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Chemo-Sonic Pretreatment Approach on Marine Macroalgae for Energy Efficient Biohydrogen Production

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Abstract: The core objective of this analysis is to implement a combination of alkaline (NaOH) and sonication pretreatment techniques to produce energy-efficient biohydrogen from the marine macroalgae *Chaetomorpha antennina.* Anaerobic fermentation was implemented in control, sonic solubilization (SS) and sonic alkali solubilization (SAS) pretreatment for 15 days. In control, a biohydrogen production of 40 mL H_2/gCOD was obtained. The sonicator intensities varied from 10% to 90% for a period of 1 h during SS pretreatment. About 2650 mg/L SCOD release with a COD solubilization of 21% was obtained at an optimum intensity of 50% in a 30 min duration, in which 119 mL H_2/gCOD biohydrogen was produced in the anaerobic fermentation. SAS pretreatment was performed by varying the pH from 8 to 12 with the optimum conditions of SS where a SCOD release of 3400 mg/L, COD solubilization efficiency of 26% and a maximum biohydrogen production of 150 mL H_2/gCOD was obtained at a high pH range of 11 in the fermentation. The specific energy required by SS (9000 kJ/kgTS) was comparatively higher than SAS (4500 kJ/kg TS). SAS reduced half of the energy consumption when compared to SS. Overall, SAS pretreatment was found to be energetically favorable in a field application.

Keywords: COD solubilization; chemo sonic pretreatment; biohydrogen; specific energy

1. Introduction

Recently, a lot of environmental issues have been raised owing to the usage of fossil fuels. It motivates researchers and scientists to predict prompt remedial action to create a proper substitute for fossil fuels [1]. Furthermore, most countries extract energy from many natural resources such as wind, hydropower and solar power. Biomass is a significant potential source of energy among these energy resources [2]. As a photosynthetic organism, marine macroalgae has the promising potential to act as a bioresource for biofuel production. Since it is associated with the green color type of marine macroalgae autotrophs, it is a rich source of biopolymers such as protein, carbohydrates and lipids, which are responsible for more biofuel production [3]. Furthermore, the lack of lignin content makes the marine macroalgae even more appropriate for an effective anaerobic fermentation process [4]. Marine macroalgae is a collection of rapidly growing plant organisms that can grow to substantial sizes in marine environments such as rock surfaces. The median photosynthetic activity of this marine macroalgae was 6–8%, much higher than that of earthbound biomass (1.8–2.2%) [5].

The circular economy involves energy recovery from trash and residues, which can fulfil the material and energy cycle. A very promising pathway toward sustainability is the biogas–biohydrogen chain. It can be transmitted into the natural gas grid, used as a vehicle fuel, or transformed into electricity-generating units. It is produced from a variety of different substrates, such as crop leftovers, algae, animal wastes, organic portion of municipal solid wastes and sludge [6,7]. Anaerobic fermentation is the sustainable way of

extracting or generating bioenergy from macroalgae since it is adaptable to this process [8]. Hydrogen (H$_2$) is one of the various fuel sources that evolved from igniting hydrogen-holding elements such as natural gas, oil and coal. However, with regards to excessive energy content, hydrogen has an enormous energy density than other surviving sources of fuels such as methane and ethanol [9]. Figure 1 shows the global hydrogen production in the last ten years and its market value based on the report of GHR, 2021 [10].
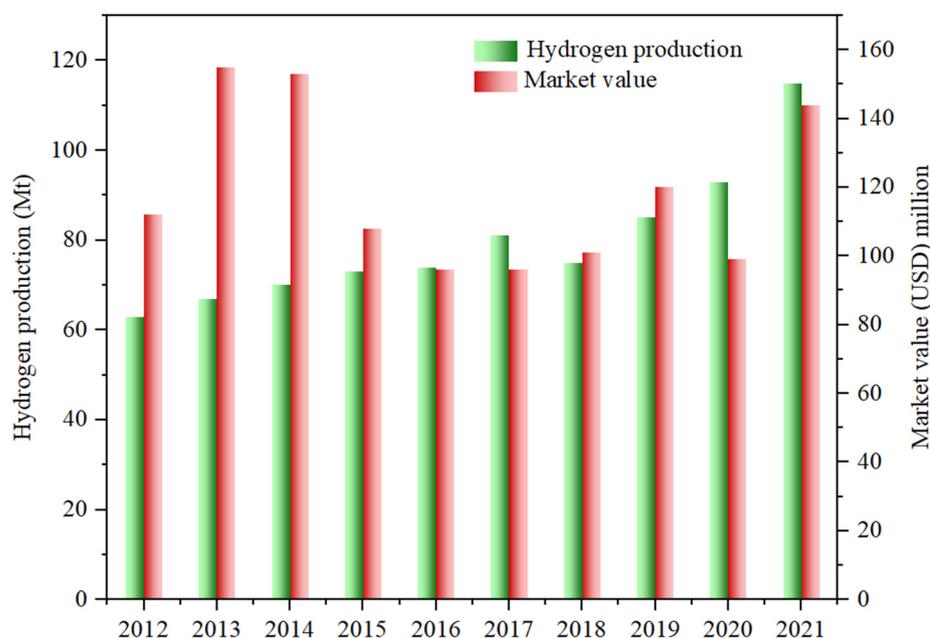

Figure 1. Global hydrogen production in last ten years and its market value.

Additionally, hydrogen is a high potential energy supplier for various utilities such as an ignition engine fuel for vehicles, rocket propellant, fuel cells for power supply and reactor coolant [11]. Furthermore, hydrogen generation makes way for the ecological crisis in the surrounding environment, such as global warming, melting glaciers and icebergs, rising ocean water levels and air pollution. Therefore, the bio-based hydrogen generation method is considered more appropriate than the standard fuel generation source. Moreover, the output of biohydrogen using substrate biomass has quite a few benefits such as uncomplicated performance, outspread accessibility of energy resources such as residues of food, vegetables, fruits, fauna manure such as cow chip, unbiased carbon source and cost worthy in its functions [12]. When specific requirements are met, the bioeconomy can advance toward sustainability: (i) the resource base is sustainable; (ii) processes and products are sustainable and (iii) transport of materials is viable [13]. Furthermore, customer satisfaction with bio-based products is also necessary to assess the influence of green premiums and the significance of sustainability certification [14]. However, these factors affect various industries because they are essential when considering sustainability as a factor that facilitates market success and a competitive advantage source [15]. In this perspective, the use of biological resources to replace non-renewable resources, escalating the use of biomass and reducing biowaste are excellent examples of a circular bioeconomy, which can be crucial in achieving sustainable development goals (SDGs) [16]. Marine macroalgae and biohydrogen satisfy all the mentioned criteria of the bioeconomy. Therefore, both marine macroalgae and biohydrogen are bio-economically feasible. Hydrolysis is the primary step of the anaerobic fermentation at which the cell cleavage occurs, a rate-limiting factor of the fermentation process. It is a difficult stage in anaerobic fermentation since the cell wall of the biomass may be more vital to rupture [17]. To augment the hydrolysis phase, the structural integrity of the biomass can be degraded through various pretreatment techniques and biopolymers such as proteins, carbohydrates, lipids and starch present in the biomass come out for the oxygen-free fermentation process [18]. Therefore, for this purpose, various

