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Utilization of Seawater in the Pretreatment and Saccharification of Seaweed

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Abstract: The growing need to cater to the energy demand coupled with the urge to mitigate greenhouse gas footprint in the energy sector has led to the exploration of biofuels, such as bioethanol, as a renewable and sustainable source. However, the desire for freshwater in bioethanol production is extremely high and hence it is considered as a high water footprint product. With the scarcity of ever-depleting fresh water resources and its huge consumption in biorefineries, it has become difficult to use fresh water for bioconversions. The use of abundantly available seawater as a substitute for freshwater was suggested to reduce the water footprint of bioethanol production. Furthermore, bioethanol production using seaweeds or macroalgal resources has shown great promise and significance in global sustainable development. Therefore, in the present study, pretreatment and saccharification of *Chaetomorpha* sp., a green seaweed, was carried out using seawater based reaction medium. The optimal pretreatment conditions were obtained using the microwave at 6.681% substrate loading, 1.487% NaOH and 7.724 min duration, which resulted in a maximum reducing sugar yield of 0.196 g/g. These findings reveal that seawater can produce comparably digestible solids to those of fresh water and can be used as an alternative to fresh water usage in biofuel production.

Key words: Seawater, seaweed, pretreatment, reducing sugar yield.

Introduction

Burgeoning energy consumption and ever depleting fossil fuel reserves have escalated numerous environmental constraints, among which global warming and the release of various harmful gases are of prime concern. Fossil fuels such as coal, oil, natural gas or petroleum are available in near exhaustive proportions and would end up soon if they are indiscriminately used. This has impelled the scientific community to explore substitutes of fossil fuel which are not only infinite but also renewable and sustainable (Daroch et al., 2013). Biofuels are recognised as potent substitutes for fossil fuels with great potential to alleviate energy and economic crisis. Among various categories of biofuel, bioethanol is one of the widely accepted substitutes for gasoline (Li et al., 2014). Based on the various categories of feedstock used, bioethanol can be classified into three generations. The first generation bioethanol utilises edible feedstock such as sugarcane juice, sugarbeet, starch, corn but such substrates often raise food versus fuel issues (Yanagisawa et al., 2013). The second generation bioethanol is produced from agro-industrial wastes and agricultural residues such as rice straw, wheat straw, and sugarcane bagasse. These substrates often contain lignin which shields the

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carbohydrate portions cellulose and hemicellulose—and prevents subsequent bioconversion. The third generation bioethanol production is derived from seaweed, which is regarded as a potential substrate for biofuel production owing to their high carbohydrate content (Dev et al., 2019; Wei et al., 2013). The present study focusses on the utilisation of seaweed, *Chaetomorpha*, as prospective biomass that can resolve the bioenergy crisis through renewable and sustainable bioethanol production.

Bioethanol is regarded as a high water footprint product due to its extensive requirement of fresh water which imparts additional pressure on the ever depleting fresh water reserves of the planet. Reports suggest that it takes around 1388 to 9812 L of water to produce 1 L of bioethanol with the majority consumed in the cultivation of crops (Gerbens-Leenes et al., 2009). Therefore, with the increasing concern of water scarcity, the surplus of fresh water cannot be ignored for bioethanol production. These circumstances have reinforced the need for the exploration of non-potable water resources, which can supplement the necessity of water in bioethanol production (Dev et al., 2019). In this context, it should be mentioned that seawater constitutes 97% of the world's water supply and covers almost 71% of the earth's surface. With such abundance, the infinite seawater resources can be exploited to ameliorate the issue of fresh water requirement in biofuel (bioethanol) production. There are numerous instances that prove the theory of using seawater mediated approach has been used for solid state fermentation for production of enzymes like L-glutaminase, chitinase, cellulase; chemo-enzymatic conversion of 5-HMF production; anaerobic fermentation for biomethane production and anaerobic digestion for lipid production (Almardeai et al., 2016; Chandrasekaran et al., 1999; Dev et al., 2019; Grande et al., 2012; Indira et al., 2016; Jiang et al., 2018; Prabhu and Chandrasekaran, 1997; Suresh and Chandrasekaran, 1998; Zaky et al., 2018). These studies have indicated the efficacy of seawater in a wide array of bioconversions. Furthermore, recent investigations have revealed that seawater can be successfully used as a reaction media for pretreatment, saccharification as well as fermentation in bioethanol production with a wide range of substrates (Ren et al., 2016; Zaky et al., 2018).

