Studies on evaluation of surfactant coupled sonication pretreatment on *Ulva fasciata* (marine macroalgae) for enhanced biohydrogen production

A.V. Snehya [a], M.A. Sundaramahalingam [a], J. Rajeshbanu [b], S. Anandan [c], P. Sivashanmugam [a,*]

[a] *Chemical and Biochemical Process Engineering Laboratory, Department of Chemical Engineering, National Institute of Technology Tiruchirappalli, Tamilnadu, India*
[b] *Department of Life Sciences, Central University of Tamil Nadu, Neelakudi, Thiruvarur, Tamilnadu, India*
[c] *Department of Chemistry, National Institute of Technology Tiruchirappalli, Tamilnadu, India*

ABSTRACT

Biohydrogen production from marine macroalgal biomass by advanced pre-treatment strategies is considered a clean energy technology. The present study focuses on investigating the effects of sonication pre-treatment (SP) and saponin coupled sonic pre-treatment (SSP) on *Ulva fasciata* for enhancing the production of biohydrogen. The SP and SSP were optimized to improve the hydrolysis process during digestion. The optimized time and sonication power were found respectively as 30 min and 200 W. A high concentration of biopolymer release was noticed in SSP than SP at optimized conditions. The surfactant dosage in SSP was optimized at 0.0036 g/g TS. The effect of SSP process was assessed by estimation of COD (Chemical Oxygen Demand) and SCOD (Soluble Chemical Oxygen Demand) release. The study revealed that, at a specific energy of 36,000 KJ/Kg TS, the SCOD release was higher in SSP (1900 mg/L) than SP (1050 mg/L). The SSP process could improve the COD solubilization to 15 % more than the SP. Carbohydrate and protein release are also more in SSP than SP. The use of biosurfactants significantly reduced the energy utilization in the hydrolysis process. The SSP pre-treated *Ulva fasciata* biomass has yielded a higher biohydrogen of 91.7 mL/g COD which is higher compared to SP (40.5 mL/g COD) and Control (9 mL/g COD).

1. Introduction

For the past two decades, the use of non-renewable fossil fuels is depleting due to industrialization and overpopulation. The worldwide energy demand in 2040 is expected to be increasing by 30 % when compared to the present scenario. In India, the primary energy sources are coal, natural gas, nuclear, solar, wind, hydro, electric, and biofuels. The energy crisis faced by India could be compromised by converting renewable organic matters into biofuels such as hydrogen [1] and bio-methane [2]. Among these, hydrogen is considered a high energy fuel and is used for industrial applications such as food processing, refining, and rocket fuel, etc [3]. Hydrogen can be produced by chemical, electrochemical, and biological processes [4]. However, biohydrogen production (BHP) is a sustainable source of energy, due to the advantages of high energy efficiency and zero-carbon emission [5]. The microbes can be used in an economical way for the production of biohydrogen by different methods such as photo fermentation (PF) (Ghiasian, 2019) and dark fermentation (DF). Among these two fermentations, DF is more suitable than PF due to its low initial investment, medium operational

cost, high productivity, and less consumption of light energy [6]. The various substrates used for biohydrogen production by DF were lignocellulosic waste, food waste, fruit and vegetable waste, industrial sludge, municipal solid waste, dairy waste, etc [7,8]. The third-generation biofuels depend on algal sources for the generation of energy [9]. In that macroalgae is an efficient source for biohydrogen production than microalgae because of its multicellular nature and is composed of high carbohydrate content with low lignin [10]. Furthermore, a circular economy approach is needed in seaweed refineries for maximum utilization of biomass [11]. The review also reveals the characteristics, merits, demerits, and utilization of marine seaweeds for various applications. The advancement in BHP using marine macroalgae is paving a new way in the fuel production sector [9,12].

Ulva fasciata, a green alga found in the coastal regions, especially in the intertidal rocks and tide pools, causes environmental problems such as eutrophication, fouling, and its presence in water bodies leading to exhaustion of dissolved oxygen. Interestingly, it is rich in carbohydrates, proteins, amino acids, with low lignin, etc. The feasibility of using *Ulva fasciata* for biohydrogen production by dark fermentation has to be

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explored for better hydrogen yield. The long intact cell wall and presence of polyphenols may act as a barrier for the hydrolysis step during digestion. Hence, various pre-treatment methods were adopted for improving the hydrolysis process and that involves physical, chemical, biological methods [13]. Many researchers have investigated the pre-treatment of marine macroalgae using physical methods including thermal [14], mechanical disruption by ball milling [15], dispersion [16], microwave treatment [17], etc. The chemical methods like alkaline, acid, and ozone approach are also used for pre-treatment. A study of BHP by acid pre-treatment of *Padina tetrastromatica* for the removal of phenolic compounds, yields a maximum biohydrogen production of 78 mL/0.05 g VS [18]. The biological pre-treatment process by enzymes and microbes is effective but expensive and quite slow process [19]. Neha Srivastava et. al (2020) reviewed the possibilities of using nano-material for enhanced biohydrogen production [20].