pretreatment techniques, such as physical, chemical, mechanical and biological ones, are incorporated [19]. Ultrasonication is a method of generating acoustic waves used to disrupt the cell wall of the biomass [20]. The sonicator gives rise to high-intensity ultrasound waves through a probe over the substrate kept in a beaker with water inside the apparatus. These high-intensity sonic waves are generated with the help of an intensity generator during the sonication process. These high-intensity sonic waves initiate the pressure wave formation and due to these pressure waves, cavitation develops. This cavitation collapses the cell wall of marine macroalgae species and disrupts it [21]. Energy exhaustion is a primary concern because the mechanical (sonication) pretreatment consumes much energy (electric current) to disrupt the biomass cell wall [22]. In order to overcome this problem, additives such as alkali and surfactants can be added, making the operation process of sonication energetically feasible [23]. Microwave–surfactant, microwave–acidic and disperser–ozone were the combinative pretreatment techniques used to solubilize marine macroalgae until now [24]. However, there are no published studies on marine macroalgae (*Chaetomorpha antennina*) solubilization using the sonication and alkali (NaOH) combination. Therefore, the marine macroalgae were solubilized in this study using a novel technique called alkali-assisted sonication. The objectives of this research are (1) to optimize the solubilization conditions for SAS for energy-effective performance; (2) to perform kinetic analysis for SS and to analyze its efficiency; (3) to assess the beneficial impact of this SAS pretreatment; (4) to evaluate the effect of this SAS pretreatment on the production of biohydrogen; (5) to perform an energy analysis of SAS in terms of field applicability.

2. Materials and Methods

2.1. Marine Macroalgae Sample

The marine macroalgae biomass species *Chaetomorpha antennina* was collected from ennore, a marine area of chennai (13°12′23.4864″ N, 80°19′38.0100″ E), Tamil Nadu, India. The marine macroalgae were entirely washed with water to detach the residue particles. The cleaned sample was shade-dried and sliced into pieces of less than 2 cm in size for the convenience of pretreatment. This biomass was kept in a refrigerator for the subsequent study [24].

2.2. Biomass Pretreatment

2.2.1. Sonic Solubilization (SS)

SS pretreatment was implemented to rupture the cell wall of the biomass. The operation mechanism utilized a sonicator (Model VCX130, New Town, CT, USA) instrument with a frequency of 20 kHz and a maximum power input of 130W. A beaker of 1L capacity volume filled with water and substrate sample was taken for this pretreatment. The substrate and water ratio taken for pretreatment was 1:50. The sonication power intensity and the time duration varied from 10 to 90% and from 1 to 60 min, respectively. The sonic probe produces the combined effect of pressure waves and cavitation. This effect results in the marine macroalgae cell wall weakening for enhanced solubilization and biopolymers release. The only drawback of this SS pretreatment was that it consumed more electrical energy to solubilize the marine macroalgae. The samples were taken and examined for a regular period.

2.2.2. Sonic Alkali Solubilization (SAS)

The solubilization of the substrate by SAS was carried out by adding alkali "sodium hydroxide (NaOH)" with an optimum condition obtained from SS pretreatment. The pH of the sample varied from 8 to 12. SAS pretreatment is appropriate for the following reasons: (1) the mechanical (sonication) pretreatment gives high and efficient output within a quick session compared to physical and biological pretreatment methods. (2) Alkali (NaOH), when added to sonication, are divided into cations (Na^+) and anions (OH^-). Cations transform into bubbles, clash with the cell wall of marine macroalgae and break it. Anions settle over the marine macroalgae cell wall surface and weaken it. This phenomenon

accelerates the solubilization process of marine macroalgae and more biopolymers are released in a short duration. The samples are taken at a regular time interval and subjected to analysis.

2.3. Anaerobic Fermentation Study

Anaerobic fermentation was performed for control, SS and SAS, into which anaerobically digested sludge (inoculum) taken from a wastewater treatment plant was added at a ratio of 9:1 in serum bottles of 250 mL volume capacity for three days. To suppress the fermentation within the acetogenic phase and to compute the volatile fatty acids (VFA) produced, a methanogenic phase obstructor 50 mM of 2- Bromo ethane sulphonic acid (BESA) was added to each bottle. The computation of VFA was performed to substantiate the pretreatment and biohydrogen production efficiency [25]. To remove O_2, nitrogen gas was introduced into all serum bottles. The bottles were firmly sealed by stoppers and positioned in an orbital shaker under agitation at a speed and temperature of about 150 rpm and 35 °C [26]. VFA analysis was performed through the distillation method [27].

2.4. Biohydrogen Potential Assessment (BPA)

BPA analysis was applied for control, SS and SAS to evaluate the biohydrogen production capability under moderate temperatures. The process of BPA was performed in serum bottles with a functioning volume of 150 mL. In all three serum bottles, the marine macroalgae sample (70%), inoculum (25%) and the nourishment food (5%) were taken [28]. As a point of expelling methanogens in the inoculum and enriching the microbes for hydrogen production, the inoculum was subjected to calefaction for 30 mins at 100 °C [29]. To maintain an oxygen-free environment, nitrogen (N_2) gas was filled in the remaining bottle area for 10 mins [30]. Rubber stoppers were used to seal the bottles. Finally, the bottles were kept in a shaker and incubated at 37 °C at 130 rpm. A gas chromatograph with a thermal conductivity detector and stainless column packed with Porapak Q (3.25 mm diameter, 2 cm length and 80/100 mesh) was used to calculate hydrogen production [31]. The experiments were triplicated. To estimate the cumulative H_2 yield, the modified Gompertz Equation (1) was used.

$$AH = Hl * exp\left(-exp\left(-pr(Hc - Hfb)\right)\right) \tag{1}$$

where:

AH—Increased H_2 production (mL);
Hl—H_2 production (mL H_2/g COD);
pr—Peak H_2 generation rate (mL H_2/g COD d);
Hc—Commencing phase of hydrogen production (days);
Hfb—Lag phase of hydrogen production (days).

2.5. Analytical Methods

The biopolymers proteins, carbohydrates and lipids released as a result of pretreatment were measured based on the method prescribed by Kavitha et al. (2016) [32]. In addition, total chemical oxygen demand (TCOD), soluble chemical oxygen demand (SCOD), and VFA were analyzed with the help of standard methods as per APHA (2005) [33].

