total carbohydrates were analysed in [32] and protein content was determined by multiplying the nitrogen content of dried biomass measured using a SerCon GSL elemental analyser (1000 °C combustion temperature) by a factor of 6.28 [33]. This approach is analogous to that based upon Kjeldahl digestion [33].

All biochemical measurements were made in triplicate, except protein, which was performed in duplicate.

2.4. Harvest Methods

Algal biomass was harvested using a membrane filtration system at Swansea University and is described further in [29]. One-inch stainless steel tubing was used to connect, via clamp fittings, two centrifugal pumps, a concentric tube heat exchanger, a pressure control valve, two stainless steel pressure gauges (before and after the filtration module) and a Membralox ceramic MF membrane fitted in a stainless steel module. The membrane had a 0.20 µm pore size and an area of 0.22 sq m. In addition, two 1.5-inch clamp butterfly valves were used in the system for better harvesting of the biomass. In order to avoid cavitation due to the pumps, a minimum holding volume of 5.0 L was established.

2.5. Downstream Process

The harvested biomass was centrifuged at a temperature of 10 °C at 12,800× *g* utilising a Beckman J2-21M/E centrifuge with a JA 10 rotor (Beckman, Brea, CA, USA). Each batch was suspended and washed twice with deionised water. After that, the biomass was frozen at −80 °C and prepared for the ultrasound pre-treatment.

2.6. Ultrasound Pre-Treatment

Ultrasound cavitation was applied to both species, *T. suecica* and *N. oceanica*. The pre-treatment was performed using a CPX 500, 500 Watts, 20 kHz (Cole Parmer, Vernon Hills, IL, USA) instrument. The algal solution was placed in a measuring cylinder and subjected to ultrasound cavitation corresponding to specific energy (E_s) of 10, 27, 40 and 57 MJ/kgTS. The cavitation times for the corresponding specific energy for each species were different. The volume of the used algal sample was 100 mL. The cavitation times corresponding to each specific energy for both of the species were calculated and performed accordingly. The pH and cavitation temperature were recorded after each cavitation experiment. The efficiency of cavitation was recorded by measuring the soluble chemical oxygen demand (sCOD) values. The cavitated samples with the highest sCOD efficiency were selected for the biochemical methane potential tests.

2.7. Analytical Methods

The characterisation of the two species were performed for their total, volatile solids, ash and moisture content. The elemental analysis (C, H, N, S) was also characterised using British standard methods and standard water and wastewater methods [34].

2.8. Biochemical Methane Potential Tests

Biochemical methane potential tests (BMP) were performed using automated methane potential test system equipment (AMPTSii). The digesters were prepared for the experiments with a working volume of 600 mL. The seed for the BMP tests was obtained from Severn Trent Wastewater Treatment Plant. The seed was sieved and then stored at 37 °C in a water bath until the tests. The system provided an online measurement of biogas production via its software and pH, and solids concentration was measured before and after the BMP tests. The batch BMP tests were 28–30 days in duration for all the performed

experiments. The main results from the experiments are discussed below. All experiments were performed in the mesophilic temperature of 37 °C. Alpha (α) cellulose was used as the positive standard for all experiments and the tests were all performed in triplicate. No extra nutrient solution was added to enhance the digestion.

3. Results

3.1. Growth and Optimisation of the T. suecica and N. oceanica Cultures

3.1.1. Growth Curves (Dry Weight)

The growth of the two microalgal species is shown below. Figure 1 illustrates that the two species exhibited good biomass accumulation. Both species had a short lag adaptation growth phase (day 0–4), where growth rate was 0.23 and 0.24 for *T. suecica* and *N. oceanica*, respectively. The exponential growth phase was around 7 days for both species, with the growth rate being ~0.38 for *T. suecica* and 0.42 for *N. oceanica.* The harvesting of both species was conducted at the end of the exponential growth rate. The final amount of biomass 1.2 kg (dry weight) of *N. oceanica* and 1 kg (dry weight) of *T. suecica* was harvested from both reactors by the 14th day.