Furthermore, the combinative pre-treatment methods used by the researchers are more efficient and economical for increasing the solubility and biodegradability of macroalgae for biohydrogen production [15]. The effect of thermochemical pre-treatment of *Palmaria palmata* uplifted the solubilization yield and enhanced the biomethane potential to 18% [21]. Tamilarasan et al., (2018) investigated the energy utilization kinetics of biomethane production using thermochemical dispersion aided pre-treated seagrass [22]. Margareta et. al., (2020) investigated an acid thermal pre-treatment of the *Ulva* species followed by dark fermentation [23]. A higher biohydrogen yield was noticed in the pre-treated system when compared to the control. This study also revealed the importance of pretreatment in the release of glucose, galactose, rhamnose, and xylose during hydrolysis.

Among all the above processes, ultrasonication has been proved to be an efficient method to achieve a higher solubilization rate, but its practical viability is restricted by high energy consumption [24]. To minimize energy consumption, sonication can be coupled with chemicals [25–27]. Many researchers have reported that achieving an energy ratio over 0.8 is considered to be an economically feasible and field applicable method [28]. Also, achieving an energy ratio over 0.7 is reported to be not possible with sole ultrasonication, microwave, and thermal pretreatments [28]. Few researchers reported that surfactants are amphiphilic, which reduces the interfacial tension between hydrophobic and hydrophilic phases [29,30], and when exposed to sonication its effect has been increased in aqueous solution and minimize the energy consumption [31].

The different surfactants such as sodium dodecyl sulfate (SDS), cetyltrimethylammonium bromide (CTAB), Dimethyl sulphoxide (DSMO) were used for the production of biomethane but there is a need for the eco-friendly and economically feasible process to be adopted for commercial production. Interestingly, the use of a biosurfactant, for pre-treatment has to be investigated, because of its bioavailability, low cost, and environmental compatibility. Thus, there is a need to develop an eco-friendly biosurfactant coupled sonication pretreatment process for the production of biohydrogen. Saponin, a plant-derived non-ionic surfactant that is thermostable and environmentally compactable [32]. Hence, the main objectives of the present study are to (i) investigate the bio solubility of *Ulva fasciata* by sonic pre-treatment, (ii) study the feasibility of saponin coupled sonication process in biopolymer release, (iii) compare the energy utilization for COD solubilization during SP and SSP processes, (iv) evaluate the efficacy of biohydrogen production from pre-treated SP and SSP samples.

2. Materials and methods

2.1. Substrate collection and characterization

The marine macroalgae of *Ulva fasciata* species were collected from Mampadi beach, on the southern coastal region of Tuticorin, Tamil Nadu, India. The substrate was washed with distilled water and shadow dried for four days. Then it was crushed and ground to 75-μm size. The

Table 1
Initial characteristics of macroalgal sample.

S.No.	Parameters	Composition in % (dry weight)
1	Protein	44.14
2	Carbohydrate	38.23
3	Lipids	1–2
4	Ash	13.63
5	Other substances	3–4

biochemical characterization of substrate was performed. The percentage of biopolymer concentrations such as protein, carbohydrate, lipid, and ash content were analysed and narrated in Table 1.

2.2. Pre-treatment of Ulva fasciata

Pre-treatment studies of macroalgae were carried out in two stages. In the first stage, optimization of exposure time and power utilization during SP were performed based on the solubilization of biopolymer and SCOD. In the second stage, optimization of SSP process was performed at different surfactant dosages for improving the COD solubilization. The SP was performed using 25 g/L of macroalgae at varying power inputs of 50 to 250 W using Probe sonicator, Model No Pro 250, Labman. During SP, 2.5 g of samples were diluted with 100 mL distilled water in a 500 mL beaker and placed in a sonicator with a probe depth of 2 cm for effective disruption. The samples were taken at regular time intervals of 10 min for a period of one hour [33]. The parameters analysed in the experiments were substrate solubilization and biopolymer release, with respect to specific energy. For SSP, 0.01 g/ml of algal samples were taken in a beaker and subjected to the synergic action of surfactant and sonication. During this process, the dosage of saponin used was in the range of 0.0012 to 0.018 g/g at optimized exposure time and input power. The samples were taken at regular intervals of time, to analyse the COD solubilization and biopolymer release.

2.3. Energy utilization by SP & SSP

Specific energy is the essential parameter for the evaluation of mechanical disintegration performance [25] and it was found using equation1.

$$SE = \frac{IP X t}{V X TS} \tag{1}$$

were, SE: Specific energy (kJ/kg TS); IP: Input Power(W); t: Exposure time to pre-treatment (min); V: Volume of sample for pre-treatment (ml); TS: Total solid concentration.