2.6. Statistical Analysis

One way analysis of variance (ANOVA) ($\alpha = 0.05$) approach was made to assess the deliverables of the experiment. The differences between experimental deliverables during the pretreatment could be subjected to statistical significance analysis if the p-values were less than 0.05. To be precise, for *p*-values < 0.05, the difference between SCOD release averages was statistically significant. On the contrary, for *p*-values > 0.05, the difference between SCOD release averages was not statistically significant [34].

2.7. Specific Energy for Sonication (SES)

Specific energy (SE) is considered for the measure of vital energy required by the sonicator to solubilize the cell wall of marine macroalgae. The SE was calculated using the subsequent Equation (2):

$$SES\ (kJ/kg\ TS) = (P_D \times S_T)/(Vs \times TS) \tag{2}$$

where:

SES—Specific energy for sonication;
P_D—Power used for disruption of the biomass cell wall (kW);
S_T—Sonication treatment time (s);
V_S—Volume of the sample (L);
TS—Total solids (kg).

2.8. Energy Analysis

One prominent contemplation in the massive scale biofuel production is the energy employed in the entire process. From an economic angle, minimum input energy should exhibit the uttermost output energy, which will be profitable [35]. This investigation studied the energy required to treat 1 kilogram of marine macroalgae biomass sample to produce H_2 gas. The total net energy that has been dominated was calculated using Equation (3).

$$N_E = O_E - I_E \tag{3}$$

where:

N_E—Net energy (kWh);
O_E—Output energy (kWh);
I_E—Input energy (kWh).

The solubilization energy taken by the sonicator is the input energy as shown in Equation (4).

$$I_E = P_S * T_S * V_R * B \tag{4}$$

where:

I_E—Input energy (kWh);
P_S—Power utilized for the sonication process (kW/kg);
T_S—Time consumed for solubilization (h);
V_R—Reactor volume (m^3);
B—Biomass (kg/m^3).

The output energy was calculated based on various parameters such as biomass biodegradability, organic load, the volume of the reactor and hydrogen yield, as mentioned in Equation (5).

$$O_E = B_{SB} * L_{COD} * H_Y * V_R * B_{CF} \tag{5}$$

where:

O_E—Output energy (kWh);
B_{SB}—Biodegradability of marine macroalgae biomass (g COD/g COD);
L_{COD}—COD load (g COD/m^3);
H_Y—Hydrogen yield (m^3/g COD);
V_R—Reactor volume (m^3);
B_{CF}—Biohydrogen conversion factor.

By determining the optimistic and pessimistic amount of net energy, the profit and loss in the energy are confirmed in the SS and SAS processes.

The energy ratio is given in Equation (6),

$$Er = O_E/I_E \tag{6}$$

where:

Er—Energy ratio;

O_E—Output energy (kWh);

I_E—Input energy (kWh).

3. Results and Discussion

3.1. Sequel of SS in the Liberation of Soluble Organics Release

Solubilization potential was estimated by the release of soluble organics during the SS process. Figure 2 shows the release of the soluble organics for sonication intensity and period. The sonicator was operated by varying its power intensities from 10% to 90% for 1 h. During SS operation, the marine macroalgae that had to be solubilized was kept under the sonicator probe. It was subjected to the impact of high-power ultrasonic waves, which resulted in the emergence of pressure waves and cavity bubbles. This simultaneous evolution of cavity bubbles and pressure waves weakened the marine macroalgae cell wall. At each intensity, the solubilized marine macroalgae sample was taken and analyzed. In Figure 2, it was observed that the release of the soluble organics was classified into two phases, namely, the faster phase (1–30 min) and the slower phase (30–60 min). The figure shows that when the sonication pretreatment time increases, there is an increment found in the soluble organics release. In the faster phase, 1–30 min, the release of soluble organics was high up to 30 mins, but in the slower phase, beyond 30 min, the minor release was found. A steady trend was spotted in the slower phase after 30 min. This trend indicates that most of the soluble organics got unleashed within 30 min in the faster phase. For a sonication process, the pretreatment time was recognized as an ideal parameter [36]. Hence, the sonication pretreatment time of 30 min was acknowledged as an optimum pretreatment time for SS. Furthermore, the sonication intensity for pretreatment also plays an indispensable part in SS. When the release of the soluble organics was reasoned against the intensity of SS, an extraneous behavior was noticed in the release of the soluble organics. In the intensity range (10–40%), there was a minimum release found in soluble organics and the release range was 1750–2320 mg/L. This provided authentic evidence that the marine macroalgae were partially solubilized [37]. When the intensity is further increased to 50%, drastic enhancement in the soluble organics release of 2650 mg/L was obtained due to the combined effort of high-power ultrasonic waves, pressure waves and increased formation of cavity bubbles. This caused the marine macroalgae cell wall to smash and become solubilized. Increasing the intensity beyond 50%, there was no excess improvement obtained. The soluble organics release found between 50–90% was in the SCOD release range of only 2819–3010 mg/L. This marginal release was found because most of the soluble organics got released at up to 50% intensity. Hence, 50% was considered to be optimum for SS. For the soluble organics released during SS, statistical analyses were carried out via ANOVA. Table 1 represents the one-way ANOVA of variance for various intensities of sonicator on the SCOD release basis. When the intensity varied from 10% to 40%, the probability value was found to be 0.46, which was greater than 0.05. This signifies that there is no statistical difference. For intensities between 40% and 50%, the probability value of 0.013 obtained was less than 0.05. This shows that there was a considerable difference found between 40% and 50%. The mean values of SCOD release from 50% to 90% imply a lack of significant difference between them, with a probability value of 0.84, which was greater than 0.05. Therefore, considering all these outcomes, a power intensity of 50% with a duration of 30 min was considered as optimum.

3.2. Response of SE over COD Solubilization

Significant attention is given to SE regarding the economy of the process for enormous biofuel production. Figure 3 represents the solubilization of SS concerning SE. It was noticed that the solubilization trend increases with an increase in SE input for all sonic intensities. The solubilization tendency can be divided into three phases: X, Y and Z. Slower solubilization was represented by phase X, which corresponds to intensities of 10% to 40%.