Therefore, the goal of the present study is to explore the impact of seawater in microwave-assisted pretreatment and enzyme catalysed saccharification of *Chaetomorpha* seaweed. Optimisation studies were carried out to achieve the maximum reducing sugar yield. The results of the current study would further emphasise the benefits and prospects of using seawater-based reaction medium in bioethanol production.

Materials and Method

Substrate and Chemicals

The green seaweed *Chaetomorpha linum* was obtained on March 2021 from Digha, which is located across the coast of the Bay of Bengal, India. The seaweed was washed with seawater to remove the sands and sediments, following which it was dried overnight at 60°C and milled through a 5mm mesh. The milled raw material was then stored in air tight container until further use.

The chemicals used were all of the analytical grades and obtained from HiMedia (India) and Sigma Aldrich (USA).

Proximate Analysis

The moisture and ash content of the seaweed were measured using the conventional oven method and incineration method (Chemists and Horwitz, 1975), respectively. Total carbohydrate content was estimated using the phenol-sulphuric method (Dubois et al., 1956). Lipid content was estimated using the Bligh and Dyer method (Bligh and Dyer, 1959). The protein method was analysed using the Lowry method by taking bovine serum albumin (BSA) as standard (Classics Lowry; Rosebrough et al., 1951).

Preliminary Screening

Preliminary screening was carried out for the two methods of pretreatment used in the current study.

Steam-assisted – 10% of substrate loading was suspended in 2% NaOH prepared in seawater and autoclaved for 121°C for 15 min in a 250 ml Erlenmeyer flask.

Microwave-assisted – For microwave pretreatment, 10% milled seaweed was suspended in 2% NaOH prepared in seawater and pretreated in a household microwave oven (Electrolux- 2.4 GHz) at 160W for 7 min.

Upon pretreatment, the samples were washed to neutral pH 7, dried and stored prior to enzymatic hydrolysis.

Optimisation of Microwave-assisted Pretreatment (MAP) of Seaweed Using RSM-BBD

Response surface methodology based Box Behnken Design (RSM-BBD) was used to obtain optimal pretreatment conditions for *Chaetomorpha* with seawater as reaction medium. The considered, three independent, parameters were substrate loading (5-10%), NaOH concentration (1-2%) and time (1-10 min) as shown in Table 1. Design expert version 11 was used for experimental design and result analysis. All the experiments were performed in triplicate with microwave power maintained at 160 W.

Table 1: Range of each variable of the Box Behnken Design (BBD)

Factor	*Name*	*Units*	*Low*	*High*
A	Substrate loading	(%)	5	10
B	NaOH	(%)	1	2
C	Time	(min)	1	10

Enzymatic Saccharification

Hydrolysis of the untreated and pretreated seaweed was performed with citrate buffer made in seawater (pH 4.8, 0.05 M) with substrate and enzyme loading of 2.5% (w/v) and 20 Filter Paper Unit/gm (FPU/gm). Enzymatic hydrolysis was carried out for 48 hr, 50°C, 200 rpm. Reducing sugars were estimated using the 3,5-dinitrosalicyclic acid (DNSA) method (Miller, 1959).

Analytical Methods

Water Swelling Capacity

Water swelling capacity is an important criterion as it determines the surface area available to enzymes. It is calculated using the following formula:

$$\text{Water swelling capacity} = \frac{W_2 - W_1}{W_1} \quad (1)$$

where $W_1$and W_2 denote the initial and final weight of the dry and swollen samples, respectively (Jeihanipour et al., 2010).