2.4. Biohydrogen potential assay

The fermentation of macroalgae for biohydrogen production was performed by using the control and the pre-treated algal biomass (SP, SSP). The assay was performed using 500 mL borosilicate bottle with a loading rate of 80 % with an inoculum to substrate ratio of 3:1 v/v (based on VS concentration) and with rumen fluid as inoculum, at optimized conditions. The rumen fluid inoculum was heated at 100 °C for minutes to alleviate methanogenic bacteria and added to the substrate. Nitrogen gas was sparged to all the reactors before sealing to provide anaerobic condition and the reactors were wrapped with aluminium foil for facilitating dark fermentation. The reactors were kept for incubation under shaking with a speed of 100 rpm at 37 °C to enable uniform mixing during fermentation for effective hydrogen production. The biohydrogen production in the bioreactors was continuously monitored by finding the displacement of the syringe inserted on the top of the sealed bottles. The displacement volume of the syringe was noted on daily basis and the cumulative hydrogen production was also noted. The cumulative hydrogen production potential was validated using the

Gompertz equation [34].

$$BH_C = BH_P * exp\{-exp[-k(BH_{ip} - BH_{lag})]\} \quad (2)$$

where, BH_C - Cumulative hydrogen produced (mL), BH_P – Hydrogen production potential (mL), k - Maximum hydrogen production rate (mL BH_2/d), BH_{ip}- initial period (days), BH_{lag} - Lag phase (days). The collected biohydrogen was analysed using gas chromatography mass spectrum (GCMS) and test its conformity.

2.5. Analytical methods

The standard methods described in APHA (2005) were followed for the analysis of total solids (TS), total chemical oxygen demand (TCOD), soluble chemical oxygen demand SCOD [35]. Total solids (TS) in the sample after pre-treatment was determined by centrifugating the pre-treated biomass to get supernatant and the residue using centrifuge. The supernatant was dried overnight in an oven at 60 °C and TS was calculated from the solids remained after drying. SCOD was determined by incubating the supernatant with potassium dichromate in combination with boiling sulfuric acid for 2 h. The incubated sample after cooling was titrated against a standardised 0.0625 N ferrous ammonium sulphate (FAS) solution using ferroin as an indicator until sudden change of colour from blue green to reddish pink. The COD in the residue was also determined by the same method as followed for SCOD. The TCOD was found out by adding SCOD and COD in the residue. The concentration of protein before and after pre-treatment was determined by Lowry's method using Bovine serum albumin as standard after reactions with salts and Folin's phenol reagent followed by measuring its absorbance at 620 nm. The carbohydrate concentration before and after pre-treatment was estimated by the anthrone method using cellulose as standard. In this method sample was reacted with the anthrone sulfuric acid and then by measuring the absorbance at 625 nm. All the chemicals used in the present study were of analytical grades.

3. Result and discussion

3.1. Effects of sonic pre-treatment on organic release from Ulva fasciata

The outer cell wall of the macroalgae is composed of biopolymers such as carbohydrates, proteins, and with low concentrations of lignin. The presence of biopolymer in macroalgae depends on species, environment, and climatic factors [36]. From the results of algal composition, the high carbohydrate presence indicates that *Ulva fasciata* is a suitable source for biohydrogen. The C/N ratio in most of the species of macroalgae is higher than 9, which is higher than the C/N present in microalgae, thus the production of inhibitory compounds produced during the anaerobic digestion process will be less [13]. The reason behind diluting the algal biomass with water for ultrasonic pre-treatment is that the dissociation of water molecules produces H and OH radicals by a redox reaction. The products formed are more reactive forming hydrogen peroxide which chemically attacks cells by oxidation and this effect is more pronounced under surfactant coupled sonication [29–31]. Furthermore, marine macroalgae exhibit high ionic strength, so the oxidative reaction increases [37]. Moreover, the pre-treatment enhances the hydrolysis process and produces monomeric sugars from polysaccharides. The pre-treatment of marine macroalgae using the microwave for biohydrogen production has improved the release of organic matter to the system [38]. From the literature, it is noted that ultrasonication and combined methods are used to extract the phyco-biliproteins from marine macroalgae [39]. Similarly, a combinative pre-treatment method has to be adopted to improve the biopolymer release from *Ulva fasciata* for biohydrogen production.

The basic principles involved in the sonochemical transformation of algae biomass into solubilized form during sonication can be explained as follows. When the medium is exposed to ultrasound, propagation is getting started with the generation of two cycles namely compressions and rarefactions. The compression cycle creates a positive pressure on
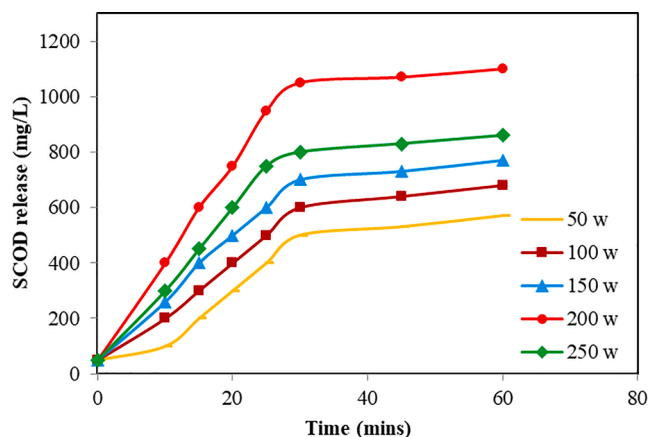

Fig. 1. Effect of sonication on SCOD release.