Phase Y represents a faster solubilization rate, ranging from 40% to 50%. Finally, phase Z extends from 60% to 90%. At a sonicator, SE input of around 1800–7200 kJ/kg TS, solubilization of about 13.46–17.84% was achieved during phase X. The amount of solubilization obtained was insignificant and can be ignored for further analysis. An effective rise in solubilization was observed in phase Y, with a maximum of 21% reached at a sonicator SE input of about 9000 kJ/kg TS for an intensity 50%. Even though the sonicator intensity and SE were increased from 60% to 90% and 9000 kJ/kg TS to 10,800 kJ/kg TS in phase Z, there was no significant increase in solubilization. To increase solubilization from 21% to 22%, for example, a sonicator SE input of 10,800 kJ/kg TS was required. As a result, it can be concluded that simply raising sonicator intensities during the SS process may waste energy. Instead, SS was found to benefit from an optimum sonicator SE input of 9000 kJ/kg TS.
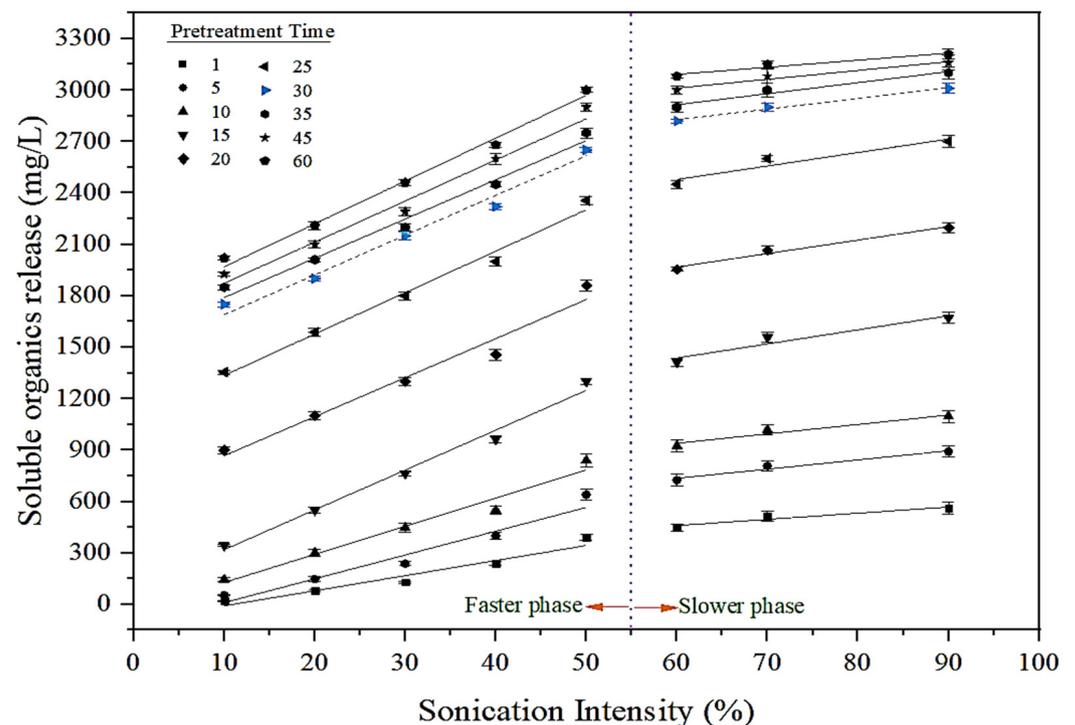

Figure 2. Soluble organics release with respect to sonication intensity.

Table 1. One-way analysis of variance for various intensities of sonicator on the SCOD release basis.

Variation Source	Sum of Squares	Degrees of Freedom	Mean Square	F Value	p-Value Prob > F	Results
10–40%	56,512	3	977,011	0.5	0.46	Not significant
40–50%	52,040,402	1	6,900,710	7.5	0.013	Significant
50–90%	40,951	3	1,052,333	0.031	0.84	Not significant

3.3. Impact of SAS in the Discharge of Organic Biopolymers

Sodium hydroxide (NaOH), as an alkaline solution, has the massive potential to fracture the cell wall's ester bond, resulting in increased cellulose decrystallization [38]. During SAS, alkali, when added to the sample, gets split into cations (Na$^+$) and anions (OH$^-$). Due to saponification, the cations get transmuted into bubble form, clash with the marine macroalgae cell wall and break it; and due to solvation, it settles in the bottom of the beaker as salts. On the other hand, the anions settle over the cell wall and make it squashy, which makes the sonication process even more rapid and comfortable. This results in the reduction of energy consumption by the sonicator. Thus, the alkali (NaOH) acts as an excellent energy-saving additive and intensifies the sonication pretreatment even more

effectively [39]. In the present study, alkaline (NaOH) was combined with the SS process to enhance the solubilization capability of the previous certainties. Figure 4 signifies the soluble organics and biopolymer release at various pH levels. The alkali was added by differing its pH from 8 to 12. The sonicator was operated at 50% of power intensity and 30 min of duration, which was optimized in SS, and the sample's pH was varied. During the operational time of SAS, for every 5 min, the solubilized biomass sample was taken and examined for each pH from 8 to 12. From the figure, it was understood that the patterns of soluble organics (SCOD) and biopolymers (protein, carbohydrates and lipids) show two divergent phases: an accelerated and a slow phase. The accelerated phase occurs from pH 8 to pH 11, where a soluble organics release (2900–3400 mg/L) was obtained. This proves that the combinative pretreatment was very effective as more SCOD were released in SAS (3400 mg/L) compared to SS (2650 mg/L), as presented in Figure 2. This massive increase in the release of soluble organics during the accelerated phase could be due to the combined action of SAS, which prompts the fracturing of marine macroalgae cell walls and the release of intercellular components. It is similar to the work of Kumar et al. (2017) [40], where the SCOD release of 1603 mg/L was obtained from microalgae via combined pretreatment of sonication and electrolysis. The slow phase lies from pH 11 to pH 12, where a soluble organics release (3400–3450 mg/L) was obtained. A significant hike was found in the release of soluble organics between pH 8 and 11, but in the slow phase beyond pH 11, a minimum rise was noted in the release of soluble organics. This makes it evident that almost all the soluble organics got released within pH 11 and it was adequate to solubilize the marine macroalgae cell wall. Therefore, increasing the pH level beyond 11 will increase chemical cost rather than marine macroalgae solubilization. From Figure 4, it is evident that at optimum solubilization of 21%, SAS consumed less SE (4500 KJ/kg TS) compared to SS (9000 kJ/kg TS), which shows that SAS is more energetically feasible than SS.
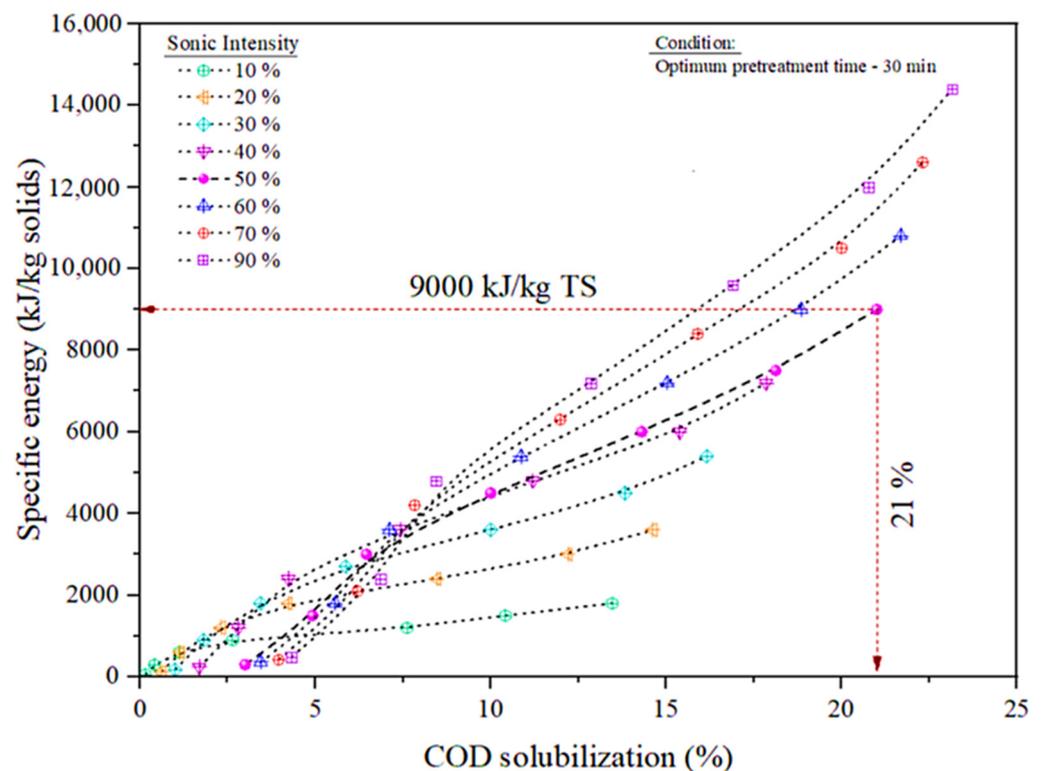

