Bioresource Technology 350 (2022) 126904

Contents lists available at ScienceDirect

Bioresource Technology

journal homepage: www.elsevier.com/locate/biortech

Surfactant induced microwave disintegration for enhanced biohydrogen production from macroalgae biomass: Thermodynamics and energetics

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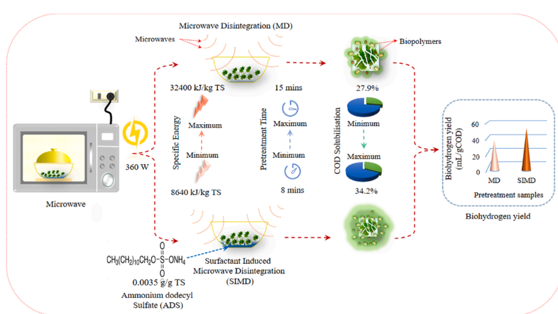
HIGHLIGHTS

- Combined treatment of microwave – surfactant enhance the macroalgal solubilisation.
- Maximum of 34.2 % algal biomass solubilisation achieved in SIMD.
- SIMD obtained a maximum hydrogen production of 54.9 mL H_2/g COD.
- Energy ratio of 1.04 achieved at SIMD pretreatment.

GRAPHICAL ABSTRACT



ARTICLE INFO

Keywords:
Macroalgae
Microwave
Surfactant
Bio-hydrogen
COD solubilisation

ABSTRACT

This research work aimed about the enhanced bio-hydrogen production from marine macro algal biomass (*Ulva reticulate*) through surfactant induced microwave disintegration (SIMD). Microwave disintegration (MD) was performed by varying the power from 90 to 630 W and time from 0 to 40 min. The maximum chemical oxygen demand (COD) solubilisation of 27.9% was achieved for MD at the optimal power (40%). A surfactant, ammonium dodecyl sulphate (ADS) is introduced in optimal power of MD which enhanced the solubilisation to 34.2% at 0.0035 g ADS/g TS dosage. The combined SIMD pretreatment significantly reduce the treatment time and increases the COD solubilisation when compared to MD. Maximum hydrogen yield of 54.9 mL H_2 /g COD was observed for SIMD than other samples. In energy analysis, it was identified that SIMD was energy efficient process compared to others since SIMD achieved energy ratio of 1.04 which is higher than MD (0.38).

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https://doi.org/10.1016/j.biortech.2022.126904
Received 9 January 2022; Received in revised form 18 February 2022; Accepted 21 February 2022
Available online 25 February 2022

1. Introduction

Conventional fossil fuels like petroleum, coal and natural gas are the major energy sources for many sectors such as industries, transport, and power generation. Petroleum and petro based products are major source for transportation and industries. However, they are limited, and the usage leads to many challenges such as pollution, global warming, and climate change (Bhatia et al., 2021). Pollution control and prevention are major issue of concern in current situation. So, the world is in need to find alternative and attractive energy sources which not affects the environment (Preeti et al., 2021). Many countries are encouraging the alternative energy findings from renewable sources to fulfill their global level energy demand (Banu et al., 2020; Sharmila et al., 2020). Biofuel is one of the largest existing renewable energy resources in the world. Biofuel is green energy resources and primary alternate of fossil fuels. Biofuels are derived from food crops using conventional methods, known as 1st generation biofuels or conventional biofuels. In 2nd generation biofuels, biofuels are derived from feedstock of lignocellulose biomass, waste biomass from agricultural crops, non-food crops and forestry residues using new techniques. Algal (micro and macro algae) based biofuel production is considered as 3rd generation biofuels (Nagarajan et al., 2021; Sim et al., 2021). Biofuels such as photo biological solar fuels and electro fuels are considered as 4th generation biofuels. Biohydrogen ($BioH_2$) is identified as the most probable, sustainable, and clean energy for future where it acts as a capable alternate fuel to conventional fuels (Bharathiraja et al., 2016). Moreover, hydrogen is environmentally friendly and during combustion it releases water vapour. Hydrogen is used as clean transport fuel and power generation high calorific value and high energy yield (Saratale et al., 2019). $BioH_2$ generation from biomass is important due to its sustainability and high efficiency. Anaerobic fermentation (dark fermentation and photo fermentation) and electro-microbial hydrogenation (microbial electrolysis cell and microbial fuel cell) are widely used methods to produce $bioH_2$ through biological methods (Kannah et al., 2021).

Marine macro algae have some advantages over other feedstock such as more growth when compared to plants on land, high CO_2 capture rate and negligible lignin content which makes ease biodegradable (Jung et al., 2011). *Ulva reticulata (U. reticulate),* commonly known as ribbon sea lettuce grows in marine region which are found on rocks. *Ulva* sp. is suitable for biofuel production. So, *Ulva reticulate* is considered as an alternative promising resource for the biohydrogen production. Anaerobic fermentation (AF) is a biological process of producing biohydrogen from various biomass by using various fermentative microbes under anaerobic conditions which takes place under oxygen free environment and associated with acidogenic process. AF is classified into two: photo fermentation and dark fermentation (Wang et al., 2011). AF is performed to convert the substrate into $bioH_2$ and other intermediates such as organic acids under the absence of light through anaerobes such as *Clostridium* sp. which is known as dark fermentation (Bharathiraja et al., 2016). Presence of carbohydrate makes the algae as an appropriate source for hydrogen generation. In AF, polymeric carbohydrates are converted into simple sugars. Marine biomass has rigid cell structure and the complex biopolymers (carbohydrate and protein) present within marine biomass are not easily converted into simple structures by anaerobic microbes during hydrolysis process. This limits the hydrolysis rate and extent the anaerobic fermentation. Moreover, it affects the anaerobic biodegradability during energy conversion from marine biomass. To overcome the issues, pretreatment is required to enhance the hydrolysis of marine biomass.