Results and Discussion

Proximate Analysis

Chaetomorpha linum is a filamentous free-floating seaweed with rigid cell walls and composed of crystalline cellulose in the outer lamellar part and a branched polymer of arabinose, xylose and galactose in the inner amorphous part (Schultz-Jensen et al., 2013; Wang et al., 2011). Seasonal variations and environmental gradients such as temperature, salinity, light, and water are the prime factors that determine the proximate composition of seaweeds (Fonseca et al., 2006; Lavery and McComb, 2021; Marinho-Soriano, 2006). The moisture and ash content of *Chaetomorpha* were found to be 2.18 ± 0.67% and 29.66 ± 3.1%, respectively. Although the obtained ash content is higher, it is in accordance with the previous reports of 11% – 34% for green algae (Bird et al., 2011). The carbohydrate content for seaweed was 33.12 ± 0.98%, which is similar to the reported percent carbohydrate content of *Chaetomorpha* sp. (Hessami et al., 2018). Upon pretreatment, the carbohydrate content increased to 39.46 ± 0.55%, which indicates the efficacy of pretreatment. The lipid content and protein content were 1.12 ± 0.08 % and 13.43± 0.66%, respectively.

Preliminary Screening

The seaweed was subjected to steam and microwave-assisted pretreatment using seawater as a reaction medium. The most common alkaline reagent, i.e., 2% NaOH was used for the pretreatment of the seaweed. Microwave pretreated biomass was found to yield the highest reducing sugar of 0.139 g/g in comparison to steam-assisted which yielded 0.121 g/g. Interestingly, steam-assisted pretreatment is an energy-intensive approach and requires a longer time to disintegrate the biomass. On the contrary, the microwave method is capable of enhancing saccharification yield by causing fibre swelling and fragmentation of the biomass in a short duration (Moodley and Kana, 2017). These results corroborate with the previous reports confirming the advantages of the microwave alkaline approach for thermal treatment of biomass. It was observed that microwave pretreatment exhibited a superior saccharification ratio than steam explosion pretreatment, although the former had low pretreatment temperature and lesser reaction time (Tsubaki and Azuma, 2011).

Process Optimisation

The effect of three independent pretreatment parameters, namely solid loading (A), NaOH (B) and time (C), was examined using Design expert software 11. The pretreatment parameters set at solid loading from 5-10%, NaOH from 1-2% and time from 1-10 min were assessed for maximum reducing sugar yield (RSY). The design and the experimental results of microwave-assisted NaOH pretreatment of seaweed are summarised in Table 2. The untreated biomass upon hydrolysis yielded 0.112g/g of reducing sugars whereas the biomass pretreated at the optimal condition of 6.681% substrate loading, 1.487% NaOH and 7.72 min resulted in a maximum release of 0.196 g/g reducing

Table 2: Box Behnken Design (BBD) for optimization of process parameters affecting reducing sugar yield

Std	*Run*	*Substrate loading (%)*	*NaOH (%)*	*Time (min)*	*Reducing sugar yield (g/g)*
10	1	7.5	2	1	0.152
14	2	7.5	1.5	5.5	0.188
9	3	7.5	1	1	0.093
4	4	10	2	5.5	0.178
2	5	10	1	5.5	0.159
5	6	5	1.5	1	0.112
11	7	7.5	1	10	0.192
15	8	7.5	1.5	5.5	0.183
3	9	5	2	5.5	0.167
8	10	10	1.5	10	0.187
6	11	10	1.5	1	0.12
7	12	5	1.5	10	0.177
16	13	7.5	1.5	5.5	0.182
17	14	7.5	1.5	5.5	0.186
12	15	7.5	2	10	0.185
13	16	7.5	1.5	5.5	0.189
1	17	5	1	5.5	0.162

