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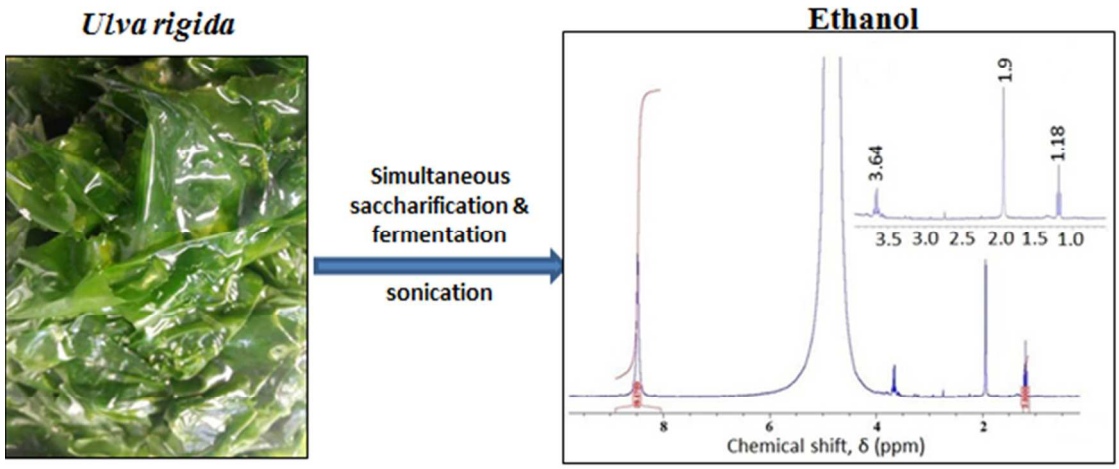

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ARTICLE TYPE

Single Step Production of Bioethanol from the Seaweed *Ulva rigida* using Sonication

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Ulva rigida, a common green seaweed, was used as a feedstock for the production of bioethanol in a simultaneous saccharification and fermentation (SSF) process carried out under sonication. Sonication provided a faster way for the simultaneous release of glucose from *Ulva rigida* and its conversion into bioethanol. Within 3 h, 196 ± 2.5 mg glucose per gram of dry weight of biomass and 333.3 ± 4.7 mg bioethanol per gram of glucose were produced in the SSF process under sonication. In addition to being fast, the process was devoid of any chemical pretreatment and involves only a single stage of sonication for the release of glucose from algae by the action of enzymes and also for the simultaneous fermentation of glucose to ethanol using *Saccharomyces cerevisiae*.

1. Introduction

Efforts to develop renewable fuel sources are at high. Biofuels are considered as clean and sustainable energy source for transportation applications.[1–3] Bioethanol is the most widely used bio-fuel for transportation application throughout the world. Use of bioethanol provides a sound way to reduce both the consumption of fossil fuels and environmental pollution.[2] Common biomass sources for biofuels include grains of various kinds and sugarcane. However, biofuel production from these crops, especially corn, has led to public criticism due to rising food prices and global food shortage. Furthermore, the ethanol production from grains has adverse environmental effects such as soil erosion, high volatile organic compounds and NO_x pollution. In addition, significant areas for plantation as well as fresh water sources are required.[4,5] As an alternative, agriculture waste has been considered as a potential feedstock.

Agricultural byproducts and industrial residues rich in ligno-cellulose emerged as promising sources for bioethanol and biopolymer production. However, due to the presence of complex lignin and hemicelluloses, lignocellulosic materials require chemical pretreatment in order to increase cellulose accessibility.[6,7] Therefore, production of bioethanol from lignocellulosic biomass is complex and has inherent environmental concerns.[8]

Seaweeds form a potential feedstock for biofuels production. The potential of seaweeds, especially, *gelidium amansi*[9], *laminaria japonica*, *codium fragile*[10] and *nizimuddinia zanardini*[11] was exploited for bioethanol production.

