Using Novel Methods (Microwave and Sonochemistry) for the Conversion of Micro and Macroalgae to Biofuels

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Israel

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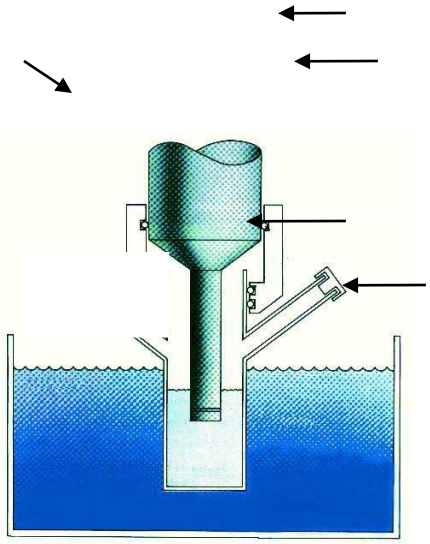
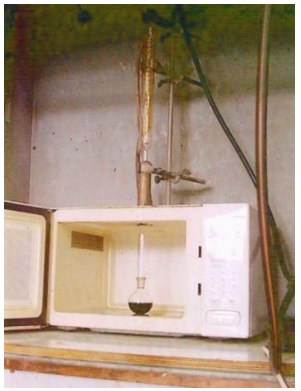
Novel methods for the fabrication of nanomaterials

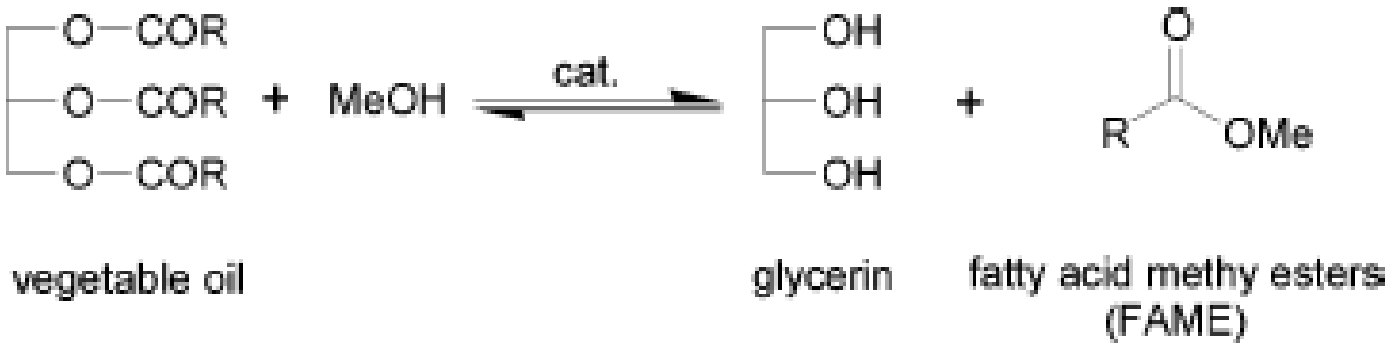
Gedaken's laboratory has fabricated a large variety of nanoparticles using 4 techniques

1) Sonochemistry, 2) Microwave radiation, 3) Sonoelctrochemistry, and 4) RAPET (Reactions under Autogenic Pressure at Elevated Temperatures)





Preparation of Biodiesel



- *Biodiesel is made from vegetable oils or animal fats by transesterification with methanol using an acid or base catalyst.*

- *The most commonly used catalysts in transesterification reaction are: NaOH , KOH , H_2SO_4.*

:Optimization of Bio-Diesel Production

Probing the New Combination of the Microwave .Irradiation of Cooked Oil and SrO as a Solid Catalyst

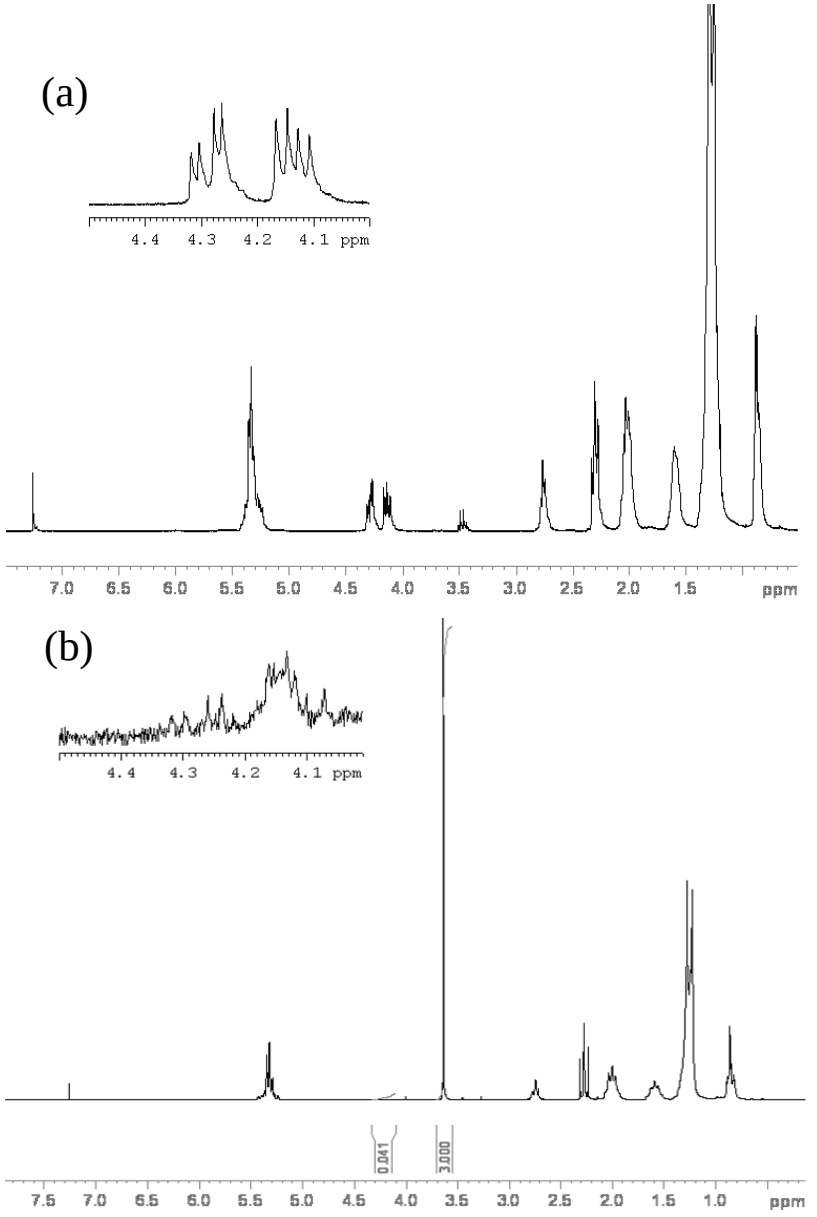
,Microwave Irradiation of a Reaction Mixture of SrO, Methanol and Oil leads to Creation of 3 Phases