Physical, chemical, mechanical, and biological methods of pretreatments are used to improve biodegradability and hydrolysis process. Among various pretreatment methods, microwave pretreatment is considered as most effective disintegration method for macro algae biomass (Yin and Wang, 2018) which breaks the algal complex structure and enhance biohydrogen generation than conventional thermal treatment (Yin et al., 2019). This pretreatment consumes high energy and demands more time for effective disintegration. So, the microwave pretreatment is combined with the other treatments such as chemicals, ultrasonication and enzymes that improve the efficiency of the treatment by reducing the energy. Optimization of time and power significantly influences the energy consumption and cost of the pretreatment. Hence, in this research, a novel attempt is made by combining microwave pretreatment with chemicals to disintegrate the marine biomass. By combinative methods, the energy demand and pretreatment time is reduced and improve the solubilisation. This is the first kind of research to account the effectiveness of combinative pretreatment of microwave with surfactant on energy efficient biohydrogen production. The technical and economical features of pretreatment process are considered before full scale implementation. For biomass disintegration, microwave pretreatment is found to be effective treatment (Kostas et al., 2017). During pretreatment, specific energy evaluation is necessary for the energy analysis of biomass pretreatment. In this work, an energy assessment is analyzed based on the laboratory results to evaluate the feasibility of a pretreatment process to be adopted for field applicability (Kannah et al., 2019). The objectives of the study are a) To assess the microwave disintegration potential on marine macro algae solubilisation. b) To optimize various surfactant dosages for effective disintegration. c) To study the effect of surfactant induced microwave disintegration (SIMD) through kinetic analysis. d) To investigate the effect of combinative pretreatments on anaerobic fermentation of macro algae biomass. e) To evaluate the biohydrogen production from macro algae through combinative pretreatments. f) To analyze the feasibility of combinative pretreatment methods in field applicability through energy analysis.

2. Materials and methods

2.1. Macro algae sampling and characterization

Ulva reticulate, a macro algae or sea weed was harvested from Kuthenkuli (8°13′01″N 77°46′48″E), Tirunelveli coastline, Tamilnadu, India. The sand particles and remains in harvested sea weed are cleaned with fresh water as the sand particles and remains may affect the pretreatment. Then the washed samples are dried in shaded conditions for 5–7 days. The samples are characterized based on dry basis (wt. %) as carbohydrate-45%, protein-38%, lipid–1–2% and ash–10%.

2.2. Microwave disintegration pretreatment

The pretreatment experiment is carried out by home usage microwave oven (company and model-IFB & 30SC2, and power output-900 W). For pretreatment, 1 L polytetrafluoroethylene (PTFE) vessel is used. During pretreatment, a PTFE cap is used as vessel cover to prevent the loss of water by evaporation. 10 gm of dried macroalgae was used in the proposed work based on the literature Tamilarasan et al. (2017) and the higher concentration may affect the pre-treatment efficiency. For pretreatment, 10 g of dried macroalgae and 0.5 L of water is taken in the vessel, then close the vessel with PTFE cap and place in microwave for treatment. the experiment was performed by adjusting the power intensity from 10 to 70 % which was under the power range of 90 to 630 W.for different pretreatment time (0–60 min). During pretreatment, the temperature is observed as 20 to 130 °C. The pretreated samples are gathered and then centrifuged to 15,000 rpm at 10 min. Then, supernatant of centrifuged sample is collected and analyzed for organic release in terms of SCOD and biopolymer (carbohydrates and protein).

2.3. Surfactant induced microwave disintegration pretreatment

Surfactant induced microwave disintegration (SIMD) pretreatment is performed by adding ammonium dodecyl sulfate (ADS) surfactant at optimized microwave pretreatment conditions. For SIMD pretreatment, 10 g of dried macroalgae and 0.5 L of water is taken in a vessel and is performed by varying ADS dosages from 0.0005 to 0.005 gADS /gTS. Then close the vessel with PTFE cap and place in microwave for treatment. After pretreatment, pretreated samples are collected and analyzed for organics release.

2.4. Anaerobic fermentation of macroalgal biomass

The effect of combined pretreatment in volatile fatty acid (VFA) production is assessed by conducting fermentation test for the samples (untreated and pretreated). AF test is performed for 72 h. In serum bottles, samples, and inoculum (anaerobic digested sludge) are taken as 9:1 ratio. To eliminate the methanogenic microbes, the inoculum is heated for 30 min at 102 °C and subsequently add 0.05 M concentration of Bromo ethane sulphonic acid (BESA) in all sample contained bottles before fermentation test. Nitrogen (N_2) gas is flushed in all the bottles to remove oxygen, later all the bottles are capped with rubber caps. The bottles are placed in a shaker at 120 rpm for 72 h at 35 °C and the samples are analyzed at 0 h after 72 h.

2.5. Biohydrogen assay

Biohydrogen assay (BHA) is conducted to evaluate the pretreatment efficiency through biohydrogen generation from marine macro algae after different pretreatments. In this study, BHA are carried out for control (untreated) and pretreated samples. Anaerobic biodegradability test is conducted to examine the hydrogen generation through a batch process (Kumar et al., 2016). Anaerobic digested sludge is used as inoculum and its characteristics as follows: pH − 6.5, total solid-18650 mg/L, total chemical oxygen demand-13450 mg/L, total suspended solids-10750 mg/L and volatile solids-7560 mg/L. Nutrients are added in this experiment contained the following ingredients: Ammonium carbonate-5240 mg/L, Sodium bicarbonate- 6720 mg/L, Dipotassium phosphate-125 mg/L, Magnesium chloride-100 mg/L, Ferrous sulfate heptahydrate-25 mg/L and Copper sulfate pentahydrate-5 mg/L (Kumar et al., 2019a). The anaerobic digested sludge was suggested to be a suitable inoculum for the generation of hydrogen since it predominantly contains fermentative microbes such as *Clostridium sps*, *streptococcus sps*, and *Enterobacter sps*. These microbes impart a significant role in hydrolysis of substrates and production of biohydrogen. The samples and inoculum are taken as 3:1 ratio in 300 mL bioreactor. After adding the substrates and inoculum, N_2 gas is flushed in the reactor to maintain the anaerobic condition. Then, the reactor is sealed with a rubber cap to make it airtight, and then place on a shaker at 150 rpm. The produced biogas is measured through inserting a needle in the cap of the reactor using syringe displacement method. The produced gas pressures and the plunger displaced volume is noted. The gas samples were taken daily and analysed for biohydrogen. Hydrogen gas was measured using a gas chromatograph, model no-4890 D (Agilent Cooperation, USA) furnished with a thermal conductivity detector (TCD). A six feet stainless column packed with Porapak Q (80/100 mesh) and the argon act as carrier gas with a flow rate of 25 mL/min). Temperature of injection port, over and detector was maintained as 120℃, 35℃ and 120℃, respectively. The produced biohydrogen and its kinetics is estimated by Ist order kinetics through following modified Gompertz equation (Banu et al. 2020),

$$C_h = S_h * exp\left(-exp\left(-k(x - x_c)\right)\right) \tag{1}$$

where,

C_h – cumulative bioH_2 (mL H_2/g COD),
S_h – Specific bioH_2 production potential (mL H_2/g COD),
k - Maximum bioH_2 production (mL H_2/g COD/d),
x – Digestion time (days) and
x_c – Time of inflection (days).