R^2 = 0.9897
Adjusted R^2 = 0.9764
Predicted R^2 = 0.8725

Table 3: ANOVA table of microwave-assisted NaOH pretreatment of seaweed

Source	*Sum of squares*	*df*	*Mean square*	*F-value*	*p-value*	
Model	0.0142	9	0.0016	74.62	< 0.0001	Significant
A-Substrate loading	0.0001	1	0.0001	4.00	0.0855	
B-NaOH	0.0007	1	0.0007	34.22	0.0006	
C-Time	0.0087	1	0.0087	412.89	< 0.0001	
AB	0.0000	1	0.0000	2.32	0.1714	
AC	1.000E-06	1	1.000E-06	0.0474	0.8339	
BC	0.0011	1	0.0011	51.61	0.0002	
A^2	0.0007	1	0.0007	32.69	0.0007	
B^2	0.0002	1	0.0002	7.92	0.0260	
C^2	0.0024	1	0.0024	113.03	< 0.0001	
Residual	0.0001	7	0.0000			
Lack of Fit	0.0001	3	0.0000	3.96	0.1084	Not significant
Pure Error	0.0000	4	9.300E-06			
Cor Total	0.0143	16				

sugars. The carbohydrate content increased from 33.12 ± 0.98% to 39.46 ± 0.55%. The study demonstrated that using seawater as a reaction medium, microwave-assisted NaOH pretreatment of seaweed can result in a substantial increase in reducing sugar yield. The quadratic equation, describing reducing sugar yield as a function of substrate loading (A), NaOH (B) and time (C) is represented by the following equation (2).

$$\text{RSY (Y1)} = 0.1856 + 0.00325\mathbf{A} + 0.0095\mathbf{B} + 0.033\mathbf{C} + 0.0035\mathbf{AB} + 0.0005\mathbf{AC} - 0.0165\mathbf{BC} - 0.0128\mathbf{A}^2 - 0.0063\mathbf{B}^2 - 0.0238\mathbf{C}^2 \quad (2)$$

where RSY is the reducing sugar yield (g/g)

Furthermore, the model accuracy, model significance, model fitness and interaction between predicted and observed values were statistically examined by analysis of variance (ANOVA). The lower *p* value of 0.0001 relates to the significance of the model. The high *R* squared value of 0.9897 and adjusted *R* squared value of 0.9764 for reducing sugar yield further validate the model adequacy. This also suggests the best fit of the model. Comparative analyses of the *F*- values indicated the significance of parameters on the response. From individual terms' *F* value, time-variable was found to have a profound effect on the response variable whereas substrate loading indicated the least effect on the response. The quadratic term (C^2) shows a strong effect on response whereas the interaction factors AB and AC have the least impact on reducing sugar yield.

Influence of Solid Loading, NaOH and Time on Reducing Sugar Yield

The 3D response surface plots obtained from feeding the experimental values into design expert 11 were visualised to study the interactive effect of solid loading, NaOH and time on reducing sugar yield. The response surface graphs of reducing sugar yield are shown in Figure 1. For plotting the graphs, two independent parameters were considered while the third parameter was kept constant. The release of reducing sugars was in the range of 0.093g/g to 0.192g/g. The maximum reducing sugar yield was obtained at substrate loading of 7.5%, NaOH concentration of 1% and 10 min pretreatment time.

A closer examination of the response surface plots indicated the presence of visible elongated maxima running along the axis of NaOH and time variables as observed in Figure 1a. This suggested that the interactions between NaOH and time have significant contributions in generating reducing sugar yield, which was also validated by the higher *F*-values of NaOH (34.22) and time (412.89). Further, the interaction plot of NaOH and time was more elliptical in nature (Figure 1a) and was statistically significant as indicated by the lower *p*-values (0.0002). On the other hand, the substrate loading had the least impact on the reducing sugar yield, which was also confirmed by its lower *F*-values (4.00) and circular nature of the response plots (Figure 1c). Further, the interaction of substrate loading and other variables were found too weak (Figure 1b-c)