The option of marine algae has not yet been well explored although they offer numerous advantages. The growth rates of seaweeds far exceed those of terrestrial plants. The ethanol production potential is higher due to the low concentrations of crystalline components (like lignin). Their cultivation does not necessarily encroach on agricultural land required for food crops. They can act as bioremediation crops to lower the eutrophication impact on inshore waters.[12] In addition the need for fresh water supply is also reduced.[13, 14]

Although seaweeds have been tested for ethanol production, it has been difficult to obtain high concentrations of ethanol. This could be due to inefficient methods of hydrolysis and fermentation processes, which form important steps in making seaweeds the next bioethanol feedstock.[5, 15]

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The green macroalga *Ulva* (Chlorophyceae) is a common seaweed abundantly found worldwide that also thrives in eutrophicated coastal waters providing a potential aquatic energy crop due to its high potential growth rates and relatively high content of carbohydrates. Even though a variety of seaweeds were explored as potential feedstocks for bioethanol production, the seaweed *U. rigida* has only been described to a limited extent.[1, 5, 14–20]

Most of these studies used pretreatment processes like thermo chemical treatments using dilute acid, that were generally followed by enzymatic treatments.[15, 21–23] However, this process might have the disadvantage of generating hydroxymethyl furfural (HMF) and other furfurals due to the harsh pretreatment conditions. The presence of toxic residues in the hydrolysate could also decrease alcohol fermentation yields.[24, 25] Simultaneous saccharification and fermentation (SSF) is an industrially adoptable and attractive option for the production of ethanol. The benefits of combining the enzymatic hydrolysis together with the fermentation include: the reduced number of operations needed, the reduced end product inhibition of the enzymatic process, and high ethanol yields that are produced using SSF compared to separate hydrolysis and fermentation.[23, 26, 27] The overall process of bioethanol production can be rate-limited by each of these steps, particularly step 1 (pretreatment).[28] Thus, the present methodology could reduce the processing time of *Ulva* to bioethanol by eliminating the requirement of chemical pretreatment step.

Ultrasonication has been applied widely in various biological and chemical processes. Ultrasound assisted hydrolysis has been shown to intensify and to improve the efficiency of the process and considerably reduce extraction time and energy used. Ultrasound is a green extraction technique.[29, 30]

The application of sonication process (40-35 kHz for 5-30 min) prior to or during enzymatic hydrolysis have shown to enhance enzyme activity and have significantly increased the enzymatic conversion of starch materials to glucose.[31, 32] Moreover, the application of mild ultrasonic conditions has shown to accelerate the process of glucose fermentation and increase the overall ethanol yield.[33, 34] However, despite of the above advantages, the application of sonication in order to accelerate enzyme hydrolysis of biomass and a subsequent increase in the production of ethanol (SSF process) is scarce.

In this study, the feasibility of producing bioethanol from the marine alga *Ulva rigida* in a single step was evaluated. The process involves the SSF of Ulva under sonication conditions without the requirement any chemical pretreatment. The objective was to develop an energetically, and environmentally viable and fast process for the production of bioethanol from *Ulva* biomass.

2. Experimental:

2.1. Materials

2.1.1. Feedstock for bioethanol production

U. rigida was obtained from a seaweed culture collection at IOLR (Israel Oceanography and Limnological Research) and dried at 70 °C for 48 h in an oven. The dried samples were grounded into a fine powder (to pass through a 1 mm sieve) using a coffee grinder, and then stored in an air-tight labelled plastic bags in a desiccator. All values were reported relative to the dry weight of the seaweed. Mean and standard deviation values were calculated.

2.1.2. Enzymes

Commercially available enzymes, *amyloglucosidase* from *A. niger* (≥300 U/mL) (product no. A7095), *α-amylase* from *B. amyloliquefaciens* (≥ 250 units/mL) (product no. A7595) and *cellulase* from A. *niger*, (≥ 0.3 units/mg solid) (Product no. C1184), were obtained from Sigma-Aldrich. Glucose oxidase (GOx) (Product no. G3660), SigmafastTM 0-phenylene diamine dihydrochloride (OPD) tablet (P9187), Peroxidase from horse radish (HRP, product no. P8125) employed for the quantification of glucose were also purchased from Sigma-Aldrich.