2.6. Analytical methods

To determine the characteristics and for analysis**,** standard procedures (APHA, 2005) are used. An elemental analyzer is used to analyze the elemental analysis. Anthrone and Lowry's method are used for determining the carbohydrate and protein analysis, respectively. All the experiments are done in triplicates, and the results are specified as mean of the values.

2.7. Statistical analysis

One-way ANOVA analysis is used to analyze all the results obtained from the treatment. If the *p*-values obtained from the experiment are found to be less than 0.05, then the difference between the experimental results from pretreatment is determined as statistically significant. If *p*-values greater than 0.05, then the results from the pretreatment is not statistically significant.

2.8. Thermodynamic analysis study

Thermodynamic analysis is used to evaluate how energy affects performance a process. In thermodynamic analysis, mathematical equation or kinetics is used to determine the effects of energy inputs and outputs of a process. Different types of thermal methods such as hydrothermal, dry thermal and radiation thermal are available in thermal pretreatment methods to disintegrate biomass. In conventional pretreatment, more time is required for the disintegration; however, the lengthy treatment time causes the recalcitrant organic composites formation that restrict the degree of degradation. In microwave disintegration, possible disintegration demands low treatment time when compared to conventional thermal treatment. Therefore, the rate of activation energy is less in microwave than conventional thermal treatment. In view of these fact, microwave is considered as better efficient disintegration thermal method than conventional thermal method. The effect of microwave disintegration on macroalgal biomass hydrolysis falls on Ist order kinetics (Eswari et al., 2017),

$$-dS/dt = KS \tag{2}$$

$$ln\,S = -Kt + B \tag{3}$$

where S-organic release, K-reaction constant and B-constant of integration.

The following equation was used to calculate A_e,

$$ln\,K = A_e/RT + ln\,B \tag{4}$$

whereA_e-activation energy in kJ/mol, R-gas constant (8.31 J K^{-1} mol^{-1}), T-Temperature in K and B-Exponential factor.

2.9. Energetic analysis

In the current study, results are obtained from the pretreatment experiments are used to estimate the energy analysis. The energy analysis from this study is used to analyse the economic feasibility of current study to full scale extent. Energy analysis are performed for microwave and combined treatment. Macroalgae biomass solubilisation from all the treatments is used as index for evaluating the effectiveness and used in energy analysis. For energy analysis, one kilogram of macro algae biomass is used. The following parameters and calculations are used in energy analysis.

2.9.1. Microwave specific energy

The consumed microwave disintegration specific energy (MDSE) for macroalgae disintegration is measured using the following equation (Kumar et al., 2018),

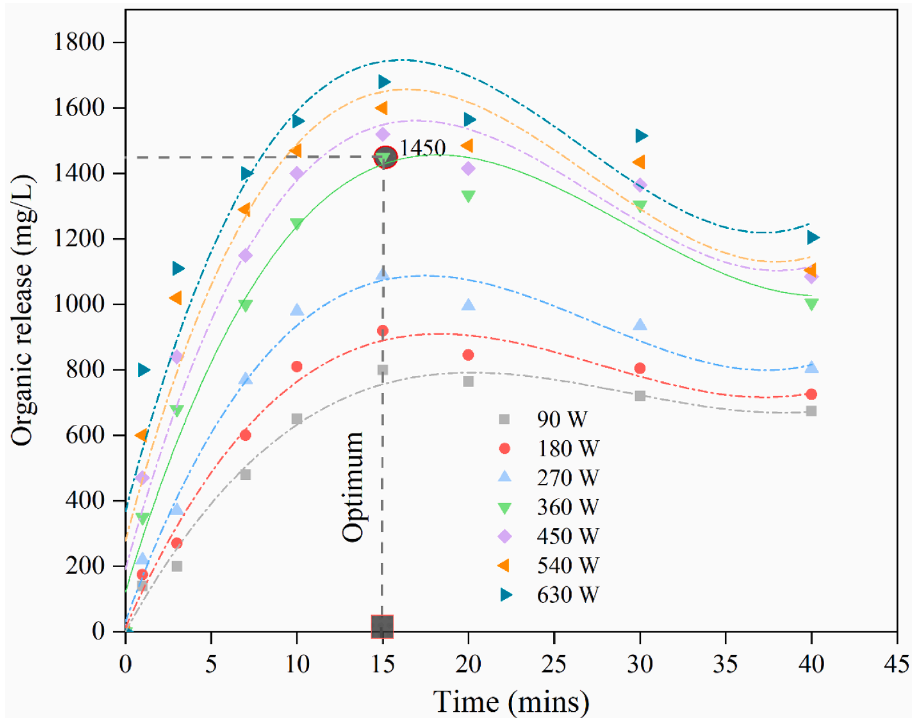

Fig. 1. Effect of microwave pretreatment on organic release.

$$MDSE\,(kJ/kg\,TS) = (M_p{*}D_t)/(V{*}S) \tag{5}$$

where,

M_p –Microwave power (kW),
D_t – Disintegration period (sec),
V-Macroalgae sample volume (L) and
S-Biomass solids initially used (kg TS)

2.9.2. Input energy

Input energy (I_e) is the energy spent or consumed by microwave during the macroalgae disintegration is calculated by following equation (Kannah et al., 2019),

$$I_e = D_p * D_t * V * M_b \tag{6}$$

where,

I_e-Input energy (kWh),
D_p-Power used for disintegration (kW/kg),
D_t – Disintegration time (hr),
V-Sample volume (m^3) and
M_b-Biomass mass (kg/m^3).

2.9.3. Output energy

Energy gained as hydrogen production from macroalgal treatment is considered as output energy (O_e) and calculated using the following equation (Kannah et al., 2019),

$$O_e = B * L * Y_h * V * CF \tag{7}$$

where,

O_e–Output energy (kWh),
B-Macroalgal biomass biodegradability (gCOD/gCOD),
L–Organic loading (gCOD/m^3),
Y_h–Yield of biohydrogen (m^3/gCOD),
V– Reactor volume (m^3),
CF–Biohydrogen energy conversion factor (1 m^3 = 3.5 kWh).