the liquid by pushing the molecules together, whereas the rarefaction cycle creates a negative pressure by pulling the molecules from one another. Due to this high negative pressure microbubbles (cavitation bubbles) are formed in the rarefaction regions. These microbubbles grow in successive cycles to reach an unstable diameter and getting collapse violently producing shock waves with a temperature of around 5000 °C and pressure of 500 atmospheres in a few microseconds. The repeated process of bubble formation, growth, and subsequent violent collapse is known as cavitation [40]. At this temperature, water molecules are decomposed into highly reactive hydrogen (H$^\bullet$) and hydroxyl radicals ($^\bullet$OH). These radicals recombine to form hydrogen peroxide and molecular hydrogen. Hence, two synchronized effects namely hydro-mechanical shear forces and the oxidizing effect of $^\bullet$OH are produced [41]. These two combined effects cause cleavage of biomass and discharge of components within the biomass into the liquid phase. The chemical effect also depolymerizes released cell wall components namely cellulose, protein, and lignin, and facilitates their transport into the liquid phase [42] and increases the SCOD level.

The pre-treatment of *Ulva fasciata* was performed using an efficient probe sonicator by varying the power input in the range of 50 W to 250 W and an exposure time of 10 to 70 min. The SCOD release with sonication time and power consumption is presented in Fig. 1 During the process, the SCOD release by the macroalgae has increased drastically in the range of 10–25 min and stabilized within 40 min. In Fig. 1, the SCOD release was achieved in two phases, the first phase is a drastic release and the second one is a moderate release. During the first phase, the SCOD release is found to be higher in the power range of 200 W (1050 mg/L) than the other. Further increase in power input, decreases the SCOD release. The SCOD release for 150 W and 250 W at 30 min is found to be 700 mg/L and 800 mg/L respectively. The SCOD level decreases as the sonication power increases by 200 W to 250 W. This may be due to the effect of operational parameters such as temperature, pressure, pH on cavitation, and mechano-acoustic effects. The increase in pressure may cause lower cavitation, due to low viscosity and rise in temperature during sonic pre-treatment [43]. Furthermore, an increase in sonic power of 50 to 250 W may cause an increase in cavitation and vapour pressure of the fluid. The bubble collapsing decreases due to the increased pressure, temperature and it may affect the SCOD release [38]. In the second phase, the release of organic matter after 30 min is found to be consistent. This may be due to the presence of a high concentration of intracellular substances. A study on pre-treatment of waste activated sludge by ultrasonication reveals that an increase in suspended solids concentration decreases the dissolvable organic release, sludge lysis rate, and sonication efficiency [44]. Also, the increase in retention time will decrease in biopolymer release due to the change in temperature and pH [45]. From Fig. 1, it is observed that the macroalgae disintegrate faster in sonolysis pretreatment for 30 min at 200 W with a high SCOD
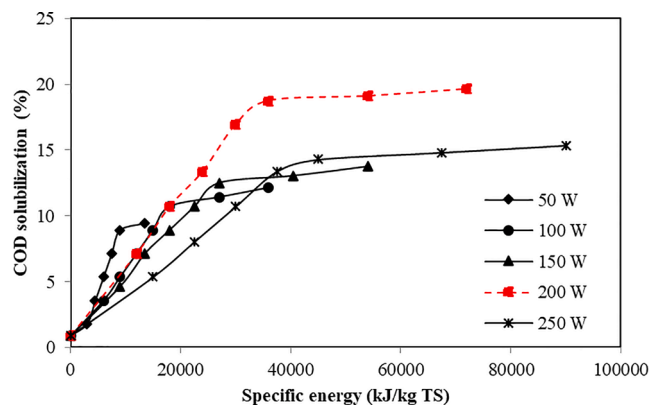
Fig. 2. Influence of specific energy on COD solubilization.

Fig. 3. Effect of SSP on SCOD release and COD solubilization.

release of 1050 mg/L. Hence, the optimum exposure time of 30 min is used for subsequent studies in this research.