2.1.3. Yeast

Bravo instant dry yeast, 125 g pack is procured from supermarket. The commercial Baker's yeast (*Saccharomyces cerevisiae*) procured is stored in a refrigerator and subsequently used for the fermentation reaction. 0.5 g of yeast was used for a typical SSF process.

2.2. Analytical methods

2.2.1. Chemical composition of *Ulva rigida*

Total cell carbohydrates were extracted from the algae by mixing 20 mg of samples in 2 N H_2SO_4 solution (1 mL) and boiling the same for 1 h. The mixture was cooled and centrifuged for 10 min at 11,000 rpm to remove residual solids, and directly processed for estimation. Carbohydrate concentrations were determined by

a phenol-sulfuric acid colorimetric method modified to a microplate format, which measures absorption at 490 nm. Soluble carbohydrates were estimated relative to a glucose standard solution.[35, 36] Starch quantification was carried out following the method described by Smith & Zeeman.[37] Briefly, 20 mg of ground samples were washed twice in 80 % (v: v) ethanol, then resuspended in 200 mM sodium acetate (pH 4.8), boiled for 10 min, and incubated for 3 h with *amyloglucosidase* (6 U; Sigma) and *α-amylase* (1 U; Sigma). A solution of equivalent volume and composition was incubated without the enzymes and used as control samples. The release of glucose was determined at 450 nm using a glucose oxidase assay with Bio-Rad Laboratories (Model Benchmark) microplate reader, and compared to a glucose standard.[38] Starch content was calculated as 90% of glucose content. The cellulose content of the algae was determined using neutral detergent residue method.[39] Briefly, 1 g dry algae was treated under reflux condition with 100 mL of the neutral detergent in water solution prepared with the following constituents: 1.86 g EDTA, 0.68 g $Na_2B4O_7.10H_2O$, 3 g sodium dodecyl sulfate, 1 mL glycol ether and 0.456 g Na_2HPO_4.

Samples (50 mg) for protein analysis were digested in 1 N NaOH for 24 h at room temperature and directly quantified using the Bradford method, using bovine serum albumin as the standard with Bio-Rad Laboratories (Model Benchmark) microplate reader.[40]

The content of carbon, hydrogen, nitrogen and sulphur in the dry biomass was determined using CHNS elemental analyzer, Flash EA 1112 series, Thermo Electron Corporation, Italy calibrated with L-Cistina, Sulfanilamide, BBOT as a reference standards.

2.2.2. Glucose estimation by colorimetric enzymatic method

In order to quantify the amount of glucose in the hydrolyzate an enzymatic method was employed.[38] Typical analytical procedure comprises of adding a 78 µL of analyte to 122 µL of reaction mixture (20 µL GOx 10 U/mL, 2 µL HRP 10 U/mL, and 100 µL of 0.4 mg/mL OPD). The reaction mixture was incubated at room temperature for 30 min in the dark. Absorbance of the samples was measured at 450 nm and corresponding amounts of glucose were deduced from the standard curve obtained with different concentrations of commercial D-glucose. All measurements and experiments were conducted in triplicates.

2.2.3. ^{1}H NMR for ethanol quantification and ^{13}C NMR for sugar analysis

The ethanol produced in the SSF process was quantified using ^{1}H NMR. [41]

The yield ($g_{ethanol}/g_{glucose}$) of ethanol was calculated from the ^{1}H NMR spectrum of the analyte using HCOONa (m = 20 mg, n = 0.294 mmol) as an internal standard in D_2O (solvent, 200 µL).