Optimum Result:

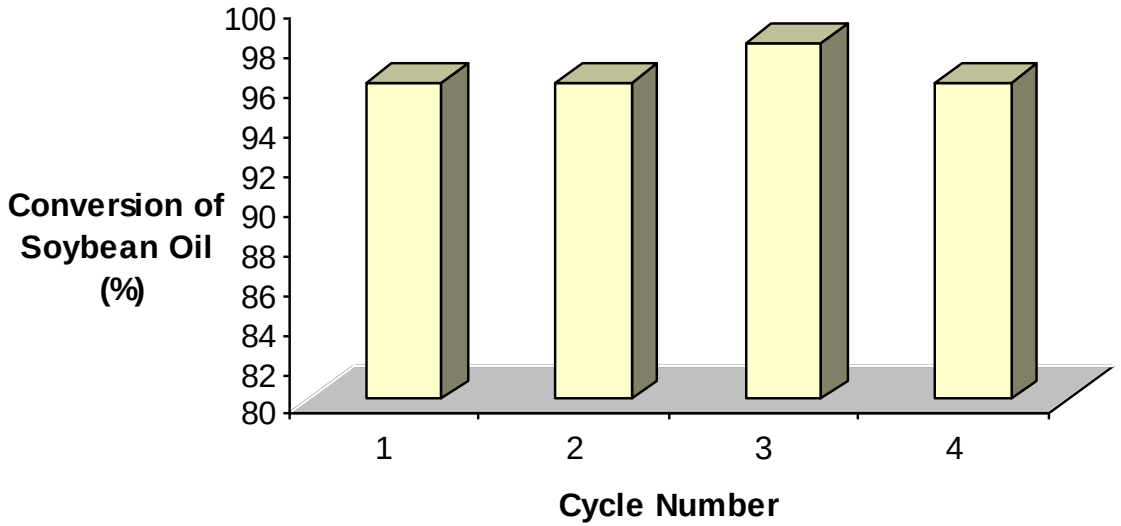
- *99% conversion in 40 seconds unstirred microwave irradiation with SrO, or in 10 seconds while stirring.*
  Amounts: Oil / Methanol / SrO
  15 gr / 4.05 ml / 0.276 gr

- *Using KOH under the same conditions leads to 83% conversion!*

- *(a) ^{1}HNMR spectrum of wastes of cooked oil. (b) ^{1}HNMR spectrum of bio-diesel production from wastes of cooked oil obtained after 40 sec unstirred, or 10 seconds for the stirred reaction using SrO as a catalyst.*



Repeated Activity of the Catalyst



The result confirm the stability and the sustained activity of SrO, which is of great importance for industrial application

- In the next stage we have investigated the bio-diesel production directly from the microalgae biomass of *Nannochloropsis* by microwave and ultrasound radiation

How were the microalgae prepared?

- **The microalgae was provided to us by Seambiotic.** This company grows the algae on seawater. The cultures were supplied with N & P and C inorganic nutrients, where the C was supplied by coal burning scrubbed flue gas containing 13% CO2. A culture of the marine Eustigmatophyte *Nannochloropsis sp*. was inoculated in outdoor raceway ponds at a concentration of less than 0.25 g/L, grown for 7 days to a concentration of 0.5 g/L. The culture was harvested using a continuous centrifuge (GEA Westfalia) and the paste was dried with a spray dryer (Anhydro) to obtain a dry powder.

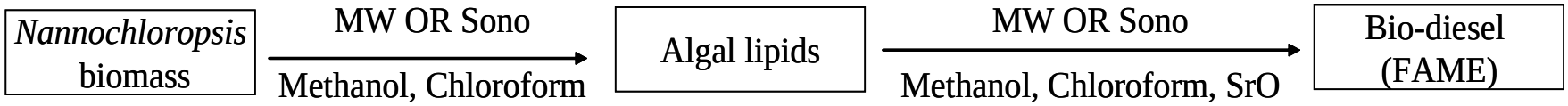
Seambiotic microalgae ponds

Seambiotic microalgae cultivation process

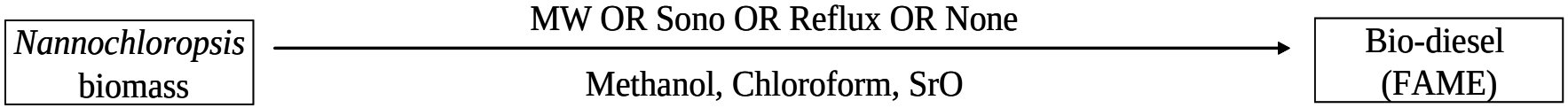
Culture Innoculation at concentration of 0.25 g/L (Raceway Pond) → Culture concentration increase to 0.5 g/L → Harvest Paste at concentration of 150-200 g/L (Continuous centrifuge) → Drying Dry algae powder (Spray drier)

Bio-diesel production from the microalgae biomass of Nannochloropsis

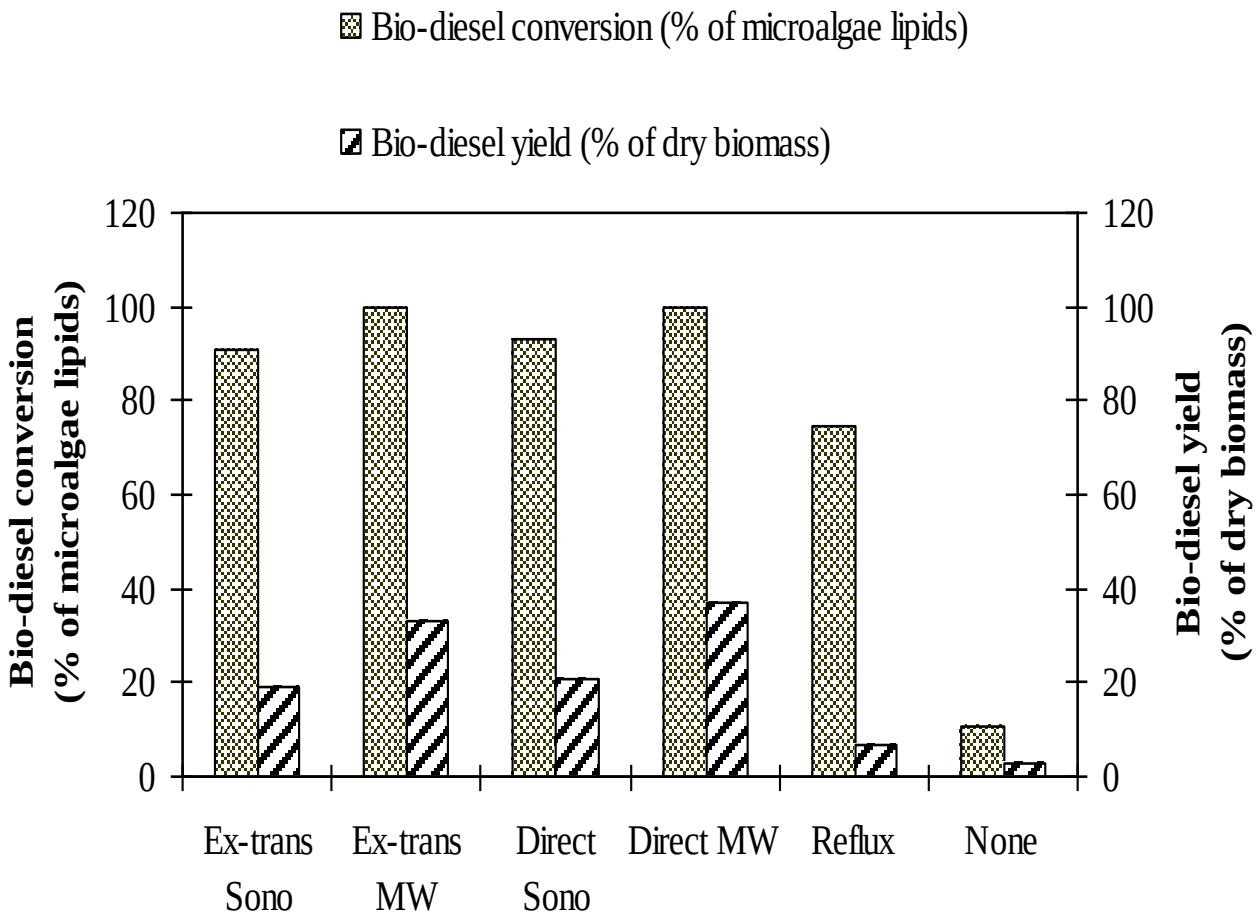
(a) A two-step reaction, namely, extraction and transesterification:



(b) A one-step direct transesterification



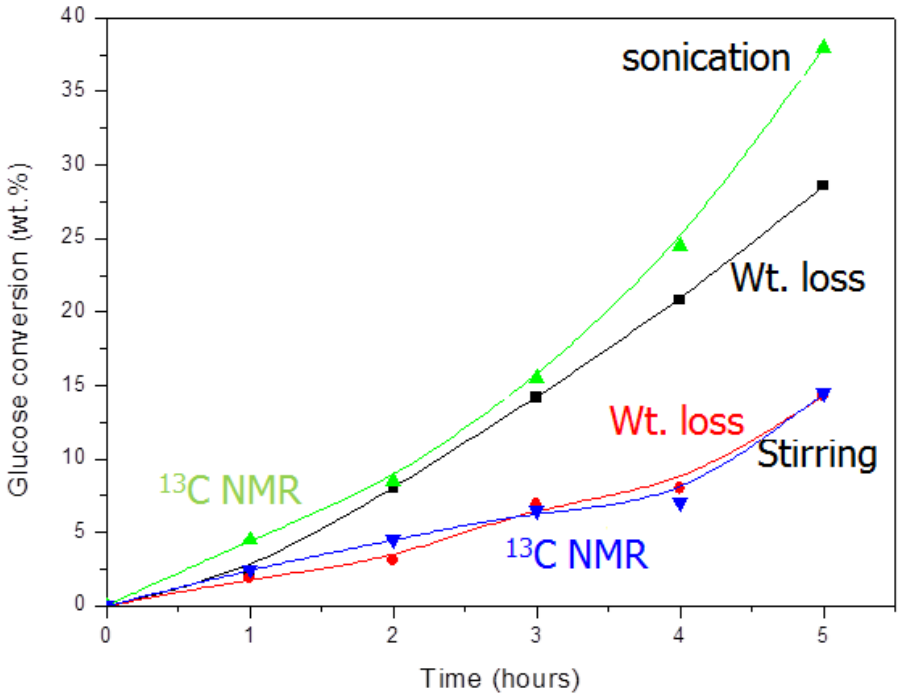
Direct transesterification reaction using microwave resulted the highest bio-diesel yield of **37.1%** with a conversion (**99.9%** conversion) of the triglyceride to bio-diesel.



Bio-diesel yield of the *Nannochloropsis* microalgae and bio-diesel conversion of the microalgae lipids using various techniques. Five techniques used in bio-diesel production: (Ex-trans Sono) extraction and transesterification reaction steps occurred separately using sonication, (Ex-trans MW) extraction and transesterification reaction steps occurred separately using microwave, (Direct Sono) direct transesterification without the initial extraction step using sonication, (Direct MW) direct transesterification without the initial extraction step using microwave, (Reflux) direct transesterification without the initial extraction step using regular reflux technique, and (None) extraction and transesterification reaction occurred without heating and stirring.

How to make the fermentation faster?

fermentation rate of glucose is accelerated using bath sonicator



Kinetics from ^{13}C NMR and from wt. loss

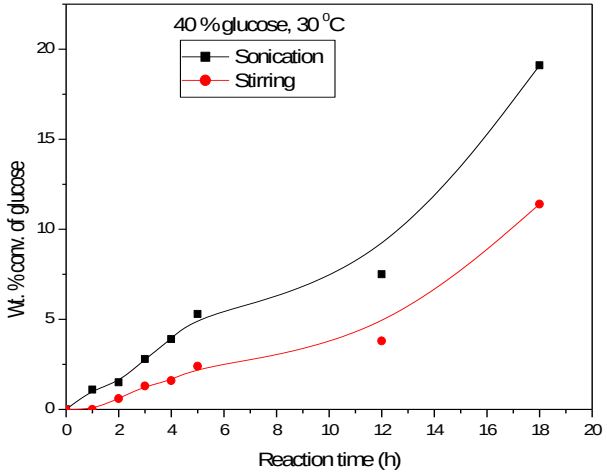
- Glucose conc. = 20 %
  Temp. = 30 °C

- sonication: 38 % conversion in 5 h
  Stirring: only 14.5 % conversion in 5 h

- $k_{sonication}$ = 15.35 x 10^{-6} sec^{-1}
  $k_{stirring}$ = 6.67 x 10^{-6} sec^{-1}

Gedanken et al., Energy and Fuels, 26, 2012, 2352

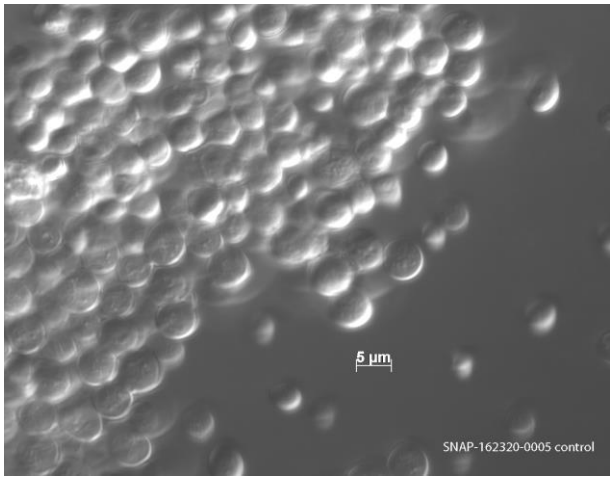
Acceleration of fermentation even at 40 % glucose concentration