3.1.1. Effect of specific energy on COD solubilization

Specific energy plays an important role during the economic analysis and scales up of biohydrogen production. The use of low-cost pre-treatment technologies gained attention for the production of biogas [22,46]. Hence, the contribution of specific energy to sonic power on biopolymer release has to be considered during pre-treatment. The sonic power influences the biopolymer release, thus improving its bio-degrading potential. In the present study, the optimization of power is achieved by keeping the biomass ratio as 1:100 and the effect of specific energy spent on the COD solubilization was analysed. Fig. 2, presents the relation of the specific energy spent on COD solubilization. The COD solubilization increases with an increase in sonic power input and with constant total solid concentration. The sonication power input will reflect on the energy utilization for pre-treatment. Fig. 2 indicates the COD solubilization of 10.17 % was achieved by spending specific energy of 18,000 KJ/Kg TS at 50 W. When the sonic power input was doubled to 100 W, by spending specific energy of 36,000 KJ/Kg TS, the COD sol-ubilization remains almost constant at 12.14%. Further rise in sonic power to 200 W at 30 min, the COD solubilization was 18.75 %, with the utilization of the same energy. This indicates that a power input of 200 W is more efficient to achieve COD solubilization than 100 W. Beyond the energy of 36,000 KJ/kg TS, the COD solubilization was found to be decreasing. This may be due to the decrease in liquefication, and con-version of soluble organic matters into gaseous substances. Similar re-sults were obtained by researchers while conducting microwave-assisted pre-treatment of macroalgae for biohydrogen production [47]. The maximum COD solubilization of 19.64 % was achieved within the en-ergy spent of 72,000 KJ/Kg TS at a sonic power input of 200 W. Furthermore, the COD solubilization was increased by 8.93% when specific energy was increased from 36,000 to 72,000 KJ/Kg TS. The optimum COD solubilization was achieved by spending energy of 36,000 KJ/Kg at 200 W. While the increase in specific energy leads to a decrease in COD solubilization. The results indicate that the increase in power input can increase the operating cost, but have less significance in bio solubility. Thus, the combined pre-treatment methods of macroalgae for biohydrogen production can be adopted to overcome these problems [48]. Hence, the optimized sonic power in this study is 200 W, which was used subsequently for further experiments in this study.

3.2. Saponin coupled sonic Pre-treatment (SSP)

The solubilization of the biomass can be improved by using surfac-tants in the pre-treatment process [49]. The surfactants are capable of improving the enzymatic saccharification process to form reducing sugars [50]. Many researchers revealed that usage of surfactants for pre-
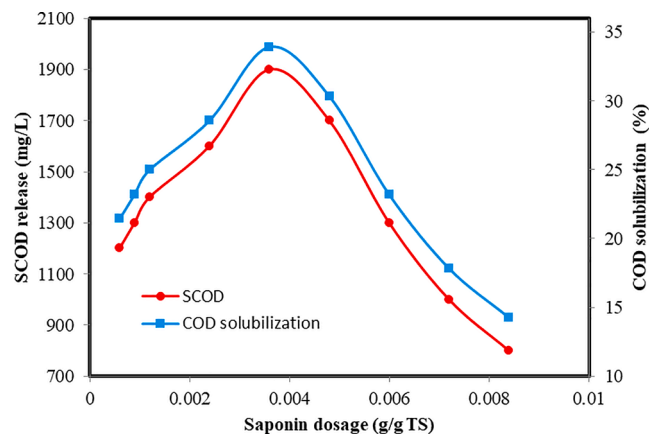
treatment of various organic sources may reduce the specific energy and be found to be cost-effective [34,51–52]. Rajesh Banu et al., (2020) studied a cost-effective surfactant aided pre-treatment method for the production of bio methanation using microalgae [53]. The carbohydrate moiety makes it hydrophilic. The surfactant was coupled with sonication to enhance the biopolymer release of the macroalgae and the energy spent for sonication can be reduced. The action of surfactant causes a decrease in surface tension and the surface area of the particle increases [54]. The combined treatment enhances the cavitation process leading to the faster release of organic compounds into the system. From the literature, the microalgae were able to release the organic matter by the usage of an anionic surfactant [45]. The effect of SSP on SCOD release and COD solubilization was studied and the results are depicted in Fig. 3. A minimum dosage of 0.0006 g/g TS was used, because of its foaming nature. Initially, at a saponin dosage of 0.0006 g/g TS, the SCOD release and COD solubilization were found to be 1200 mg/L and 21.42 % respectively. The surfactant reacts with the macroalgae and solubilizes the membrane. The membrane solubility increases at a low dosage of surfactant itself. Further increase in the surfactant dosage to 0.0036 g/g TS reveals a steep increase of COD solubilization to 33.92 % and the SCOD release was 1900 mg/L. From the literature, it is noted that the steep increase in SCOD is due to the high disintegration and defloccu-lation of macroalgae. Beyond this dosage, the SCOD release was found to be decreasing. This may be due to disruption of intracellular compo-nents and high organic loading. Moreover, the combined surfactant aided sonication process enhances the release of soluble fragments at an optimum surfactant concentration of 0.0036 g/g TS. From these
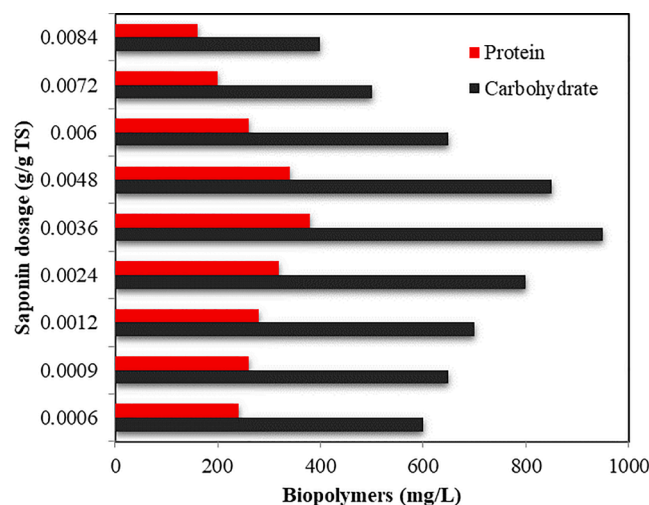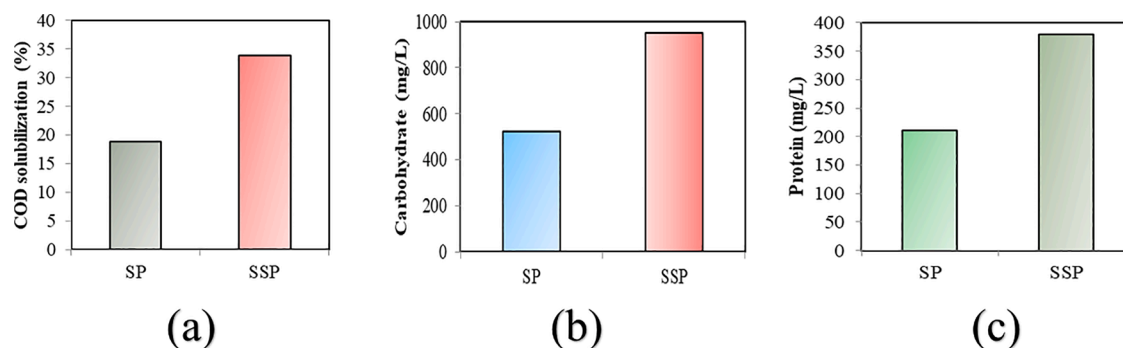