Figure 3. Solubilization efficiency of SS with respect to specific energy.
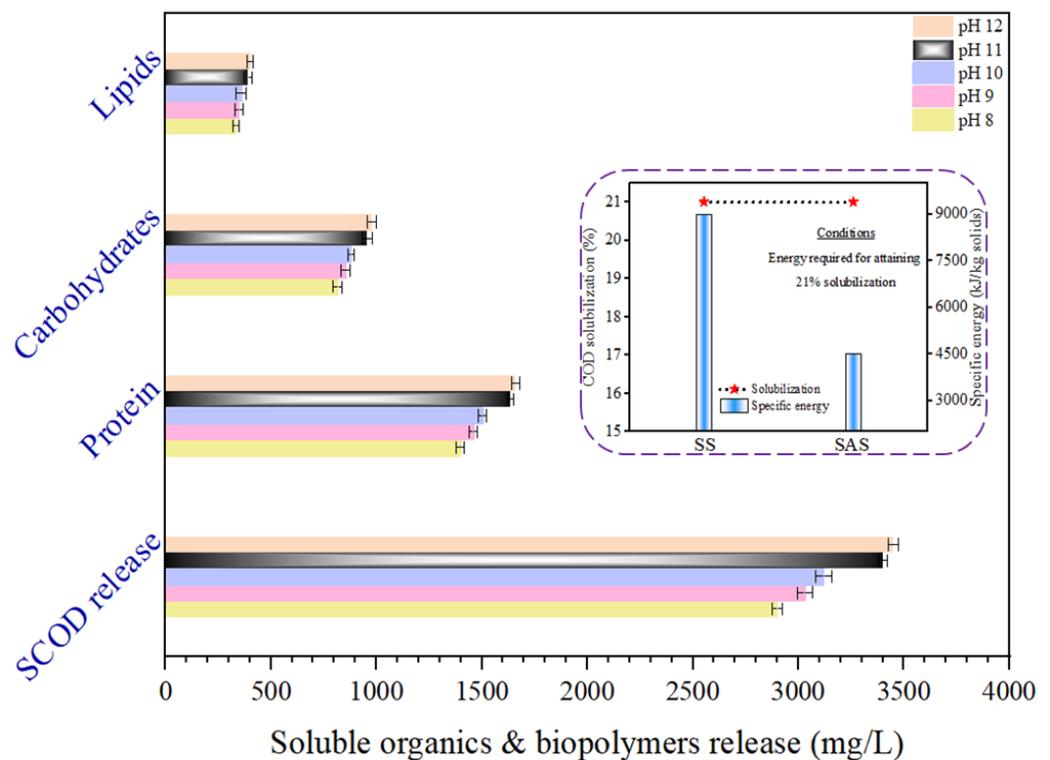
Figure 4. Soluble organics and biopolymers release in SAS.

The biopolymer's existence in marine macroalgae boosts hydrogen generation. Figure 4 elucidates the biopolymer release from pH 8 to pH 12. Indisputably, the biopolymers trend is similar to the SCOD trend and could be grouped into two phases: active and inactive. The active phase begins at pH 8 and ends at pH 11. A moderate increase in the biopolymers release was observed in this active phase up to a pH of 11, where a protein, carbohydrate and lipid release of 1637, 957 and 390 mg/L was obtained. The inactive phase begins beyond 11 where a protein, carbohydrate and lipid release of 1660, 978 and 402 mg/L, respectively, were obtained and there is no sturdy increase in biopolymers release after that, which signifies that the majority of the biopolymers got released in the pH 11. The collaborative effect of chemo sonic pretreatment makes way for effective solubilization of marine macroalgae cell wall and the liberation of biopolymers into the liquid phase of marine macroalgae. Hence from the facts mentioned earlier, it was concluded that SAS is more effective in solubilization and biopolymers release.

3.4. VFA Production in SS and SAS

The VFA investigation done for control, SS and SAS pretreated samples during anaerobic fermentation was analyzed and conveyed in Figure 5. In the commencing hydrolysis stage, the complicated hydrolytic components released during pretreatment got converted into sugars, amino acids and fatty acids. In the peripheral stage of acetogenesis, the simple monomers got transmuted into VFA [41]. Due to the biological action of microbes in the inoculum, the biopolymers got transformed into VFA [42]. Anaerobic fermentation was carried out for 72 h. At the end of 72 h, as predicted, SAS showed an enormous decrement in protein, and carbohydrate concentration from 1637, 957 mg/L to 623, 364 mg/L, which denotes the hydrolysis competence. On the other hand, SS showed a slight protein and carbohydrate concentration reduction from 1300, 760 mg/L to 498, 289 mg/L. The depletion in the concentration of biopolymers was found to be a lot less in SS compared to SAS. This made authentic evidence that highly solubilized biopolymers are easily accessible by fermentative microbes, which defines the effectiveness of combinative pretreatment [43]. It is similar to the combinative pretreatment strategy suggested by Tamilarasan et al. (2017) [44].