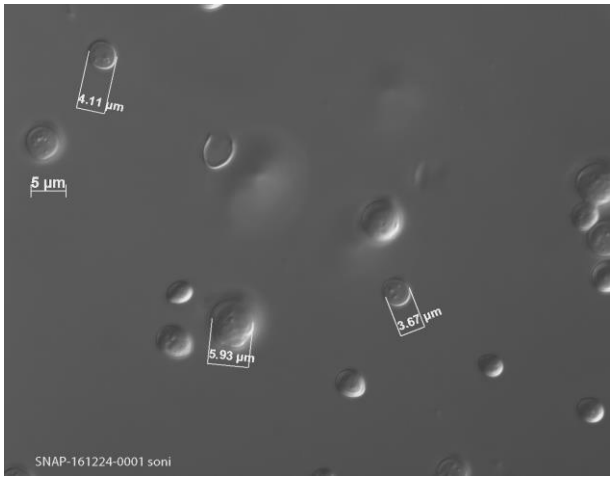


Sonication: 19 wt. % conversion in 18 h
Stirring: 11 wt. % conversion in 18 h

Glucose conversion with sonication Vs stirring

Aggregates of yeast cells Stirring





Dispersed yeast cells Sonication

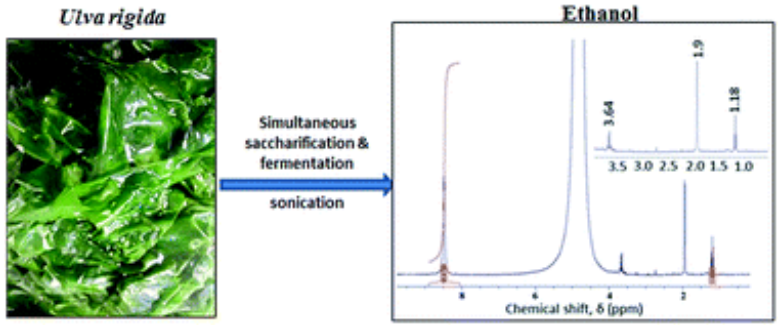
Sonication facilitated dispersion of yeast aggregates
The yeast is reusable even after sonication

What are the other avenues for improving bioethanol production process?

Single step conversion of a macroalgae to ethanol using sonication

Algea – *Ulva rigida*
Process – Simultaneous saccharification and fermentation (SSF)

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



Ulva rigida to bioethanol

Cellulose & Starch constitute the major fraction (31.4 wt.%) of carbohydrates that could yield glucose

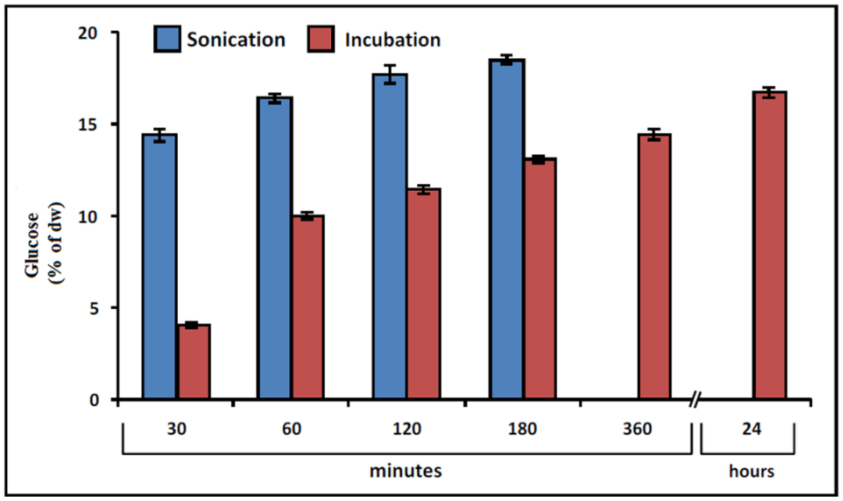
Glucose is the most easily fermentable sugar for ethanol production

Objective - selectively and fast production of glucose from the cellulose and starch components of algae and subsequently convert the same to ethanol under sonication

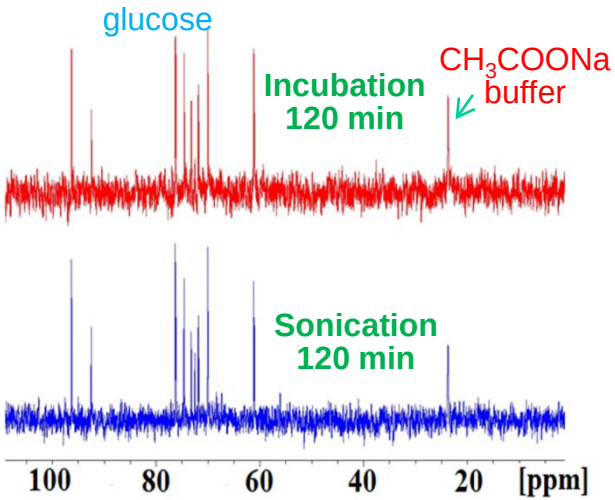
Gedanken et al., RSC Advances, 5, 2015, 16223

Enzymatic saccharification of *Ulva rigida*

Before carrying the SSF process, the saccharification process is evaluated in isolation



Sonication Vs incubation at 37 °C



^{13}C NMR spectra of hydrolyzate

1.68 g of dried *U. rigida* 40 mL of distilled water
40 mL of 200 µM sodium acetate (buffer)
(2.1 % w/v)
100 µL amyloglucosidase 300units/mL,
40 µL α-amylase 250units/mL,
0.1 g cellulase, 0.3 units/mg
All the contents were taken in a 100 mL glass media bottles with cap

Exclusive production of glucose
No other sugars produced upon hydrolysis
Use of cellulase and amylase selectively hydrolyzed cellulose and starch fractions respectively to glucose

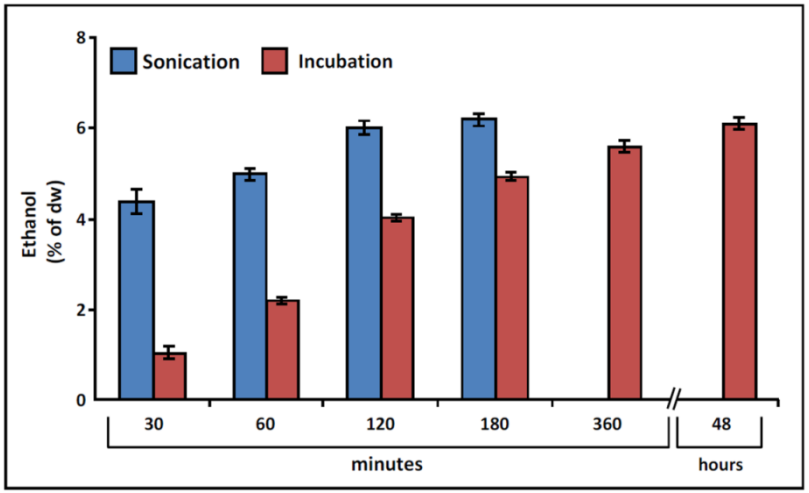
3.6 times higher yield of glucose is obtainable employing sonication during the hydrolysis stage relative to incubation