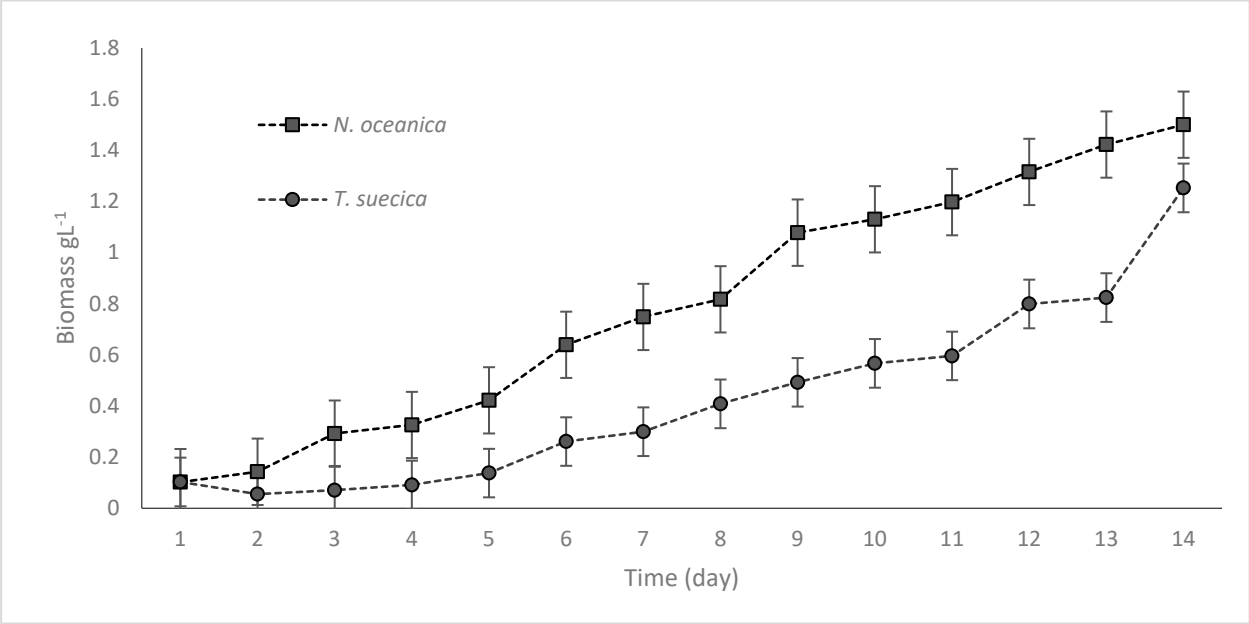

Figure 1. Growth of *N. oceanica* and *T. suecica* in 800 L tubular PBR in greenhouse conditions using F/2 commercial medium.

The growth of the two microalgal species is shown in Figure 1 and demonstrated a great biomass accumulation. Both species had a short lag adaptation.

3.1.2. Biochemical Composition

The total and volatile solids characterisations and the elemental analysis for *Tetraselmis suecica* and *Nannocholoropsis oceanica* are given in Table 1.

Table 1. The elemental analysis as well as total and volatile solids characteristics for *N. oceanica* and *T. suecica*.

Species Name	C	N	H	S	Total Solids (gTS/KgWW)	Volatile Solids (gVS/KgWW)
Tetraselmis suecica	43.17	6.54	6.78	1.43	287.41	231.67
Nannocholoropsis oceanica	56.17	6.08	8.86	0.32	39.33	7.69

The elemental analysis results show the biochemical composition of the species. Table 1 show that both the species have a high carbon content in their composition. This means that the substrates have a potential for high methane production. The values obtained in this study are comparable to those found in the literature [35]. This can be further explained in terms of C/N (carbon to nitrogen) ratios.