Fig. 4. Influence of SSP on biopolymer release.

Fig. 5. (a) Effect of SP &SSP on COD solubilization; (b) Effect of SP &SSP on carbohydrate; (c) Effect of SP &SSP on protein release.

discussions, it can be interpreted that the dosage of 0.0036 g/g TS could be considered optimum.

3.3. Influence of SSP on the release of soluble organic fractions and biopolymer release

The action of surfactants in the macroalgal cells leads to the formation of a capsulated layer and lowers the surface energy. The combinative sonication coupled pre-treatment generates high energy and accelerates the macroalgal cell solubilization. Rajesh Banu et al., (2020), explained the mechanism of the surfactant coupled sonication process for the liquefication of mixed cultures of microalgae for biomethane production [53]. The internal energy in the system disrupts the cell organelles and endo polymeric substances are released in the environment. Besides these, an increase in biopolymer release was expected during the SSP process. Fig. 4, indicates that the maximum concentration of carbohydrate and protein released at the dosage of 0.036 g/g TS were 950 mg/L and 380 mg/L respectively. Further increase in surfactant dosage to 0.0084 at the dosage of 0.0036 g/g TS reduces the concentration of biopolymer to 400 mg/L and 160 mg/L. Similar results were obtained by the researchers in the organic release of the substrate while using surfactant for biomethane production [44,49]. The study reveals that during the SSP process the increase in surfactant concentration causes the disintegration of biopolymer. The stability of bubbles decreases due to the presence of high solid content in media. The increase in surfactant concentration cannot improve bio solubilization. Thus, the dosage of surfactant in the SSP process has been optimized as 0.0036 g/g TS.

3.4. Comparison of SP and SSP

The cost-effectiveness of SP and SSP processes depends on the energy utilized and the bio solubilization of organic compounds. The performance of pre-treatment by SP and SSP was compared by finding the COD solubilization, carbohydrate, and protein concentration. Fig. 5a indicates the COD solubilization of SP and SSP respectively were 18.75 % and 33.9 %. Furthermore, there was a notable increase of COD solubilization by 80.8 % for SSP compared to SP. Similarly, the concentration of the biopolymers such as carbohydrate, and protein were found to be higher in SSP than SP. The concentration of carbohydrate by SP and SSP represented in Fig. 5b and were found respectively to be 525 mg/L and 950 mg/L. The concentration of protein (Fig. 5c) by SP and SSP process were found respectively to be 210 mg/L and 380 mg/L. The study emphasizes that in SSP, an increase of carbohydrate and protein concentration respectively by 94.9% and 80%. The synergistic effect of the saponin coupled sonication process effectively solubilizes the complex structure macroalgae resulting in an increase in the release of biopolymer. The increase in biopolymer release has a significant role in biohydrogen production. Hence, the SSP process was found to be effective than SP.
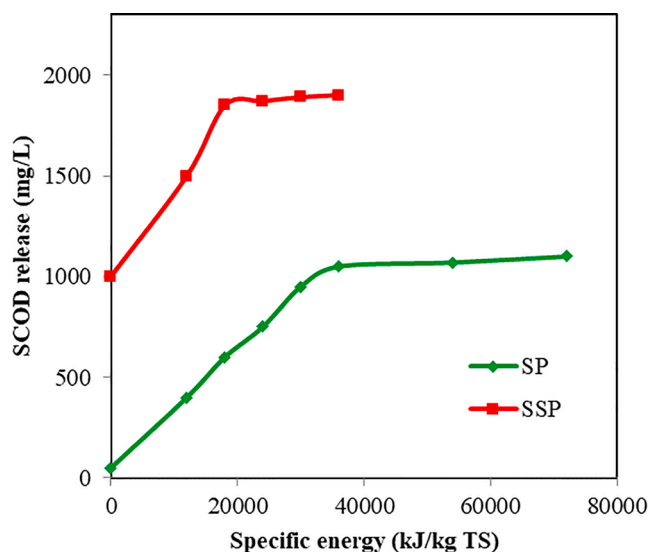