Enhancement in the release of glucose from the algae upon sonication is attributed to mechanical and thermal effects

Ultrasound improved the hydrolysis process by the reduction of the structural rigidity of the cellulose and starch components in the biomass

Ultrasound-assisted process reduced the hydrolysis reaction time by improving mixing and phase transfer, and by enhancing the diffusion of enzymes across cell membranes (algae), so that enzymes can easily reach the bulk of the substrate

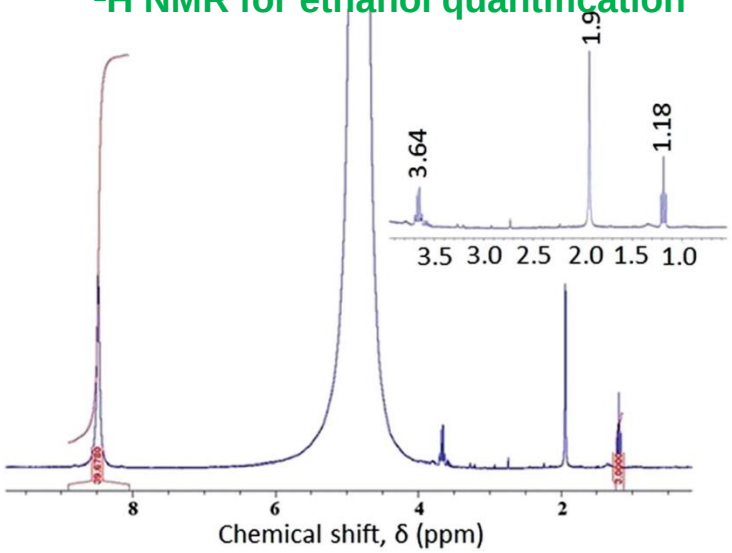
Sonication based SSF process for bioethanol production



SSF process - Sonication Vs Incubation at 37 °C

1.68 g of dried *U. rigida* in 40 mL of distilled water
40 mL of 200 µM sodium acetate (buffer)
(2.1 % w/v)
100 µL amyloglucosidase 300 units/mL
40 µL α-amylase 250 units/mL,
0.1 g cellulase (0.3 units/mg)
0.5 g of Baker's yeast
100 mL glass media bottles with cap were used

^{1}H NMR for ethanol quantification



3H (t, 1.18 ppm) and 2H (q, 3.64 ppm) - ethanol
3H, s, 1.9 ppm - sodium acetate (buffer)
1H, s, 8.5 ppm - internal standard (HCOONa)
Relative integral values of the internal standard and the ethanol peaks – measure of ethanol amount

Analogous to the saccharification process of algae with enzymes, the SSF process was also faster under sonication relative to incubation

In 30 min. the yield of ethanol under sonication is significantly high (4.3±0.3 wt.% Vs 1.0±0.1 wt.% under incubation)

Even after incubation for 48 h, the ethanol yield under incubation is only 6.1±0.1 wt.% which could be achieved in a short duration of 120 min. with the use of mild sonication

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

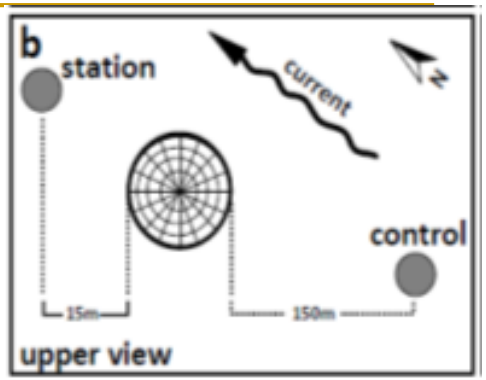
Cultivation of high carbohydrate *ulva rigida* for enhanced bioethanol production

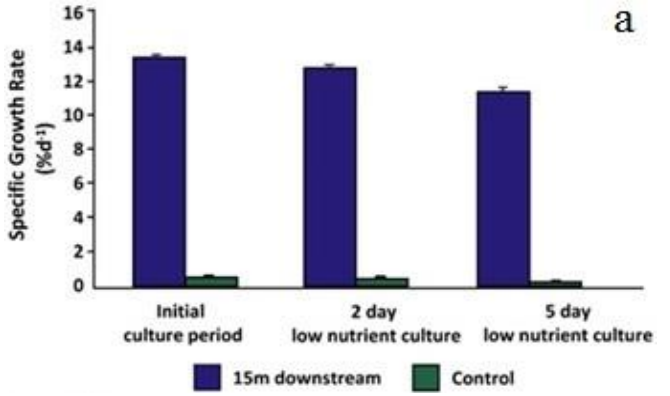
Integrated multi-tropic aqua culture

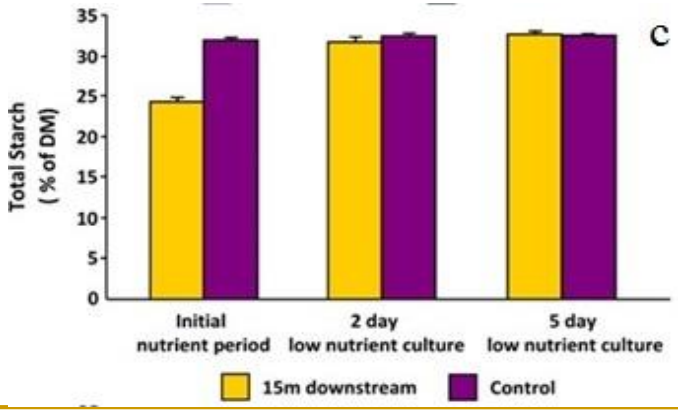
High carbohydrate *Ulva rigida*

SSF

Ethanol yield 16 wt. %







27 times higher specific growth rates (SGR) achieved under nutrient rich conditions

Availability of inorganic nutrients - most important factor controlling the growth and productivity of seaweeds

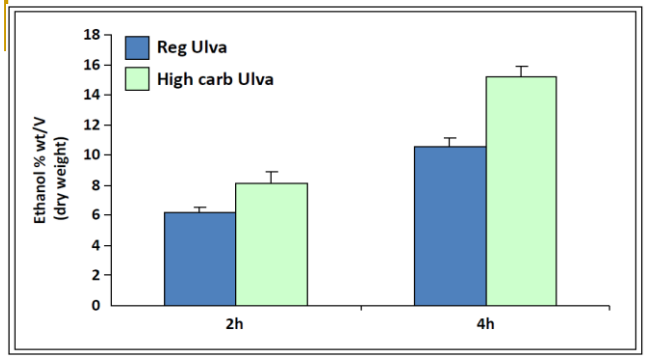
Starch content was higher (31.5 % of DM) at the control station than downstream to the cages (24% of DM)