The formula used to calculate the amount of ethanol formed in the given analyte is the following:

$n_{EtOH} = (n_{HCOONa} \times I_{EtOH})/(3 \times I_{HCOONa})$

where: n_{EtOH} = number of moles of ethanol in the analyte,

n_{HCOONa} = number of moles of sodium formate added,

I_{HCOONa} = ^{1}H NMR integral of sodium formate (1H) was set to 1,

I_{EtOH} = ^{1}H NMR integral of peak of ethanol at 1.16 ppm (-CH_3; 3H).

From n_{EtOH}, amount of ethanol (g) in the analyte was calculated as the following:

g_{EtOH} = mol. wt. of EtOH x n_{EtOH}

From the amount of ethanol in the given volume of the analyte, the wt. % of ethanol in the total volume of the product is calculated as the following:

Yield of ethanol (wt. %) = (g_{EtOH} /total volume of the product) x 100

^{13}C NMR was employed for the qualitative analysis of the hydrolyzate for the observation of fermentable sugars formed as a result of the action of enzymes (*amylase* and *cellulase*) on the algae.[34] ^{1}H-NMR and ^{13}C-NMR spectra were recorded on a Bruker Avance DPX 300.

2.3. Enzymatic Hydrolysis

2.3.1 Hydrolysis in an incubator

To 1.68 g of dried *U. rigida* 40 mL of distilled water and 40 mL of 200 µM sodium acetate (buffer, pH-4.8) (2.1 % w/v) were added. Into the suspension were added enzymes: 100 µL amyloglucosidase 300 units/mL, 40 µL *α-amylase* 250 units/mL, and 0.1 g *cellulase* 0.3 units/mg. The contents were kept in sealed 100 mL bottles (bottles with flasks, Fisher brand) in an incubator (Labtop model) at 37 °C with shaking 150 rpm for 24 h. The optimum temperatures for enzyme activity (*amyloglucosidase*, 60 °C; α-amylase, 70 °C; *Cellulase*, 45 °C) are higher than the temperature set during the SSF process (37 ˚C), this was set in order to fit the yeasts optimum activity (*Saccharomyces cerevisiae*- 25-35°C). Aliquots (0.5 mL) of samples were then taken at regular time intervals , centrifuged for 5 min at 7,500 rpm and the supernatant was stored frozen (-20 °C) for subsequent glucose analysis. The hydrolyzate was qualitatively

analysed for fermentable sugars using ^{13}C NMR and quantified using a glucose oxidase enzymatic method.[38]

2.3.2 Hydrolysis under sonication

The process of hydrolysis of algae under mild sonication was carried out in a bath sonicator (MRC Clean -01 Ultrasonic cleaner, 40 kHz ultrasound frequency, Ultrasonic power-120 W).[34] The reaction temperature (37 °C) was maintained using a Julabo cooler. All the conditions of the hydrolysis are similar to those specified above except that the reaction was carried out in a bath sonicator instead of the use of incubator.

2.4. Simultaneous saccharification and fermentation (SSF)

The reaction conditions employed for the simultaneous saccharification and fermentation are analogous to those employed in the hydrolysis of algae. In addition to the algae, enzymes, buffer and yeast (Baker's) were added to the fermentation broth. Typical constitution of the broth comprise of: 1.68 g of dried *U. rigida* in 40 mL of distilled water and 40 mL of 200 µM sodium acetate (buffer, pH – 4.8) (2.1 % w/v) taken in a 100 mL glass media bottles with cap (Fisher band). Into the suspension were added enzymes, 100 µL *amyloglucosidase* 300 units/mL, 40 µL *α-amylase* 250 units/mL, 0.1 g *cellulase* (0.3 units/mg) and 0.5 g of Baker's yeast. The SSF process was carried out in two ways: in the conventional incubator with shaking at 150 rpm for 48 h and under mild sonication for 3 h at 37 °C and at a pH of 5. The process efficiency is calculated by dividing the amount of ethanol (g) produced per gram of glucose by a factor 0.51. This is based on the theoretical value of ethanol (0.51 g) that could be obtained per gram of glucose.