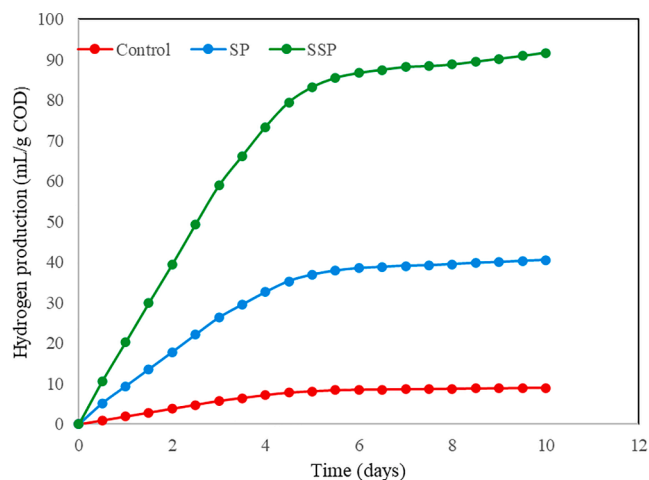**Fig. 6.** Comparison of SP and SSP on SCOD release.

Fig. 7. Effect of SP & SSP on biohydrogen production of *Ulva fasciata*.

The specific energy spent in the process indicates the feasibility of scaling up the process. From Fig. 6, SCOD release by SP and SSP without power input respectively were found to be 50 mg/L and 1000 mg/L. When the power input was increased from 1200 KJ/Kg TS to 2400 KJ/Kg TS, there was a steep increase in SCOD release (400 to 750) mg/L for SP and (1500 to 1870) mg/L for SSP. While comparing both the process, the SCOD release in SSP was higher at a rate of 1850 mg/L by spending

Table 2

Kinetics analysis of Control, SP and SSP for bio-hydrogen production

S.No.	Samples	k (mL/d)	H_L (days)	H_Y (ml of H_2 / g of COD)	R^2
1	Control	0.1	3.2	9	0.9552
2	SP	0.25	1.2	40.5	0.9537
3	SSP	0.45	0.8	91.7	0.9545

Where k is the maximum hydrogen production rate, H_L is the No. of days required to complete the lag phase, H_Y is the hydrogen yield, R^2 is the coefficient of determination

energy of 18,000 KJ/Kg TS. Besides, the SCOD release in the SP process was very less at a reaction time of 15 min. The energy required for the SSP process was 3600 KJ/Kg TS for the release of 1900 mg/L of SCOD within 30 min. The SSP depicts the peak concentration at very low levels of specific energy whereas SP consumes almost double the energy requirement compared with SSP. The maximum SCOD release by SP process is nearly half of the maximum SCOD release by SSP process. Hence, SSP is a better choice when compared with SP in terms of SCOD release.

3.5. Biohydrogen production

Biohydrogen production potential of untreated (control), SP, and SSP samples were evaluated and indicated in Fig. 7. From this figure it is visualised that the hydrogen generation starts increasing gradually from first day to fourth day. The observed biohydrogen production of the 4th day in the control, SP, SSP respectively were 7.2 mL/g COD, 32.6 mL/g COD, 73.4 mL/g COD. The reason for gradual increase in hydrogen production is due to availability of substrate to microbes. It is also inferred that a higher biohydrogen production of 91.7 mL/g COD was observed in SSP than SP. This could be due to higher liquefaction achieved by synergetic biosurfactant coupled sonication. The maximum biohydrogen production in control was 9 mL/g COD, and this is due to the slow hydrolysis process demanding the need for pre-treatment of substrate for higher biohydrogen production. When compared with other pre-treatment processes, the surfactant coupled sonication process produced biohydrogen within an incubation of 6 days. Beyond six days there is no increment in hydrogen production, which is attributed due to effect of non-availability of substrate to microbes. The statistical data collected were validated using the modified Gompertz model. The

kinetic parameter obtained from first modelling for different samples is presented in Table 2. The coefficient of correlation value (R^2) was determined and is almost in the range of 0.9537 to 0.9552 suggesting the best fit.