In contrast, the untreated control sample did not manifest a major decrement; instead, a build-up was spotted in the biopolymer's concentration. In control protein, the carbohydrate concentration was increased from 160,110 mg/L to 180,130 mg/L respectively. The reason behind this is that the biopolymers are not solubilized since there was no pretreatment in control; hence, the microbes try to break the marine macroalgae cell wall and release the biopolymers. This release was found only using disintegration instead of fermentation. The increased VFA production should have a higher hydrogen yield at the end of fermentation process. The VFA production analysis was performed to validate the effectiveness of biohydrogen production in the fermentation process. The utmost liberation of VFA during fermentation intensifies biohydrogen production [45]. Figure 5 clearly states that among control (110 mg/L), SS (860 mg/L) and SAS (1800 mg/L) after 72 h of anaerobic fermentation, SAS showed higher VFA production compared to SS and control due to the alkali sonication impact and effective utilization of pretreated and hydrolyzed biopolymers by acetogenic microbes. From the findings, SAS presents effectiveness in VFA production, hence proving that SAS will yield more hydrogen at the end of the fermentation process.
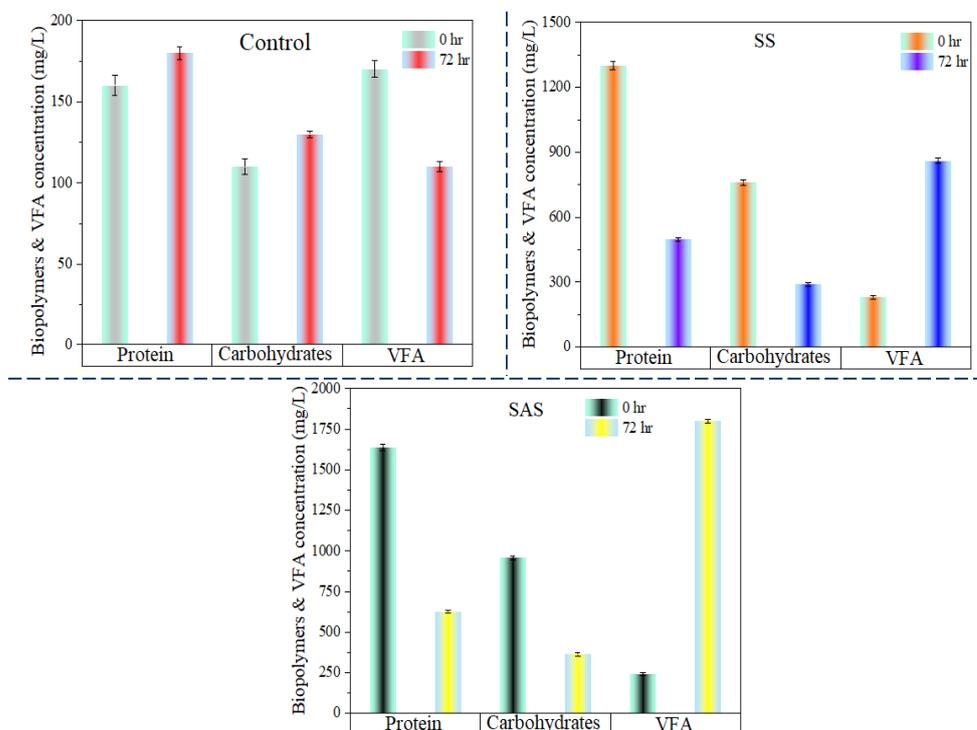

Figure 5. VFA production in control, SS and SAS.

3.5. Biohydrogen Potential Assay (BPA)

Figure 6 signifies the biohydrogen production in control, SS and SAS. From Figure 6, it was unquestionably understood that the biohydrogen generation got varied with control, SS and SAS. Biohydrogen analysis was done for 15 days. Regardless of augmentation in biohydrogen generation concerning increasing days of fermentation, the generation rate of biohydrogen was less in control (40 mL H_2/g COD) in comparison with SS (119 mL H_2/g COD) and SAS (150 mL H_2/g COD) on the eighth day of fermentation. This is due to the certainty that the microbes in inoculum are more comfortable in the biological degradation of marine macroalgae to generate hydrogen when the biomass is in soluble form than solid form. SAS sample has more effectiveness in biohydrogen production than control and SS because the alkali and sonication gave an impressive hydrolysis effect. Hydrogen-producing microbes' subsequent utilization of acetogenic elements enhances biohydrogen generation [46]. Owing to the combined pretreatment method imposed over the marine macroalgae, the anaerobic culture media had a very suitable approach to liberating biohydrogen. At the same time, depending upon the composition, solubilization

efficiency and pretreatment conditions, the biohydrogen production potential may vary for different substrates. In this condition, the released solubilized compounds, especially proteins and carbohydrates, declined as there was a rise in VFA and biohydrogen production due to the effective hydrolysis and consumption of biopolymers by the microbes. The commencement of biohydrogen fermentation starts with the biopolymer's biodegradation. The proceedings of biopolymers degradation by the fermentative and hydrogen-producing microbes resulted in the emergence of biohydrogen. The biopolymers which were solubilized got exploited by fermentative microbes as a source of energy and electrons [47]. Then, the hydrogen-generating microbes use these compounds and transmute them into biohydrogen. Anaerobic microbes in the inoculum can easily access the biopolymers in the marine macroalgae via this combinative pretreatment. The inoculum (anaerobic sludge) comprises microbes that effectively utilize the solubilized biopolymers and convert them into monosaccharides, thus escalating biohydrogen production [48]. In the preliminary stage, the third day of the operation, the biohydrogen production was low for all samples. This may be due to the instantaneously unadaptable condition of the microbes in the environment. After the third day in the augmented stage, there was a steady increase in the biohydrogen generation where control, SS and SAS showed a biohydrogen production of 5, 75 and 106 mL H_2/g COD, respectively. This rising scenario of biohydrogen in the augmented stage guarantees an effective proliferation and fermentative action of microbes. The eighth day of fermentation begins with the sound stage where control, SS and SAS showed a biohydrogen production of 40, 119 and 150 mL H_2/g COD respectively, beyond which there was no rise in biohydrogen production since a stable range was observed. The summary of this stable stage shown in Figure 6 shows that the biohydrogen producers have unreservedly exploited the solubilized substrates. A maximum biohydrogen yield of 150 mL H_2/g COD was obtained in SAS than SS 119 mL H_2/g COD and control 40 mL H_2/g COD. This is due to the chemo sonic pretreatment that makes the biopolymers in the marine macroalgae easily approachable to the anaerobic microbes in the inoculum sludge, which is essential for biohydrogen production. Table 2 signifies the kinetics constants accomplished through Gompertz modeling of control, SS and SAS samples. SAS shows an uttermost hydrogen production potential and rate (150 mL H_2/g COD and 0.91 mL/d) in correlation with SS (119 mL H_2/g COD and 0.67 mL/d) and control (40 mL H_2/g COD and 0.47 mL/d) expressing the combinative potency of sonication and alkali [49]. It is witnessed that the SAS has a very short preliminary stage (1.5 days) in comparison with control (3.7 days) and SS (2.6 days). An excellent fit was observed in exploratory data as the correlation coefficient of 0.995 was obtained. A similar range of fit was obtained in the work of Tamilarasan et al. (2018) [50]. Based on the above points, it was proved that SAS is more effective in biohydrogen generation than control and SS. Table 3 shows biohydrogen production from different species of marine macroalgae with various combinative pretreatments. From a sustainability point of view, marine macroalgae have emerged as prospective sources for biobased products and biofuel.