2.9.4. Net energy

Net energy (N_e) is the factor that shows difference of output and input energy in a process. Positive net energy denotes the gain in energy and negative net energy denotes the loss of energy. Net energy was calculated by (Kannah et al., 2019),

$$N_e = O_e - I_e \tag{8}$$

where,

N_e–Net energy (kWh),
O_e–Output energy (kWh),
I_e–Input energy (kWh),

2.9.5. Energy ratio

Energy ratio (E_r) is the ratio of output and input energy and it was calculated based on (Passos et al. 2012),

$$E_r = O_e/I_e \tag{9}$$

Energy ratio value more than 1 indicates the net energy production.

3. Results and discussion

3.1. Microwave disintegration

Microwave disintegration (MD) pretreatment makes tear in macroalgae cell structure and releases the inner molecules which leads to effective hydrolysis. Microwave disintegration is done by thermal and athermal effects whereby increase the temperature of the medium and alter the structures present in the medium (Yu et al., 2009; Banik et al., 2003). Researchers reported that heat produced in microwave breaches from inward to outward direction that easily disintegrate the structure (Tyagi and Lo 2013; Xiao et al., 2012). Microwave disintegration improves the biogas production while compared to conventional heating. But it consumes more energy that makes the treatment unattractive, thereby combined with other treatment could reduce the energy requirement and also enhances the treatment.

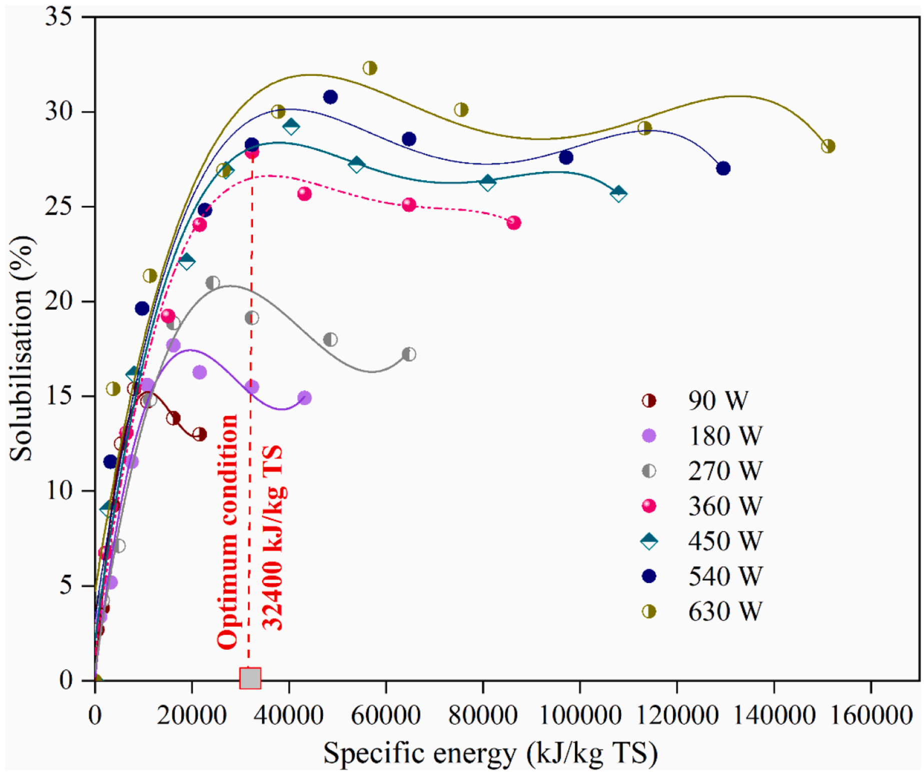

Fig. 2. Effect of specific energy during microwave pretreatment on solubilisation.

3.1.1. Impact of microwave disintegration on soluble organic release

Organic release is used as response factor to recognize the microwave treatment efficacy. The effect of microwave power (90 – 630 W) on organic release at various treatment time (0 – 40 min) is shown in the Fig. 1. The microwave irradiation can damage the complex and rigid structure of macroalgae cell wall and release the inner molecules. Fig. 1 shows the released soluble organics pattern with respect to time and is classified as disintegration (0 – 15 min) and mineralization stage (15 – 40 min). In disintegration stage, the organics that gets released will start to increase when treatment time increases. At 15 min time, the greater organic release is obtained. This rapid release stage which phase up to 15 min can be due to the hydrogen bond breakage that improves the cell membrane disintegration and release the molecules rapidly into the aqueous medium. Athermal effect of microwave results the possible breakage of hydrogen bonds due to the aligning of electromagnetic field (Wang and Li, 2016). Beyond 15 min, there is a decrease in organic release which occurs due to mineralization by evaporation. This is because when treatment time increases, the temperature of aqueous medium increases and evaporation may occur (Eskicioglu et al., 2006). Therefore, 15 min pretreatment time is considered as optimum time for the microwave.

However, the microwave power also influences the biomass solubilisation. Uma rani et al. (2012) stated that increasing the microwave power significantly increases the solubilisation. From the Fig. 1, organic release at various microwave power exhibits a hasty release followed by a gradual release, when microwave power is increased. The marginal release of organics, 730 and 1090 mg/L were obtained for 90 and 270 W, respectively at an optimum time of 15 min. This insignificant increase is probably due to the minor potential of microwave power level that induces less disintegration in algal biomass. Therefore, MP ranges from 90 to 270 W does not considered for further studies. Beyond 270 W, there is a considerable rise in release in organics is achieved. Maximum soluble organics of 1450 mg/L is released when the microwave power increased to 360 W. The statistical test suggests that *alpha p* (prob >F) value for organic release at 90 – 360 W is calculated to be 0.013. The result implies a significant increase in organic release between 90 and 360 W. This can be due to the increase in the power input, where the microwave breaches easily into the macro algal biomass and the thermal effects causes more breakage that enhance the organic release. Ebenezer et al. (2015) studied about microwave treatment in sludge pretreatment and achieved 28% COD solubilisation due to the microwave thermal effect. Beyond 360 W, insignificant increase in organic release is obtained. Marginal increase of 1680 mg/L is obtained at the maximum microwave power of 630 W. There is no significant organic release but the microwave energy consumption is more beyond 360 W and hence not considered. The statistical test suggests that *alpha p* value for organic release at 450 – 630 W is calculated to be 0.48. The result implies an insignificant increase in organic release between 450 and 630 W.