High nutrient concentrations were found to alter the proximate composition in seaweeds and caused a shift to lower levels of carbohydrates such as starch

After two days of culture manipulation at the low nutrient site, the starch contents bounced up and levelled with the values of the control site

Gedanken et al., RSC Adv 5, 2015, 59251

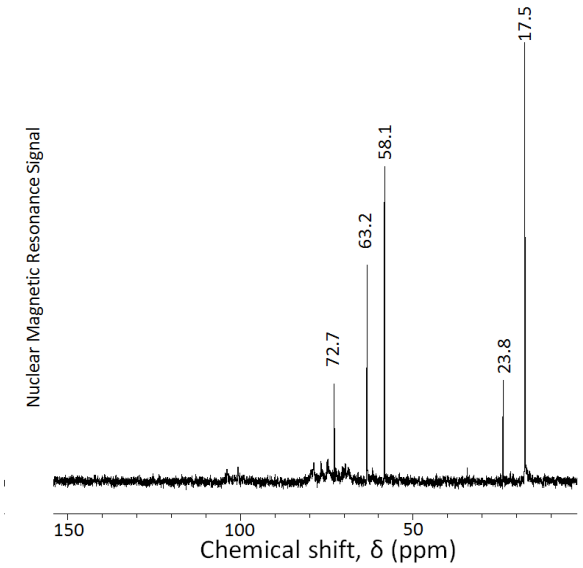
Bioethanol yield – regular *ulva* Vs high carbohydrate *ulva*



Effect of tailoring the carbohydrate content of *ulva rigida* on the ethanol yield (15 wt.% solid consistency, middle enzyme loading)

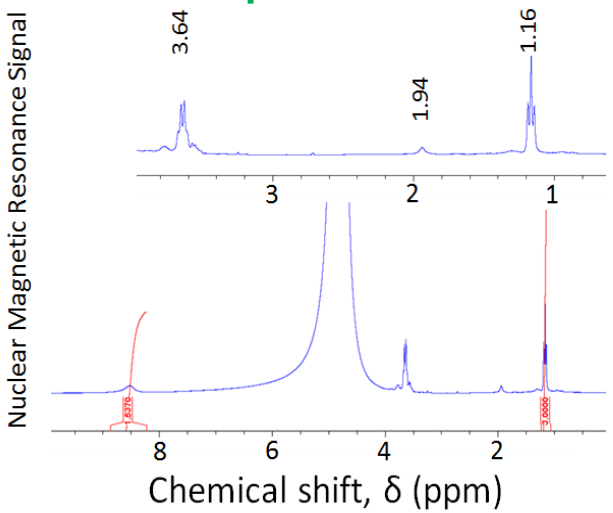
Enhanced ethanol yield with high carbohydrate algae cultured algae (16 wt. %) Vs as received *Ulva rigida* (8 wt. %)

Qualitative and quantitative analysis of fermentation products



^{13}C NMR spectrum of aliquot of sample collected from the fermentation (SSF) broth under optimal reaction conditions

No fermentable sugars are detected (in the region of 60-100 ppm)
SSF process is effective for the conversion of glucose



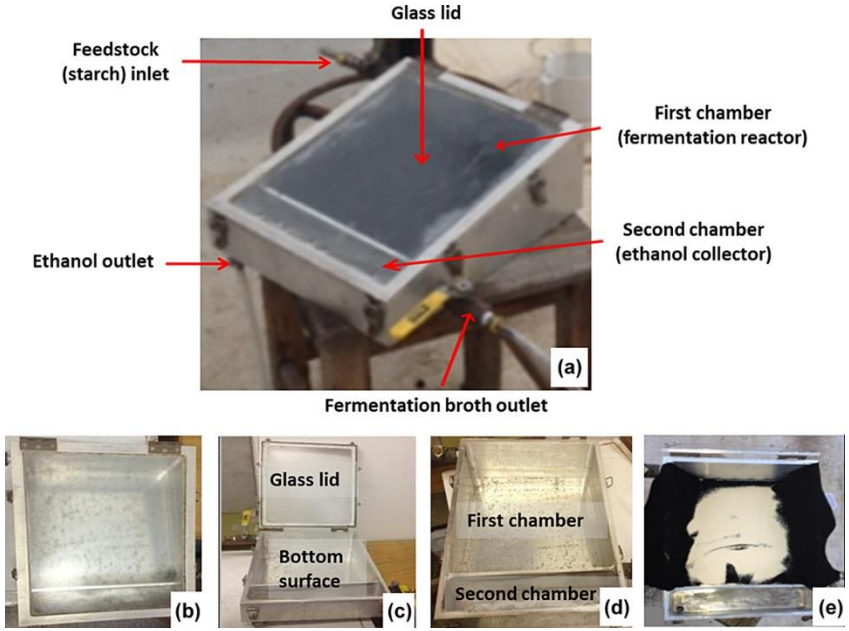
^{1}H NMR spectrum of aliquot of sample collected from the fermentation (SSF) broth under optimal reaction conditions

Observed ethanol yield - 16 wt.%
Process efficiency – 89 %
Expected ethanol yield: 17.8 wt.%
31.5 wt.% starch yield 35 wt.% glucose
35 wt.% glucose should yield 17.8 wt.%

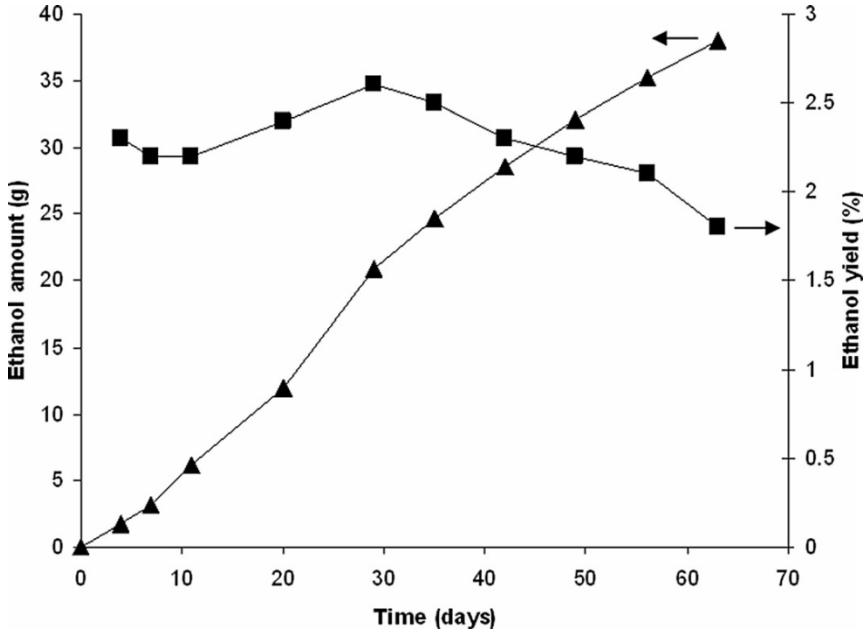
Process efficiency improved from 65 % to 89 % using high carbohydrate *ulva*