In anaerobic digestion, the carbon to nitrogen ratio is an important parameter as it can significantly impact the volume and quality of the biogas produced in the anaerobic digestion process. The ideal C/N ratio of organic feedstock for AD is shown to be between 25:1 and 30:1. High C/N ratios favour higher methane production; however, a low C/N ratio means that digester performance will be hindered due to ammonia toxicity. As C/N ratios have such a significant impact on the digestion process, the feedstock ratio is often adjusted via co-digestion (digesting more than one feedstock) [36]. *Nannochloropsis* species has also been studied and reported as a convenient feedstock for co-digestion with organic feedstock such as corn, as it enhances alkalinity and nutrient composition in the digester [37].

3.2. Ultrasound Pre-Treatment on the Species

Cavitation tests were performed to aid algal cell wall rupture. Both species are noted for their characteristic cellular structure (including tough cell walls), which have been reported in the literature as rate-limiting during anaerobic digestion of the biomass [5]. The different specific energy of cavitation tests performed for both the species are given in the Table 2.

Table 2. The sCOD efficiency of cavitation tests performed for *N. oceanica* and *T. suecica*.

Specific Energy (MJ/KgTS)	Cavitation Time (s)	pH	Temperature (°C)	sCOD Efficiency (%)
Tetraselmis suecica				
10	5	7.48	19.1	5.13
27	13.5	7.60	19.8	3.81
40	20	7.66	20.2	4.00
57	28.5	7.67	21.2	3.96
Nannochloropsis oceanica				
10	20	6.28	19.8	14
27	54	6.36	21.6	18
40	80	6.50	24.1	17
57	114	6.52	24.2	12

SCOD is the soluble chemical oxygen demand efficiency tested for both the species after the cavitation tests. This parameter has been used in the literature specifically to determine the efficiency of the ultrasound pre-treatment, where the results show higher soluble COD at higher applied energy [9]. However, in our results, for *T. suecica*, a specific energy of 10 MJ/KgTS shows the highest SCOD efficiency of 5%, and for *N. oceanica*, a specific energy of 27 MJ/KgTS shows the highest SCOD efficiency of 18%. These algal samples were later used for BMP tests. The cells after cavitation (i.e., with no extraction of lipids) were used for BMP tests to measure biogas production potential.

3.3. Biochemical Methane Potential Test Results

The pre-treated microalgae biomass, i.e., after ultrasound cavitation, was then studied for its methane potential using BMP tests. The biomethane potential (BMP) test is one method that can be used to estimate the amount of methane that can be produced from anaerobically digested organic matter at 35 °C. The biogas production from selected micro-

algae and their residues after pre-treatment was monitored for 30 days using an automated methane potential test system (AMPTS).

For the untreated microalgae samples, *T. suecica* gave a higher specific methane production value of 0.304 LCH4/kgVS for the undiluted concentrated paste with a TS of 287.41gTS/KgWW. As the total solids concentration was high, a diluted sample of the same species with TS of 114.34gTS/KgWW was further studied for its methane production pattern to be able to compare with low TS concentrated N. oceanica. For the diluted sample of *T. suecica*, a specific methane production of 0.169 LCH4/KgVS was observed. The specific methane production value of the undiluted *T. suecica* was almost the double of the diluted sample. On the other hand, *N. oceanica* only had a very low specific methane value of 0.123 LCH4/KgVS. The lower bio-methane production of *N. oceanica* was expected because of its very low biomass (39.33 gTS/KgWW) concentration compared to *T. suecica* (287.41 gTS/KgWW).

From this it was understood that optimum biomass concentration (TS and VS concentration) is critical for microalgal biomethane production, as both of them were significantly different. The values for specific methane potential for the species is given in Table 3 and the graphs are shown in Figure 2.

Table 3. The specific methane values obtained at BMP tests.