3.1.2. Effect of specific energy during organics release and solubilisation of algal biomass

Similar to microwave power, inadequate organic release of 730 to 1090 mg/L is achieved at microwave disintegration specific energy (MDSE) spent of about 4050 to 12150 kJ/kg TS and this release can be ignored for the further analysis. At optimum microwave power, maximum organics of 1450 mg/L was is released at 32400 kJ/kg TS MDSE. The *alpha p* values through statistical analysis (one-way ANOVA) for organic release at microwave power between (90 – 360 W) up to 32400 kJ/kg TS is estimated as 0.04. The *alpha p* values through statistical analysis for organic release at microwave power between (450 – 630 W) up to 32400 kJ/kg TS is estimated as 0.8. This implies the significant increment in organic release up to 90 – 360 W.

Beyond 360 W, insignificant rise of organic release is achieved even if there is an increase in the microwave specific energy. Perhaps, organic release has increased from 1450 (360 W) to 1680 mg/L (630 W) at 15 min is stable which demands 56700 kJ/kg TS MDSE. From the above results, it can be identified that the further energy is spent during the insignificant organic release and leads to less biomass solubilisation that makes the process uneconomical and unattractive. Therefore, 32400 kJ/kg TS is optimum microwave specific energy that has spent during

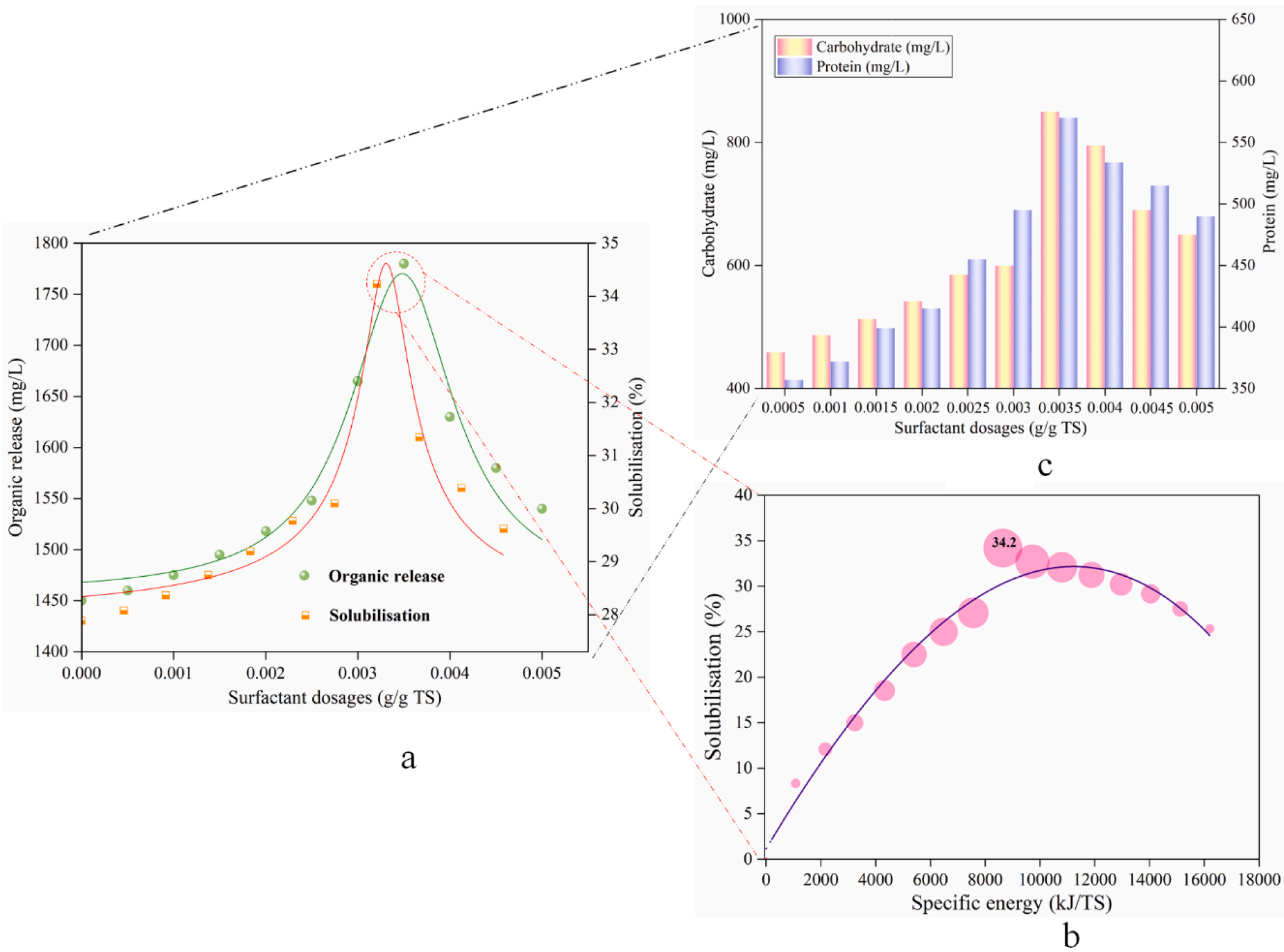

Fig. 3. Effect of surfactant dosages on microwave pretreatment.

microwave pretreatment. Beyond 32400 kJ/kg TS, the energy spent is more but without any improvement in solubilisation which leads to the treatment uneconomical.

Fig. 2. shows that the solubilisation of macroalgae biomass at microwave disintegration specific energy (MDSE) conditions. From the figure, it is identified that the biomass solubilisation increases when specific energy increases. At optimum microwave power condition, 7.7% of solubilisation is found at MDSE of 2160 kJ/kg TS. A steady rise in biomass solubilisation is detected when there is an increase at the microwave specific energy from 2160 to 32400 kJ/kg TS. Maximum biomass solubilisation of 27.9% is attained at 32400 kJ/kg TS. Microwave irradiation disintegrates the cell structure (Kostas et al. 2017) and leads to greater release in organics which is the reason behind the maximum solubilisation. Beyond 32400 kJ/kg TS, solubilisation started to decrease due to the organic loss by evaporation. From the above discussion, clearly identified that maximum solubilisation of 27.9% is attained at 15 min pretreatment time with MDSE spent 32400 kJ/kg TS. Therefore, 32400 kJ/kg TS is the optimal MDSE applied for the microwave disintegration.