Solar-Energy Driven Simultaneous Saccharification and Fermentation of Starch to Bioethanol for Fuel-Cell Applications

SSF of starch:
starch solution (1.6 L, 5 wt. %); Baker's yeast (75 g);
amylo glucosidase (2.5 mL), α-amylase (2.5 mL)



Solar reactor for conversion of starch to bioethanol

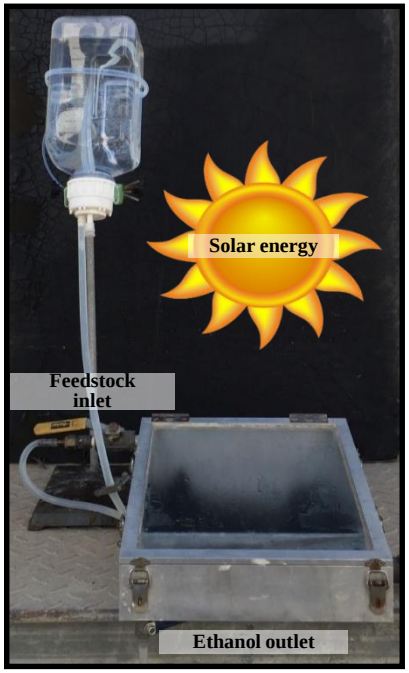


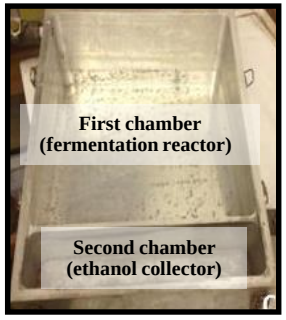
Solar energy driven bioethanol production from starch as a function of time

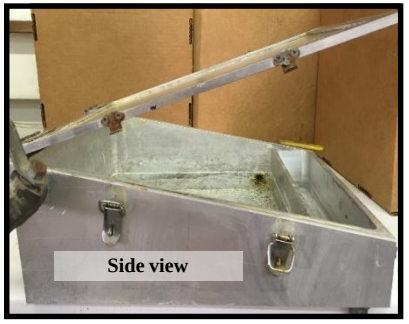
2.5 wt. % ethanol collected daily (ca. 25 mLday^{-1}
38 g ethanol was collected over 63 days
Ethanol yield 84 wt. % of the theoretical yield


Top glass lid
Bottom surface

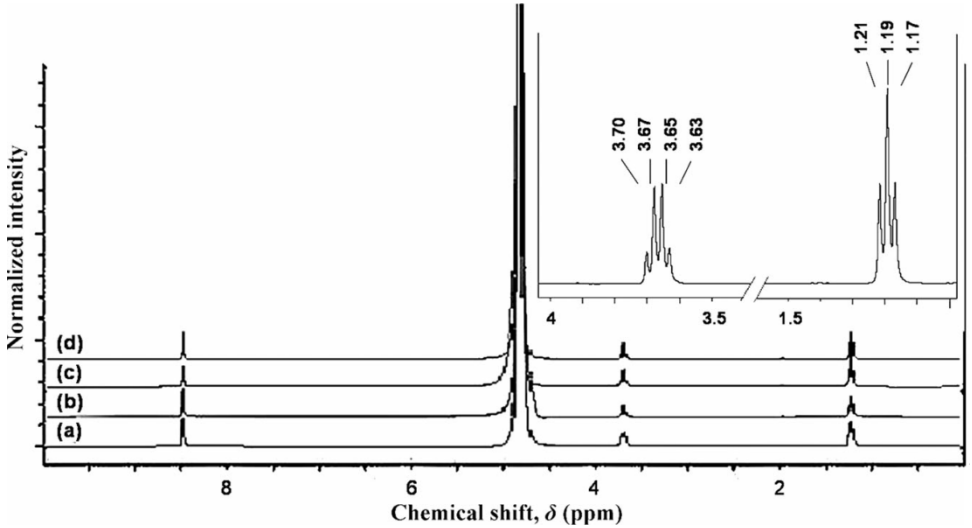

Baker's yeast on
activated carbon cloth


Solar energy
Feedstock
inlet
Ethanol outlet

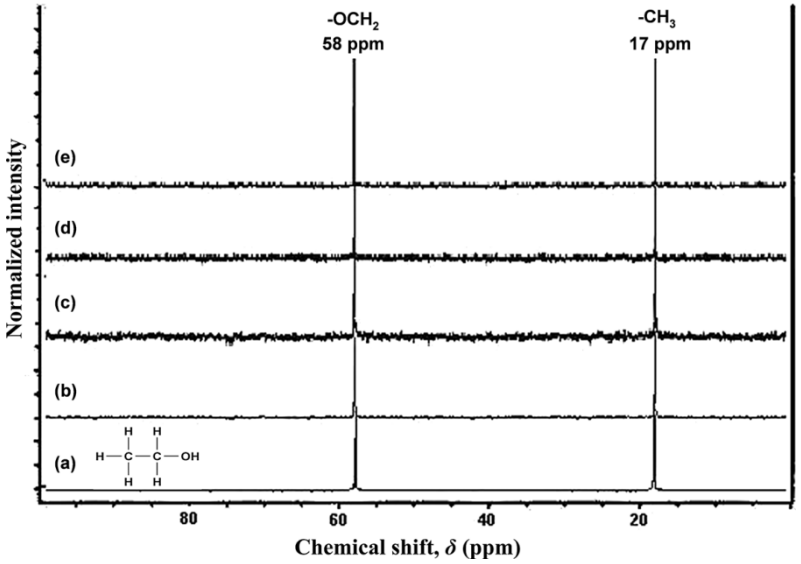

First chamber
(fermentation reactor)
Second chamber
(ethanol collector)


Side view

Analysis of products of SSF of starch



^{1}H NMR spectra of the starch fermentation product on the (a) 7th, (b) 14th, (c) 21st, and (d) 28th day
Inset shows the ethanol peaks—
a 3H (t) at 1.2 ppm and a 2H (q) at 3.7 ppm
Singlet peak at 8.4 ppm is the internal standard, HCOONa, and the peak at 4.8 ppm is the solvent