3.6. Energy Interpretation

The overall energy consumed for the operation of the marine macroalgae (1 kg) accounted for energy interpretation. Figure 7 depicts the overall energy interpretation between SS and SAS, which includes optimum condition, total energy spent, energy gained through biohydrogen production, net energy, and energy ratio [28,45,51,52]. For effective pretreatment accomplishment, the exhausted input energy should be compensated by the output biohydrogen production. In the evaluation aspect, the output biohydrogen and input sonication energy of SS and SAS observed at an optimum setup were considered. Solubilization efficiency of 21% was kept as an indicator to derive the energy constants for the appraisal of SS and SAS pretreatment energy efficiency [51,52]. The energy consumed by SS (0.1 kWh/kg solids) and SAS (0.05 kWh/kg solids) was determined based on all these specifications. The output biohydrogen production energy of (0.09 kWh/kg solids) was obtained for both SS and SAS since the SCOD solubilization efficiency was taken as

21% to derive the energy parameters. Net energy and energy ratio are the two fundamental factors that conclude the energy competence and pretreatment efficiency [2,45,53,54]. The net energy (−0.01 kWh) and energy ratio (0.8) for SS were less compared to SAS, where the net energy (0.04 kWh) and an energy ratio of (1.8) were obtained. It is proclaimed that the SAS pretreatment process would benefit when there is an energy ratio greater than 1. This is similar to the work of Rajesh Banu et al. (2020) [55]. This certifies that combinative pretreatment of SAS was a more energy valuable pretreatment than SS.
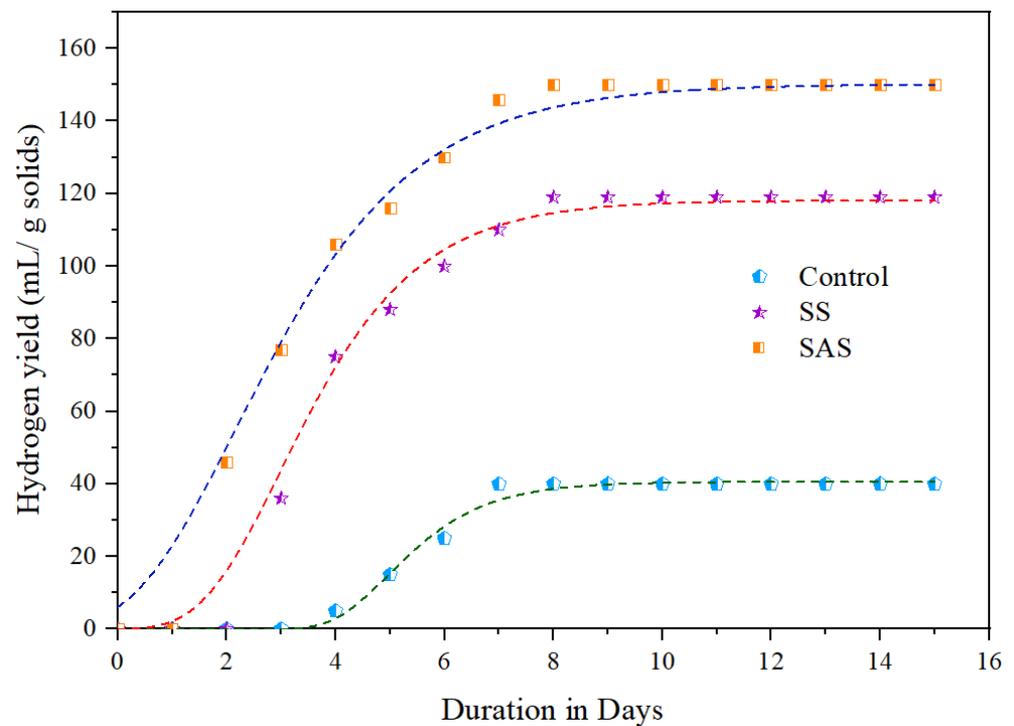

Figure 6. Biohydrogen production in control, SS and SAS.

Table 2. Kinetic analysis for various solubilized samples through Gompertz modelling.

S. No.	Samples	K (mL/d)	H_P (mL)	H_l (days)	R^2
1	SAS	0.99	150.1	1.5	0.995
2	SS	0.67	119	2.6	0.986
3	Control	0.47	40	3.7	0.983

Table 3. Biohydrogen production from different species of marine macroalgae with various combinative pretreatments.

S.no	Marine *Macroalgae Species*	Pretreatment	Operational *Parameters*	Hydrogen *Yield*	Reference
1	Ulva reticulata	Surfactant coupled with disperser pretreatment	Disperser—10,000 rpm, time—30 min, Surfactant—80 dosage (21.6 mg/L)	ΔY: 63 mL H_2/g COD	[30]
		Acidic-hydrogen peroxide coupled with microwave pretreatment	Microwave power—40%, time—10 min, pH—5, H_2O_2 concentration—0.024 g/g TS,	ΔY: 63 mL H_2/g COD	[28]