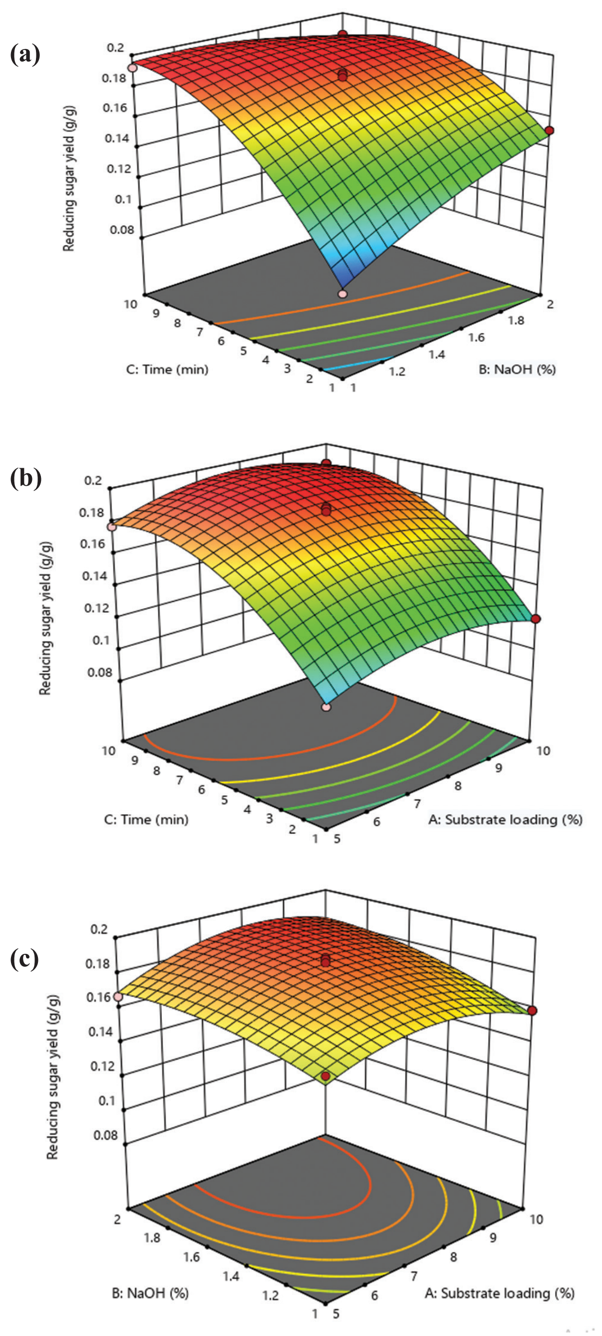

Figure 1: The influence of process parameters on reducing sugar yield during microwave-assisted NaOH pretreatment of seaweed.

and yielded higher p-values (0.1714 and 0.8339), which indicate a lack of substantial effect on the response variable. Therefore, based on the analysis of the 3D plots, it can be concluded that an increase in NaOH and time resulted in higher response and optimum levels of these parameters was essential for generating maximum reducing sugar yield.

Water Swelling Capacity

The water swelling capacity of the untreated and pretreated seaweed was measured. The pretreatment greatly enhanced accessible surface area as evident by the increase in water swelling capacity of the untreated and pretreated seaweed value from 0.67 g/g to 1.66 g/g, respectively. These results correlate with the enhanced digestibility of the pretreated biomass.

Conclusion

The findings of the current study revealed that a combination of NaOH and seawater can synergistically promote the release of digestible solids from seaweed in a microwave-based approach and indicated that alternative water resources can substitute fresh water requirements in biorefineries. Under optimal pretreatment conditions of 6.681% substrate loading, 1.487% NaOH and 7.724 min duration, 0.196 g/g reducing sugar were determined. These findings have the potential to accentuate the use of seawater in biorefineries which could simultaneously alleviate the burden on the freshwater resources of our planet.

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Conflict of Interest

The authors declare no conflict of interest.

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