Mass balance analysis has been carried out to compare the COD removed with that of produced hydrogen during hydrogen fermentation [55,56]. Fig. 8 represent the mass balance analysis of samples control, SP and SSP. In SSP, the added COD (influent COD) to the hydrogen fermentor was 1.12 g (for 200 mL of macroalgae substrate) out of which around 45.3% of the COD was removed as effluent COD with a value of 0.5075 g. Also, 0.0105 g of the added COD was utilized by the microbes for biomass growth which was around 1% of the added influent COD. A total of 0.602 g of COD was converted to hydrogen gas in the reactor which constituted COD consumptions of 1.9 g/L SCOD (0.38 gSCOD/ 200 mL of substrate added to the fermentor). This implies that 53.75% of the influent COD was utilized for hydrogen production and showed 100% balance in SSP. In SP, the added COD (influent COD) to the hydrogen fermentor was 1.12 g (for 200 mL of macroalgal substrate) out of which around 68.4% of the COD was removed as effluent COD with a value of 0.766 g. Also, 0.007 g of the added COD was utilized by the microbes for biomass growth which was around 0.625% of the added influent COD. A total of 0.347 g of COD was converted to hydrogen gas in the reactor which constituted COD consumption of 1.05 g/L SCOD (0.21 g SCOD /200 mL macroalgal substrate added to the fermentor). This means that 30.98% of influent COD was utilized for hydrogen production and showed 100% balance in SP. In control, the added COD (influent COD) to the hydrogen fermentor was 1.12 g (for 200 mL of macroalgal substrate) out of which around 96.88% of the COD was removed as effluent COD with a value of 1.085 g. Also, 0.001 g of the added COD was utilized by the microbes for biomass growth which was around 0.089% of the added influent COD. A total of 0.034 g of COD was converted to hydrogen gas in the fermentor which constituted COD consumptions of 0.035 gSCOD/L (0.007 gSCOD/200 mL macroalgal substrate added to the fermentor). This implies that 3.13% of the influent COD was utilized for hydrogen production and showed 100% balance in control.

4. Conclusion

The feasibility of producing biohydrogen from a green marine
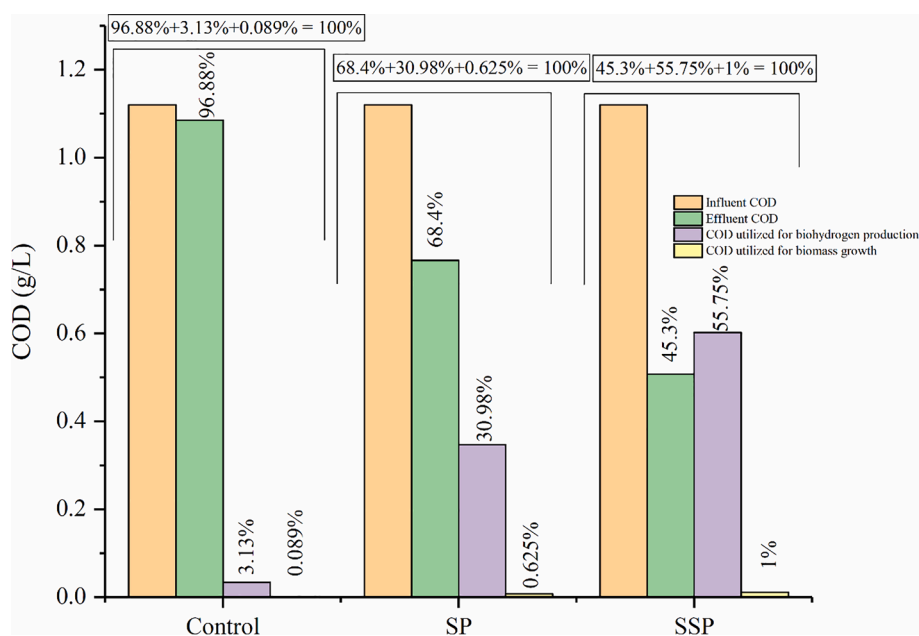

Fig. 8. Mass balance analysis of samples control, SP and SSP.

macroalga, *Ulva fasciata* was evaluated by SP and SSP process. In SP, pre-treatment time and input power were optimized as 30 min and 200 W respectively. The surfactant dosage in SSP was optimized to 0.0036 g/g TS. The SP and SSP treatment processes were optimized to improve the hydrolysis process during digestion. By comparing these two processes, a high concentration of biopolymer release was found in SSP than SP at optimized conditions. The effect of the SSP process on pre-treatment was assessed by estimation of COD solubilization and SCOD release. The study revealed that, at a specific energy of 36,000 KJ/Kg TS, the SCOD release was higher in SSP (1900 mg/L) than SP (1050 mg/L). The SSP process could improve the COD solubilization to 15 %, more than the SP. The use of biosurfactants reduced the energy utilization in the hydrolysis process. The pre-treated *Ulva fasciata* biomass was subjected to bio-hydrogen assay in which a higher biohydrogen yield of 91.7 mL/g COD was observed for SSP when compared to SP (40.5 mL/g COD) and control (9 mL/g COD). These conclude that the surfactant coupled sonication process is more efficient and could be adopted for scaling up of the pilot plant process to arrive at a further optimum operating condition for commercial plant and study the actual economics of the process.

CRediT authorship contribution statement

A.V. Snehya: Investigation, Validation, Writing – original draft. **M. A. Sundaramahalingam:** Formal analysis, Data curation. **J. Rajesh-banu:** Resources, Writing – review & editing. **S. Anandan:** Visualization, Writing – review & editing. **P. Sivashanmugam:** Conceptualization, Supervision, Methodology, Writing – review & editing.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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