Substrate	Specific Methane Potential (LCH_4/kg VS)
Tetraselmis suecica (as received)	0.304
Tetraselmis suecica (diluted, untreated)	0.169
Tetraselmis suecica (diluted, cavitated)	0.165
Nannochloropsis oceanica (untreated)	0.123
Nannochloropsis oceanica (cavitated)	0.101

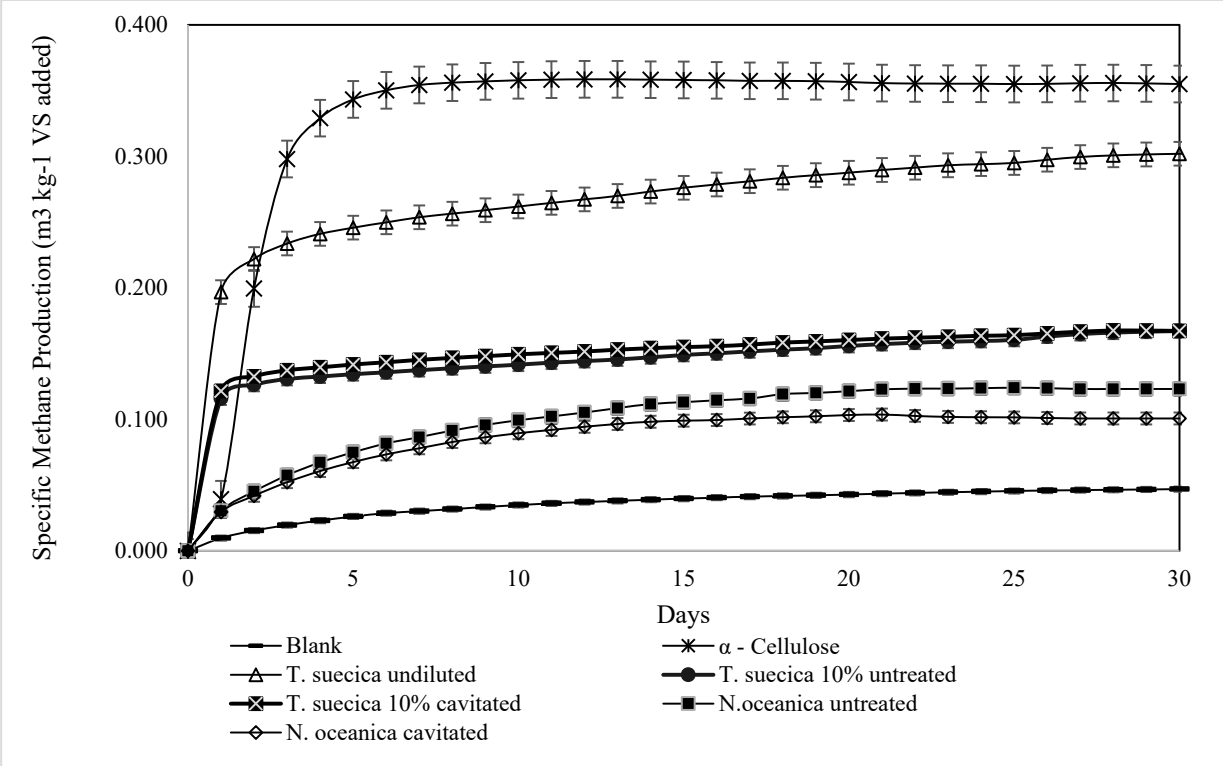

Figure 2. Specific methane production of the species during biochemical methane potential tests.