3.2. Surfactant induced microwave disintegration

3.2.1. Effect of surfactant dosages on organic release at optimum microwave condition

In microwave disintegration, the released organics in aqueous medium might cause cluster formation which sequentially affect the pretreatment efficacy. Adding chemical such as ADS (surfactant) in microwave treatment, could adsorbed on the surface of disintegrated biomass and forms an organic layer around the biomass which significantly reduce the surface tension of hydrophobic compounds and enhance the solubility to the aqueous phase, thus prevents the biomass from aggregation (Yi et al., 2013). For further process, the experiment is performed under the optimum MD conditions (microwave power – 360 W) and through organics and biopolymer releases.

Fig. 3a depicted the effect of surfactant on release of soluble organics at microwave optimum condition. It is observed from the figure that the organic release and solubilisation exposes two patterns: an increase and decrease phase. In increase phase, the rapid release of organics starts from the ADS dosage of 0.0005 to 0.005 g ADS/g TS and noted a linear increment in organic release of about 1780 mg/L at 0.0035 g ADS/g TS. Xiao et al. (2017) achieved more SCOD (organic release) in surfactant combined microwave compared to microwave alone, similarly in this study also surfactant induced microwave disintegration (SIMD) can release maximum release of 1780 mg/L compared to microwave alone (1450 mg/L). Then, in decrease phase, organics release started to decrease from dosage 0.004 to 0.005 g ADS/g TS and maximum organics (1540 mg/L) released in 0.005 g ADS/g TS. By statistical analysis, the *alpha p* value of one-way ANOVA for organic release between 0.0005 and 0.0035 g ADS/g TS is estimated as 0.02. This showed a significant increase in the organic release between 0.004 and 0.005 g ADS/g TS. Further, the *alpha p* value for the organic release between 0.004 and 0.005 g ADS/g TS is 0.2 ($p < 0.05$) which indicates that the difference is not significant.

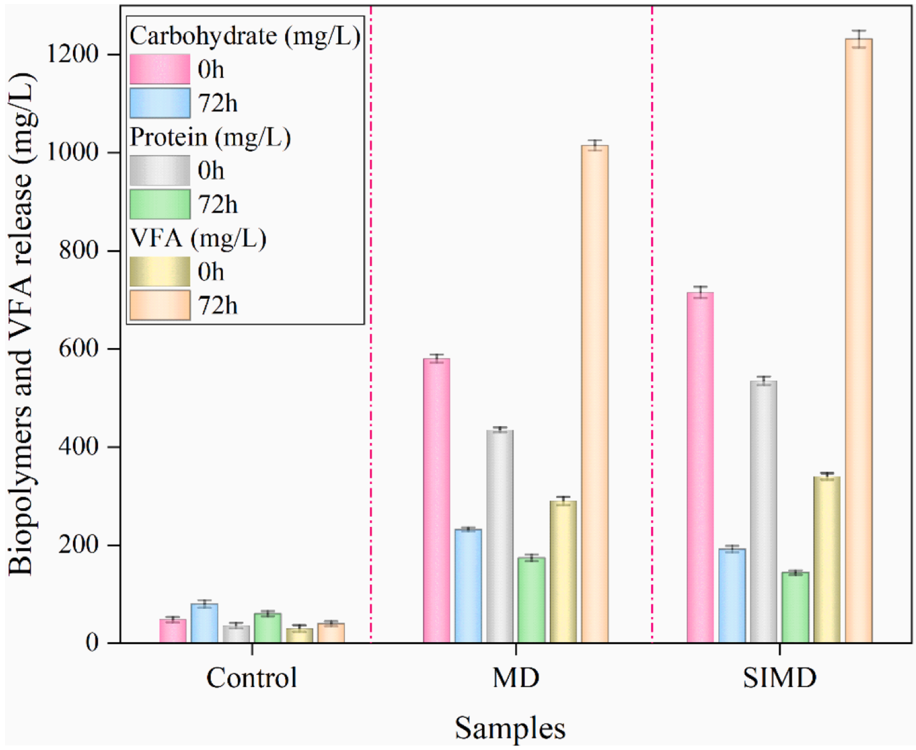

Fig. 4. Effect of surfactant aided microwave pretreatment on anaerobic fermentation.

3.2.2. *Effect of organic release and biomass solubilisation at optimum surfactant induced microwave disintegration*

The organic releases exhibit two different phases: a disintegration phase (1080 to 8640 kJ/kg TS) and mineralization phase (9270 to 16200 kJ/kg TS). The organic release can increase due to the proper disintegration and reaches the maximum release of 1780 mg/L at 8 min with the specific energy spent of 8640 kJ/kg TS. When pretreatment time increases, the energy consumption also increases but mineralization that takes place affect the release. For analyzing the organic release, linear regression modeling is performed and observed a linear trend pattern in organic release. As predicted, the rate of reaction during the disintegration phase (0.11086) is higher than the mineralization phase (-0.03704), which indicated that the microwave specific energy also affects the efficiency of the treatment. Generally, biomass disintegration by microwave improves the solubilisation by rising the temperature of the treatment. It is predicted that the combined microwave with surfactant might lead to improved solubilisation with lessened energy consumption by reduced time. Similar to organic release, solubilisation also increases from 1080 kJ/kg TS and reaches maximum solubilisation of 34.2% at 8640 kJ/kg TS. The solubilisation at optimum ADS dosage with respect to microwave specific energy is shown in the Fig. 3b. From above discussion, it is identified as 0.0035 g ADS/ g TS at optimum dosage with 8640 kJ/kg TS MDSE for surfactant induced microwave treatment.