3. Results and discussion:

3.1. Chemical composition of *Ulva rigida*

Carbohydrates are the algal key component in the process of hydrolysis for the production of fermentable sugars. Therefore, the algal chemical composition could be used as an indication to the extent to which the algae could be hydrolysed to produce fermentable sugars. Seaweeds are highly diverse in terms of the kind of carbohydrates involved in carbon storage and cell structure. Most seaweeds commonly use starch for energy storage but storage compounds may also include molecules other than starch, depending on the species, such as ulvan in the green *Ulva*. While cellulose and starch could be hydrolysed to glucose which could be easily metabolized by *Saccharomyces cerevisiae*, the ulvan fraction does not contribute to the formation of glucose.[42]

The chemical composition of *U. rigida* on dry weight basis was summarized in Table 1.

Table 1. Proximate analysis of *Ulva rigida*

Proximate composition	Relative % on dry weight basis
Carbohydrate	37±3.9
Cellulose	23.8 ±1.2
Starch	7.6±1.1
Protein	6.2±0.9
Carbon	28.1±1.2
Nitrogen	4.5±0.7
Hydrogen	5.5±1.3
Sulphur	2.3±0.4

The total carbohydrate was found to be 37 ± 3.9 with a starch content of 7.6 ± 1.1 %. These results are in agreement with the previous reports on the composition of *Ulva sp*. [21] Cellulose content of the algal samples were found to be 23.8 ± 1.2 % of dry weight. Siddhanthe et al., reported a cellulose content of 20 wt.% in *Chaetomorpha aerea*. Ventura et al., observed a hemicellulose and cellulose content of 20% wt. in *Ulva lactuca*.[43, 44]

Thus, based on the cellulose and starch contents of *Ulva*, nearly 31 wt. % of the biomass could be hydrolysed to fermentable sugars. Even though, the structural carbohydrate component ulvan is known to yield monosaccharides such as rhamnose, major fraction of fermentable sugars (glucose) is obtained from the cellulose and starch fractions.[45–47] The hydrolysis conditions are so designed as to hydrolyse enzymatically the cellulose and starch components of the algae and produce glucose as the sole fermentable sugar. The focus of the study was to selectively produce glucose from algae as glucose is the sugar that could be fermented to ethanol more easily using common yeast strain like Saccharomyces cerevisiae, compared to any other C5 or C6 sugars generated from the biomass.

3.2. Enzymatic hydrolysis of *Ulva rigida*

Without any chemical pretreatment, dry biomass of *U. rigida* was subjected to enzymatic hydrolysis in order to obtain fermentable sugars, the reaction was carried out in two ways, namely, in a bath sonicator and in an incubator.

The progress of the saccharification process at 37 °C was monitored by evaluating the amount of glucose produced with time. Values of glucose yield (wt. %) as a function of time of enzymatic hydrolysis was shown in **Fig. 1**.

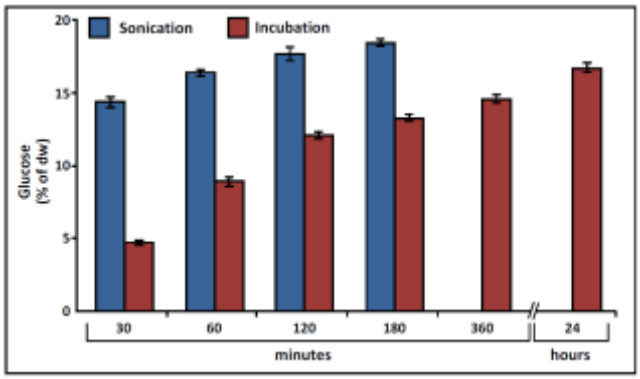

Fig. 1. Glucose yield as a function of time under sonication Vs incubation at 37 °C (replicate no. n = 3; error bars indicate standard deviation, SD)