Table 3. *Cont.*

| S.no	Marine *Macroalgae Species*	Pretreatment	Operational *Parameters*	Hydrogen *Yield*	Reference
2	Laminania Japonica	Heat pretreatment using autoclave	Temperature—121 °C, Duration—30 min	ΔY: 83.45 ± 96 mL/g	[51]
		Sonication pretreatment	Frequency—20Khz	ΔY: 23.56 ± 4.56 mL/g	[51]
		Thermal pretreatment	Temperature—170 °C Duration—20 min	ΔY: 109.6 mL/g	[52]
		Microwave combined with acidic pretreatment	Temperature—140 °C, Duration—15 min, H_2SO_4—1%	ΔY: 28 mL/g	[45]
3	*Padina tetrastromatica*	Acidic pretreatment	Sulphuric acid— 1% *v/v* of H_2SO_4	ΔY: 78 ± 2.9 mL/ 0.05 gVS	[53]
4	*Chaetomorpha antennina*	Surfactant coupled with microwave pretreatment	Microwave power- 0.36 KW, Duration—15 min, Surfactant dosage— 0.0035 g /g TS	ΔY: 74.5 mL H_2/g COD	[2]
		Alkali (NaOH) combined with sonication pretreatment	Sonication intensity—50%, Duration—30 min, pH—11	ΔY: 150 mL H_2/g COD	This study
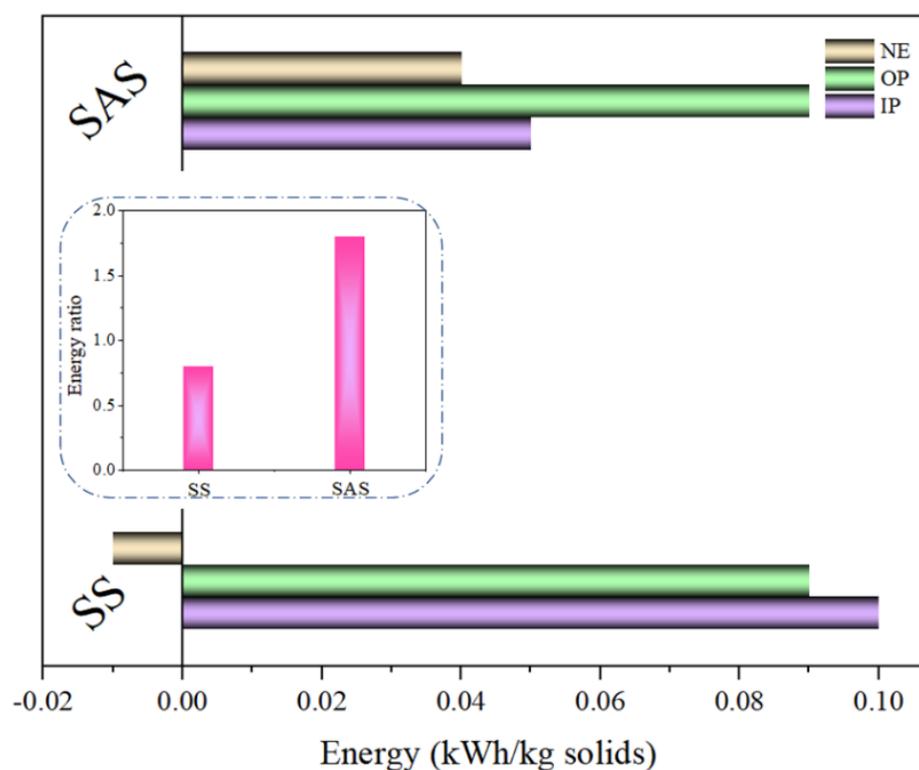
Figure 7. Energy interpretation in SS and SAS.

4. Conclusions and Future Areas of Research

An exploration was made to generate energy-efficient biohydrogen from marine macroalgae by utilizing chemo sonic pretreatment. SS liberated a SCOD release of 2650 mg/L and COD solubilization of 21%, which was lesser than SAS in which a SCOD release of

3400 mg/L and COD solubilization of about 26% was obtained. In comparison with control (40 mL H_2/gCOD) and SS (119 mL H_2/gCOD), SAS (150 mL H_2/gCOD) showed maximum biohydrogen production. pH 11 was the appropriate range for alkali with 50% sonication intensity and 30 min duration for energy-efficient biohydrogen production. VFA production was higher in SAS (1800 mg/L) when compared to SS (860 mg/L) and control (110 mg/L). SAS stated net energy of 0.04 kWh/kg of marine macroalgae biomass and an energy ratio of 1.8, which was effective when compared to SS, in which net energy of −0.01 kWh/kg and an energy ratio of 0.8 was obtained. Hence, chemo sonic pretreatment was regarded as a promising pretreatment approach for biohydrogen generation from marine macroalgae.

Marine macroalgae have emerged as prospective sources for biobased products and biofuel, making them the most viable and desirable biofuel sources. The development of commercial bio-refinery technologies, which primarily utilize marine macroalgae as feed, may be restricted by a distinct lack of practical concepts that must be addressed before its prototype can be successfully sold. Numerous lab-scale experiments are currently being performed, however, it is uncertain whether these technologies could be implemented in the near future. The efficacy of the bioprocess and output of the bioproduct should be reviewed as a result of the scale-up process in order to keep records of losses that happened. Other challenges include species selection as well as conventional microorganisms' role in hydrolysis, conversion and utilization of particular polysaccharides. The development of marine macroalgal biorefineries may be limited by its inability to scale up the biotechnologies which is now being used to conduct ongoing research. Freshwater utilization rises as the biorefinery process progresses, which leads to a freshwater shortage worldwide. The feasibility of using saltwater in a specific biorefinery process has been demonstrated in some research, but it has not yet been verified in a comprehensive marine macroalgal biorefinery process, which entails a number of interrelated processes and activities.

It is essential to identify the spectrum of potential bioproducts and biofuels for each marine macroalgae variety that may be grown sustainably, as well as the best, most comprehensive and unified bioprocessing methods. This information can depend on the long-term sustainability and financial benefit of the green economy. The marine macroalgal sector develops if all bioprocessing steps and the range of potential bioproducts are maintained in a centralized system that can be accessed globally. A strong collaboration between academics and industries which comprises environmental engineers, marine scientists, skillful laborers and economists should yield effective methods for biofuel production from marine macroalgae. The organization of the bioeconomy in a particular nation could undergo a dramatic change in the following decades due to the effects of global warming. As a result of the rising temperatures brought on by climate change, research has revealed potential changes in the geographical distribution of marine macroalgae in diverse coastal environments. Shifts in marine macroalgal distribution affect the infrastructure, locations, employment opportunities and overall viability of marine macroalgal biorefineries in the bioeconomy. Therefore, it is essential to model and predict the transformation of the commercially significant marine macroalgal species under climate change. Better macroalgae collection techniques to have a high yield of biofuels via genetic alteration will be the future of algal biology. The marine macroalgae chosen for biofuel production should suit all the environmental requirements so that they can be considered as a sustainable feedstock. The bioprocessing characteristics of each marine macroalgal species, such as life cycle evaluation, energy and energy-based modeling, should be accurately examined using various eco-friendly techniques. This could help for better sustainable biorefinery development.

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Data Availability Statement: Not applicable.

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