3.2.3. *Effect of surfactant dosages on biopolymer release*

Macroalgae contain high number of biopolymers such as carbohydrate and protein, which is suitable for hydrogen production. Fig. 3c depicts the effect of surfactant dosages on soluble components (carbohydrate and protein) release. Resembling as organic release, the release of biopolymers also exposed an important increase up to 0.0035 g ADS /g TS. It is observed that the release of carbohydrate and protein increases linearly with an increase in ADS dosage up to 0.0035 g ADS/g TS and found to be 880 and 560 mg/L. Synergistic effect of surfactant induced microwave treatment on macroalgae is the cause for this linear increase in biopolymer release. The concentration of biopolymers exhibited the increment due to the combined effect of pretreatment. Kumar et al. (2018) stated that the similar release of soluble components during chemo-disperser treatment of marine algae. It is also observed that beyond 0.021 g/g TS ADS dosage, the concentration of carbohydrates and proteins starts to decrease and noticed as 690 and 530 mg/L, respectively at 0.027 g/g TS.

3.3. *Thermodynamic analysis*

Many studies described about the advantages of thermal disintegration methods over other methods of biomass disintegration. Various thermal process such as hydrothermal, dry thermal and radiation thermal are available for biomass disintegration (Ruiz et al., 2015; Passos et al., 2013). In conventional treatment, more time required for the disintegration, however the lengthy treatment time causes the recalcitrant organic complex formation that restrict the degree of degradation (Uma Rani et al., 2012). In microwave disintegration, possible disintegration demands low treatment time when compared to conventional thermal treatment. Therefore, the rate of activation energy is less in microwave than conventional thermal treatment. In view of these facts, microwave is considered as better efficient disintegration thermal method than conventional thermal method. By using first order kinetic model, the analysis is performed through the biomass solubilisation. Based on the equation (2.3), the microwave effect on macroalgae biomass hydrolysis is calculated.

In this study, organic releases from MD and SIMD is used to analyze the thermodynamic study. During MD and SIMD at different microwave power intensity (90 to 630 W), the temperature may vary from 20 to 120 °C, then the curve falls in Ist order kinetics for both treatments, its efficiency is calculated. The activation energy is calculated using equation which is described in equation 2.4. In this study, the minimum activation energy is evaluated as 0.224 kJ/mol for SIMD, whereas activation energy for MD and control were evaluated as 0.354 and 0.723 kJ/mol respectively. Thus, it indicates the SIMD enhance the solubilisation by reducing the activation energy when compared to MD treatment. The activation energy produced during combined microwave

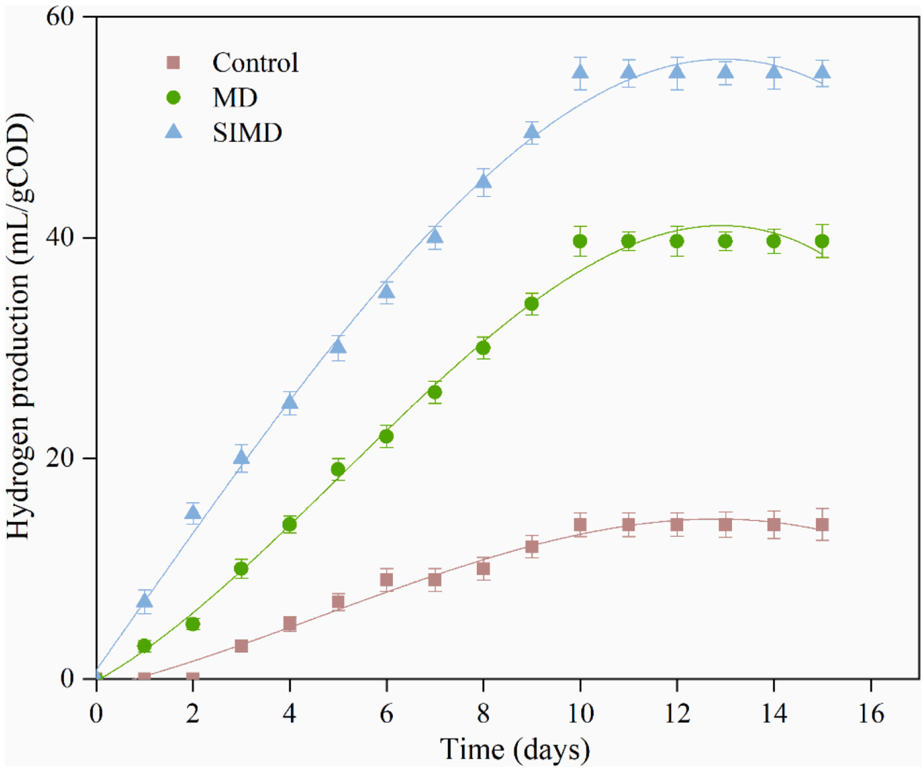

Fig. 5. Biohydrogen generation using various treated samples.

(0.224 kJ/mol) is lesser than 20.19 kJ/mol (Luo et al., 2012) in biological treatment, which implies that the microwave increase chemical reaction rate by lowering activation energy (Eswari et al. 2017).

3.4. Anaerobic fermentation

During hydrolysis, complex structures such carbohydrate and protein present macroalgae are converted into simple sugars and peptides. Later, these simple structures are converted into volatile fatty acids (VFA) (Sambusiti et al., 2015). In SIMD, biomass gets disintegrated by microwave effect and releases organics into aqueous medium. Then the surfactant reduces surface tension and reduces the aggregation of biopolymers which improves solubilisation and leads to effective hydrolysis (Bhuyan, 2010). Then the released organics are utilized and produce VFA by fermentative microbes. It is observed from the Fig. 4 that carbohydrate concentration in MD and SIMD exhibit a sharp decrease from 0th h (580 and 715 mg/L) to 72nd h (232 and 192 mg/L).

Similarly, protein in MD and SIMD also showed sharp decrease from 0th h (435 and 535 mg/L) to 72nd h (174 and 144 mg/L). By the combined effect of microwave and surfactant in SIMD, carbohydrate and protein concentration are higher, so it can be easily utilized by the microbes (Banu et al., 2019), then converted into VFA. Moreover, the microbes take 72 h (3 days) to utilize the maximum biopolymers in pretreated samples (Pham et al., 2013). During AF, more VFA production indicates the effectiveness of treatment and more accumulation of VFA indicates the potential hydrogen production (Xia et al., 2016). SIMD exhibited more VFA accumulation (340 mg/L) compared to MD (290 mg/L) and control (40 mg/L). Then the biopolymers in samples are converted into more VFA and found to be 1232 mg/L (SIMD), which is high compared to control and MD. From this, it is clearly identified that hydrolysis rate is comparatively high in SIMD sample, which resulted in more soluble organics production in 0th h and its consequent utilization for VFA generation at 72nd h. Therefore, higher release of carbohydrate and protein in SIMD increased the VFA production. Thus, SIMD process enhances the fermentation and produce more hydrogen.