A short saccharification period of 30 min with the enzymes in a bath sonicator resulted in the release of glucose as high as 14.4±0.32 wt.% and the corresponding glucose yield value in the hydrolyzate in the incubator was only 4.7 ± 0.19 wt.%. Thus, a 3.10 times higher yield of glucose was obtainable employing sonication during the hydrolysis stage. The glucose yield value raised from14.4±0.32 to 17.7±0.45 wt.% as the time of saccharification varied from 30 to 120 min under sonication. Conversely, the variation of glucose yield was from 4.7 ± 0.19 to 12.1±0.23 wt.% when the saccharification process was carried out in an incubation for 120 min After 24 h of saccharification in the incubator, the yield of glucose was 16.7±0.27 wt.% which was equivalent to the yield of glucose obtained at the short period of 60 min (16.4±0.28 %) under sonication conditions and lower than the glucose yield (19.6 ± 0.02) obtained in 3 h of sonication. Kim et al., reported a sugar yield of 19.4 wt.% from *Ulva lactuca*[19] and Trivedi et al., reported a sugar release value of 20.5 wt.% from *Ulva fasciata*.[21] The glucose yield value obtained in the current report (19.6 wt.%) was close to the values reported previously[16, 17] but under mild reaction conditions without either heating or using acid and in a short reaction time.

The advantage of obtaining higher amount of glucose from the algae at the given time with the use of mild sonication for the saccharification process was evident from **Fig. 1.** The enhancement in the release of glucose from the algae upon sonication was attributed to mechanical and thermal effects. Ultrasound was known to improve the hydrolysis process by the reduction of the structural rigidity of the lignocellulose and starch components in plant biomass. Moreover, ultrasound-assisted enzymatic hydrolysis was found to reduce the hydrolysis reaction time by improving mixing and phase transfer, and by enhancing the diffusion of enzymes across the algal cell membranes, so that enzymes can easily reach the bulk of the substrate.[28]

Regardless of the method employed for the saccharification process, *U. rigida* yielded exclusively glucose as the fermentable sugar. The ^{13}C NMR spectra of the aliquots of samples collected from the hydrolyzate under bath sonication (**Fig. 2A**) and incubation (**Fig. 2B**) at 120 min are depicted in **Fig. 2**.

Well resolved and intense peaks are observed in the range of 60 – 100 ppm. The peaks located at 60.9 (C6), 69.9 (C4), 71.8 (C2β), 73.1 (C2β), 74.5 (C3), 76.2 (C5), 92.4 (C1α) and 96.2 (C1β) ppm are typical of glucose. Thus, irrespective of the method of hydrolysis of the algae, the process was selective to the production of glucose as the sole fermentable sugar. In addition to the peaks corresponding to the carbon atoms in the glucose skeleton, another significant peak appeared at 23.6 ppm. This was attributed to the carbon of acetate which was used as a buffer for the enzymatic hydrolysis of algae.

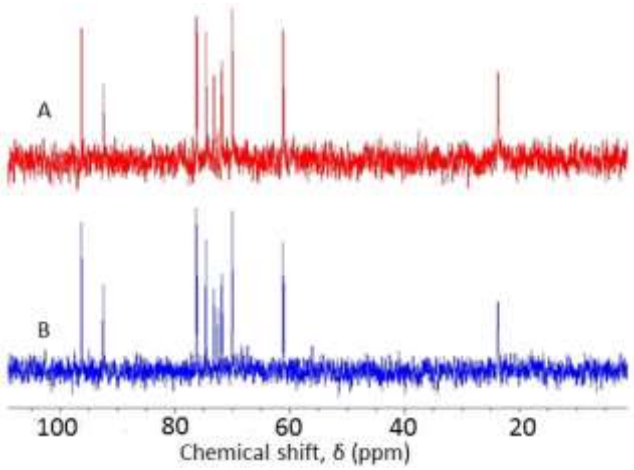

Fig. 2. ^{13}C NMR spectra of hydrolyzate of *Ulva rigida* produced under sonication (A) and incubation (B) at 37 °C at 120 min

After evaluating the hydrolysis process of *U. rigida* under sonication and incubation, further work was devoted to the simultaneous production of glucose from the algae by the action of enzymes and its conversion to ethanol by the action of yeast (*Saccharomyces cerevisiae*).