The impact of ultrasound was then observed for *Tetraselmis* (diluted) and *Nannochlorpsis* samples. For *Tetraselmis*, a specific methane production of 0.165 LCH4/Kg VS was observed; however, for *Nannochloropsis* a value of 0.101 LCH_4/Kg VS was observed for the cavitated samples. The values obtained for the BMP tests in this study were compared to those reported in the literature for both the species. For *Tetraselmis suecica*, with a lime treatment, a methane production of 337 mL/g VS was reported [38]. However, it is strictly noted that *Nannochloropsis* and *Tetraselmis* species have been studied in the literature for lipid extraction pre-treatment methods for AD rather than for ultrasound. Therefore, it has been difficult to compare our results with those reported values. Nonetheless, for *Tetraselmis sp.*, a value of 160 mL/g VS was reported without any lipid extraction pre-treatment and a production of 236 mL/ g VS was noted after supercritical CO_2 extraction. For *Nannochloropsis sp.*, a value of 357 mL/g VS was observed, while a value of 399 mL/g VS was noted after lipid extraction pre-treatment. Other values reported for *Nannochloropsis sp.*, similar to batch tests in this study, are 482 mL/ g VS where the biomass was pretreated with wet lipid extraction methods and, in the same study, *Nannochloropsis salina* produced 383 mL/g VS after hexane/isopropanol lipid extraction methods [39].

In this study, *T. suecica* had a higher specific methane potential when compared to *N. oceanica* in both untreated and cavitated biomass BMP tests. However, it should be noted that even though very small, there is a slight decrease in the values of specific methane production of the cavitated biomass for both the micro-algae. This could mean that there might be some inhibition to methane production even after the cavitation. This could be due to their biochemical compositions not favouring the optimal C/N ratios for better AD performance, indicating that the micro-algae need to be co-digested with carbon-rich feedstock such as corn [37]. This could be verified by analysing the contents after digestion, digestate, for its biochemical composition. However, due to limitations of this research, further tests on digestate were not performed. Alternatively, the applied specific energy may not be sufficient to break open the cell walls of the species to allow for better methane production. This is similar to the findings in the literature where ultrasound treatment is found to be effective depending on the energy dose and the target species. However, the method is also disadvantaged due to its high electricity consumption [40].

3.4. Scanning Electron Microscopy Results

To understand the impact of the ultrasound cavitation on cell wall rupture, scanning electron microscopy (SEM) was performed on the microalgae samples that exhibited lower methane potential, *Nannochloropsis*. The SEM images of the microalgae before and after cavitation were obtained for *Nannochloropsis* (Magnification 1000×) and are given in Figure 3.

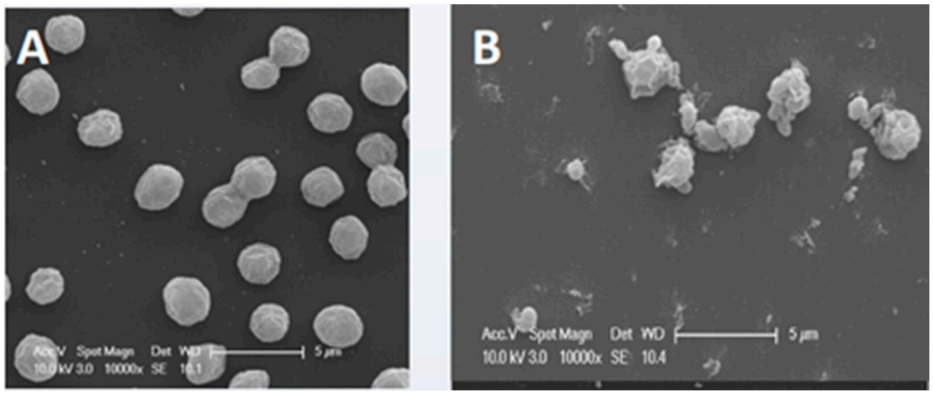

Figure 3. The microalgal cells before (**A**) and after ultrasound cavitation (**B**).

Figure 3A shows the microalgae cells before cavitation and Figure 3B shows the cells after ultrasound cavitation. From the figures it can be clearly seen that cavitation has its

effect on the cell walls, that they are disintegrated and less circular in nature than prior to cavitation; however, not all algal cell walls were completely broken by cavitation. It has been reported in the literature that the cell walls of these species can be elastic in nature and tend to get "coiled up" with ultrasonic cavitation. It is believed that as a result of cell disruption by ultrasound, the fractured cell membrane coils up, thereby retaining the cellular lipids inside them [41].