Table 1
Kinetics analysis of differently pretreated samples.

S. No	Samples	m (mL/d)	x_c (days)	a (mL H_2/ g COD)	R^2
1.	Control	0.11	4.85	14	0.9931
2.	MD	0.43	3.79	39.7	0.99638
3.	SIMD	0.58	2.9	54.9	0.99687

3.5. Biohydrogen production

Biohydrogen assay experiment is carried out for control (untreated and pretreated (MD and SIMD) samples to estimate the hydrogen production. Fermentation process gets extended and limited due to the rigid structure of biomass. Appropriate disintegration process can efficiently disintegrate the cell structure and releases the inner molecules which enhance the hydrogen production. In this study, MD and SIMD are performed to disintegrate the macroalgae biomass. Biohydrogen production for all the samples are shown in the Fig. 5. At initial stage, hydrogen generation is noticed as 5, 14 and 25 mL H_2/ g COD at 4 days. Hydrogen production is low in initial days and this is because the microbes in inoculum are not well accommodated yet and needs more time to degrade the substrate. After well acclimatized, higher hydrogen generation of 54.9 mL is attained in SIMD comparing 14 mL (control) and 39.7 mL (MD), respectively. Similar, Kumar et al. (2019b) stated that about 74.5 mL of biohydrogen produced by treating *Chaetomorpha antennina* through surfactant induced microwave process. By using modified Gompertz model, the kinetic parameters are calculated and the correlation coefficient (R^2) values falls between 0.993 and 0.996 which implies that the predicted and experimental values are good. Kinetic analysis of different samples were given in Table 1.

3.6. Energy analysis

Energy is an important factor that influences the economic feasibility of the pretreatment. In energy study, energy consumed during treatment

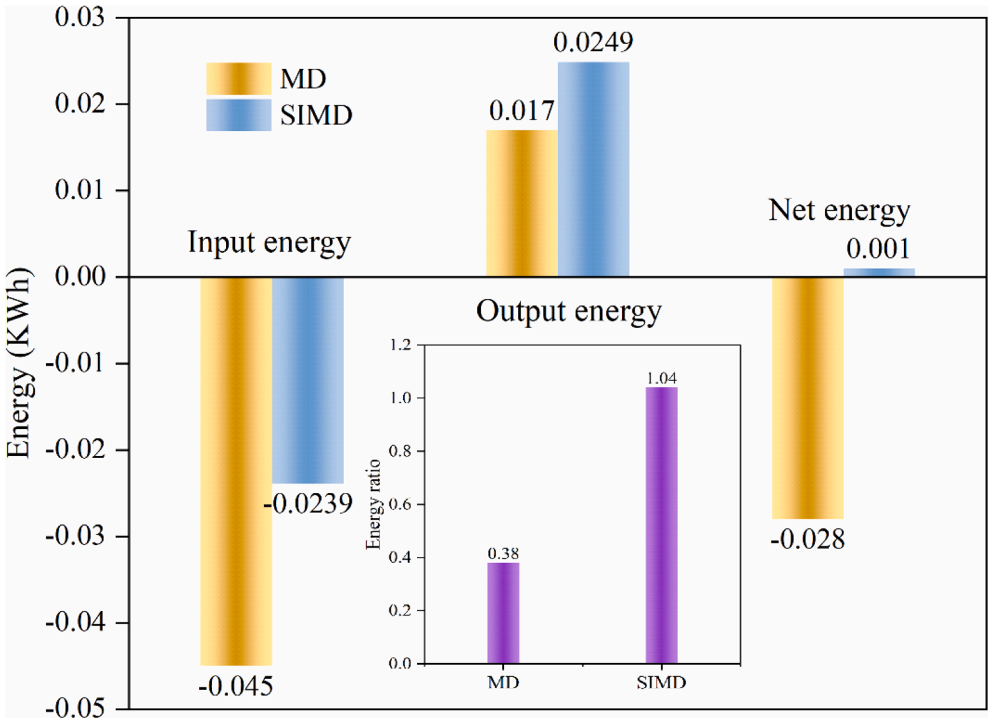

Fig. 6. Energy analysis.

as input energy and biohydrogen generation is taken as energy gain. Input energy for MD and SIMD are estimated as 0.045 and 0.0239 kWh/kg macroalgae, respectively. Microwave treatment required more time for better solubilisation therefore it consumes more energy meanwhile surfactant induced microwave consume less energy as it requires much less time when compared to MD for better efficiency. In contrast, the output energy assessed as 0.0249 kWh/kg macroalgae in SIMD is high when compared to MD (0.017 kWh/kg macroalgae). Net energy is calculated by the output and input energy. Net energy obtained as 0.001 kWh/kg macroalgae in SIMD, whereas in MD it is − 0.028 kWh/kg macroalgae. SIMD achieved energy ratio of 1.04 which is higher than MD (0.38). It is concluded by the above discussion that SIMD process is more efficient than MD process. Fig. 6 illustrates the energy analysis of MD and SIMD.

4. Conclusion

The present study examined about the biohydrogen production from marine macroalgae through surfactant induced microwave disintegration. SIMD showed higher biomass solubilisation (34.2%) using lesser energy (8640 kJ/kg TS) compared to MD. At the optimal conditions, SIMD samples showed higher biohydrogen production of 54.9 mL H_2/g COD compared to MD (39.7 mL H_2/g COD). Energy analysis revealed that SIMD was energy efficient process with net energy of 0.001 kWh/kg macroalgae. This clearly indicates that SIMD was an effective treatment process for enhancing biohydrogen production. Future research was further required to improve the production of Bio hydrogen and its implementation in large scale.

CRediT authorship contribution statement

M. Dinesh Kumar: Investigation, Writing – original draft. **V. Godvin Sharmila:** Validation, Resources. **Gopalakrishnan Kumar:** Resources. **Jeong-Hoon Park:** Resources. **Siham Yousuf Al-Qaradawi:** Resources. **J. Rajesh Banu:** Conceptualization, Supervision, Data curation, Validation.

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.

Acknowledgement

The work is supported by the funding from UGC-STRIDE project on climate change under the title “Application of bio char on agro ecosystem for mitigating climate change and GHG emission” at Central University of Tamil Nadu, Tiruvarur, Tamil Nadu, India.

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