3.3. Simultaneous saccharification and fermentation (SSF) process

The SSF process used was similar in all aspects to that of the saccharification process mentioned above except that in addition to the algae, enzymes and buffer, the yeast is also taken in the same reaction vessel and all the constituents were subjected to either sonication (mild) or incubation at 37 °C with 150 rpm shaking. It should be noted that the yeast is stable under the sonication conditions.[34]

The progress of the SSF process was monitored by evaluating the amount of ethanol produced as a function of time during sonication or incubation (**Fig. 3**). Aliquots of samples were collected from the fermentation broth at regular intervals of time and analyzed by ^{1}H NMR using HCOONa as an internal standard. Analogous to the saccharification process of algae with enzymes, the SSF process was also found to be faster under sonication relative to incubation conditions. In a short duration of 30 min the yield of ethanol under sonication was significantly high (4.3±0.26 wt.% Vs 1.0±0.13 wt.% under incubation). The value increased steadily and reached a saturation at 180 min (6.2±0.13 wt.% Vs. 4.9±0.1 wt.% under incubation). Even after incubation for 48 h, the ethanol yield was only 6.1±0.13 wt.% which could be achieved in a short duration of 120 min with the use of mild sonication. The acceleration in the SSF process by the action of sonication could be due to the possibility of generation of fresh surface on the yeast cells by the faster removal of ethanol and CO_2 formed as the metabolites during fermentation.

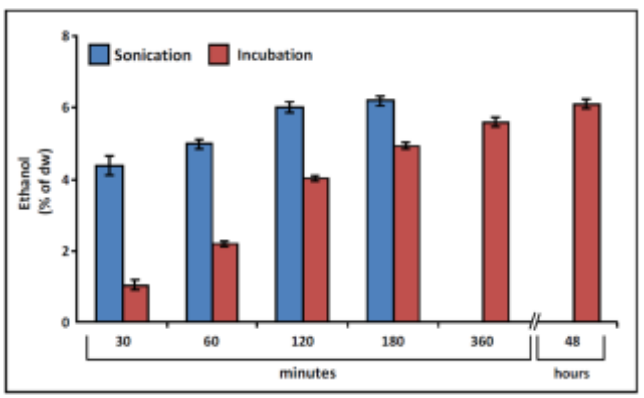

Fig. 3. Ethanol yield as a function of time in the enzymatic hydrolysis of *Ulva rigida* under sonication Vs incubation at 37 °C (replicate no. n = 3; error bars indicate standard deviation, SD)

As a representative example, the ^{1}H NMR of the aliquot of sample from the fermentation broth at 120 min (under sonication) was shown in **Fig. 4**.

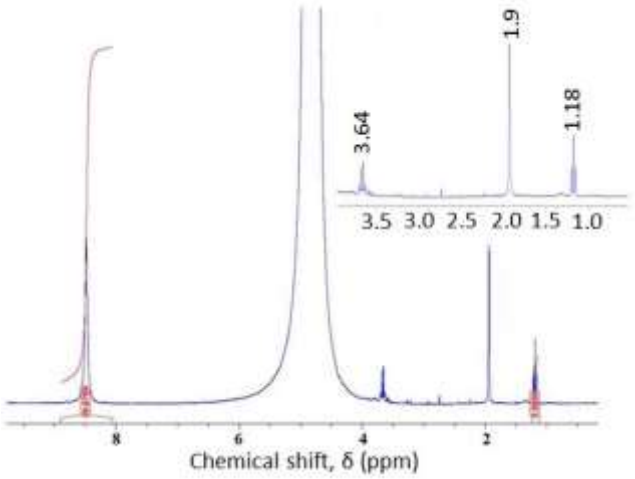

Fig. 4. ^{1}H NMR spectrum of the aliquot of sample collected from the fermentation (SSF) broth under mild sonication at 120 min