4. Discussion

Ultrasound pre-treatment is argued in the literature as both a lipid extraction technique and a pre-treatment method. Very recent research on using ultrasound for microalgal disruption has also shown that ultrasonic frequency, intensity and duration affect cell disruption [42]. Earlier in the SCOD efficiency it was observed that the two species had higher soluble COD at two different applied energies, where *T. suecica* had a SCOD efficiency of 5% at a specific energy of 10 MJ/KgTS, while *N. oceanica* had a SCOD efficiency of 18% at a specific energy of 27 MJ/KgTS. This could mean that with the increased SCOD efficiency, the cell wall was ruptured, and cellular material was released. However, from an AD perspective, for our study results, it could also mean that ultrasound energy was insufficient to achieve adequate cell disruption for increased specific methane production or that it resulted in the release of material that are inhibitive for AD. Therefore, additional pre-treatment could be needed to allow better digestion of the cell wall residues. This research is important as it provides greater understanding of pre-treatment requirements for microalgae and microalgal anaerobic digestion. This is particularly important as there is increasing commercial demand for bioactive ingredients from species such as *T. suecica* [43] and *N. oceanica* for particularly high-value compounds, e.g., Vitamin D extraction [44].

This study is also relevant for anaerobic digestion research as it improved our understanding of the characteristics of algae residues after the extraction of high-value lipids and their impact on biomethane potential. *T. suecica* was found to be a better substrate for methane production potential. In this study, it was found that there is a decrease in the values of specific methane production of the cavitated biomass for both the micro-algae species. In this study, the BMP tests were performed in triplicate with the blanks also triplicated during the BMP tests. The range of standard deviation values observed throughout the various BMP experiments are in between 0.01–0.04. The values indicate that the experimental results have been consistent and reliable. Therefore, the BMP results clearly indicate that ultrasonic cavitation alone was not sufficient to produce improved methane production from the tested micro-algae species. Moreover, these results can be useful for utilising marine species such as *T. suecica* in conventional biological AD systems for innovative purposes such as for removing harmful chemical compounds such as phenol, as in [43,45]. Furthermore, these results can also be utilised for ongoing research into biorefineries using marine algal strains such as *T. suecica* that can be grown on ammonia-rich anaerobic digestate such as food waste, as coupling microalgae cultivation and wastewater remediation has been evidenced to be more economically feasible than the application of microalgae in the biofuel market [46]. Finally, these results show that further research is still required to explore the feasibility and optimization of microalgae biorefineries for circular economy.

5. Conclusions

This study aimed at growing two marine microalgae species to use microalgal biomass residues for anaerobic digestion under mesophilic conditions following ultrasound pre-treatment. This study focused on determining whether ultrasound cavitation was an optimal pre-treatment for microalgal AD. The BMP tests from this study show that marine microalgae are beneficial for biogas production. The main BMP results from this study are that among the two micro-algae species tested, *T. suecica* was found to be a better substrate for methane production potential. Contrary to increasing the specific methane production, ultrasound cavitation demonstrated a slight decrease in the specific methane production

values for both *N. oceanica* and *T. suecica* biomass residues. This was not expected, as any disruption with higher SCOD efficiency should have resulted in higher specific methane production. Lipid and fatty acid analyses could have further asserted the AD potential of released cellular material after cavitation, and were not conducted as a part of this study. Therefore, in the case of micro-algae biomass cavitation, further tests are required to ascertain the possibility of higher ultrasound cavitation energy being sufficient to break the cell wall barrier to aid better digestion performance. Further research is also required for the optimisation of the pre-treatment of microalgae and the integration of microalgal biorefinery for circular economy.

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