The appearance of 3H (t, 1.18 ppm) and 2H (q, 3.64 ppm) were typical of the presence of ethanol in the analyte.[48] The peak, 3H, s, 1.9 ppm, was characteristic of sodium acetate employed as buffer. The peak, 1H, s, 8.5 ppm, was typical of the internal standard, HCOONa. Based on the relative integral values of the internal standard and the ethanol peaks, the amount of ethanol was found to be 6.0±0.16 wt. %. The corresponding ^{1}H NMR spectrum of the aliquot of sample from the broth under incubation at 120 min was shown in **Fig. S1.** The yield of ethanol in the afore mentioned instance was 4.0±0.07 wt. %. Trivedi et al., reported an ethanol yield of 9.2 wt. % from *Ulva fasciata* upon 36 h enzymatic hydrolysis and 48 h of fermentation.[21] Unlike the previous report, the current method offers a single stage SSF process for ethanol production. Thus, sonication is a potential route for accelerating the process of ethanol production. Moreover, reduction of the number of process stages, and the extraction time might positively effect the total process energy consumption. In principle, use of ultrasound reduces mass transfer limitations, structural rigidity, crystallinity, particle size of biomass, enzyme aggregation and there by accelerates the saccharification and fermentation.[49] Using chemical and sensorial methods of analysis, Pingret et al., demonstrated the negative effects of ultrasound on food processing. Degradation as well as modification of physicochemical properties of food products is reported by the application of ultrasound. [50, 51] In the

case of bioethanol production no such degrading effects on metabolites are observed by the use of ultrasound.[34]

A comparison of the efficiency of the SSF process under sonication and incubation conditions is shown in Table 2.

Table 2. Efficiency of SSF process: sonication Vs incubation

SSF process	*Glucose yield (g/g biomass)*	*Ethanol yield (g/g glucose)*	*Process efficiency (%)*
Sonication (t=3 h)	0.196	0.33	64.7
Incubation (t=48 h)	0.173	0.34	66.6

(Reaction conditions: biomass (dry weight) = 1.68 g; distilled water = 40 mL; *cellulase* = 0.1 g (0.3 units/mg): *α-amylase* = 40 µL (250 units/mL); *amyloglucosidase* = 100 µL, (300 units/mL); sodium acetate buffer = 40 mL)

The process efficiency was evaluated based on the ethanol yields obtained under sonication (65.5 %) and incubation (67.9 %). The lower process efficiency could be attributed to the formation of glycerol as the secondary metabolite during the fermentation (**Fig. S2**). Use of improved quality of yeast strain may lead to the selective production of ethanol from the fermentable sugars.

Based on the presented results, further research on the concept of ultrasound assisted SSF for ethanol production should focus on scale-up and process intensification: faster and more effective energy use. Moreover, the presented process could be further examined not only as a batch process, but also in a continuous mode for quick industrial adaptation. Design of special functional equipment, capital investment, and electricity consumption for the generation of ultrasonic waves are the main issues towards the industrial utility of the process. A thorough energetic and economic analysis facilitates scaling up of the process. Attempts are being made for the scaling up of several ultrasound based extraction processes.[52, 53]

Conclusion

A sonication based simultaneous saccharification and fermentation process for the production of bioethanol from Ulva rigida was developed. A maximum of 6.2 wt.% of ethanol (on dry wt. basis) was obtained under sonication in 3 h vs only 4.9 wt.% ethanol under incubation even after 48 h. The salient features of the process include (i) no requirement of chemical pretreatment of the biomass, (ii) only one stage of operation and (iii) exclusive production of glucose as the sole sugar as a result of enzymatic saccharification and (iv) faster production of glucose from *Ulva rigida* and simultaneous conversion of glucose to ethanol.

Acknowledgments

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