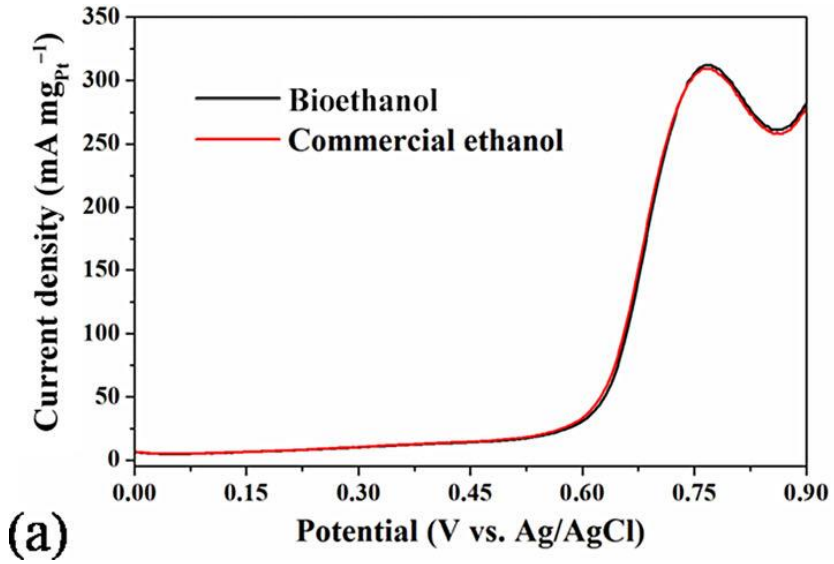


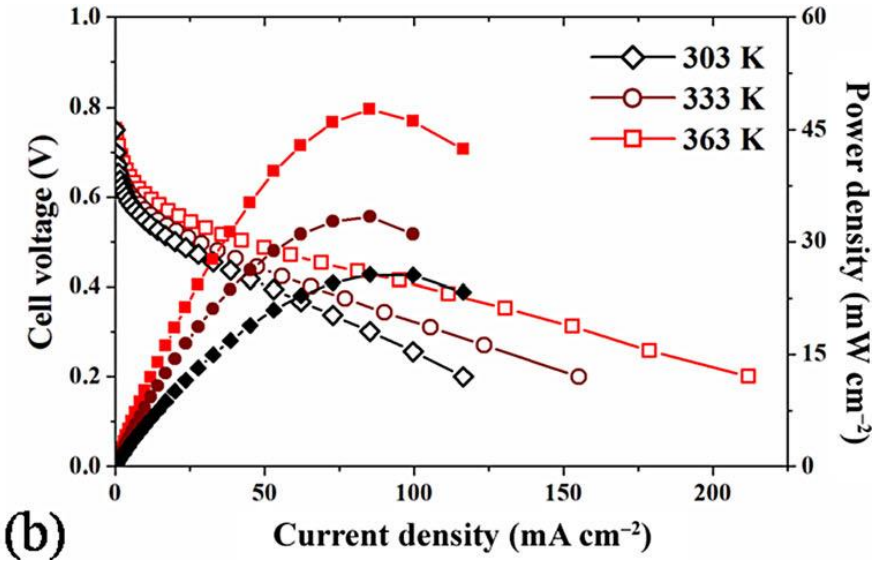
^{13}C NMR spectra of (a) authentic ethanol and the starch fermentation product on the (b) 7th, (c) 14th, (d) 21st, and (e) 28th day

No other reaction by-products (glycerol or acetic acid) were observed in the analytes indicating the purity

Intense signals seen in all four samples at 17 and 58 ppm are characteristic of ethanol
The reaction product is devoid of the reactant (starch), reaction intermediate (glucose), and the usual secondary metabolites of fermentation (glycerol and acetic acid)

Application of bioethanol for electricity generation





Linear sweep voltammograms of the Pt/C catalyst for the ethanol oxidation reaction in 0.5 M H_2SO_4 + 1.3 M C_2H_5OH (scan rate 25 mVs^{-1})

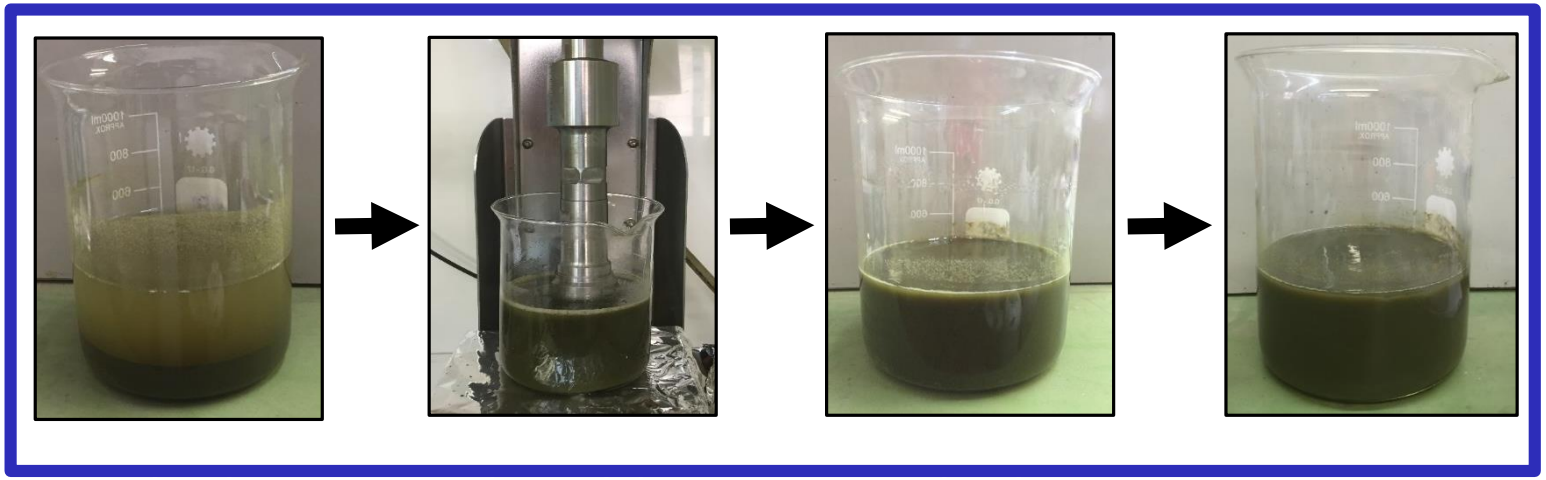
(b) polarization and power density curves at 2 $mgcm^2$ catalyst loading for Pt/C (40 wt%, E-TEK) on both the anode and cathode at different temperatures.
Anode feed: 1.3 M bioethanol at 1 $mLmin^{-1}$
Cathode feed: pure humidified oxygen at 200 $mLmin^{-1}$

A well-defined peak at +0.75 V correspond to ethanol oxidation
No additional peaks corresponding to impurities were observed The shape of
Voltammograms recorded using as-produced bioethanol and commercial ethanol were similar and the peak currents were comparable (ca. 310 mAmgPt
These aspects clearly indicate the high level of purity of the as-produced bioethanol from starch.

The open-circuit potential (OCP) of the cell was found to be approximately 0.75 V (65% thermodynamic efficiency)
The effect of temperature on the OCP was small.
Increase in cell performance with temperature was observed which is attributed to the enhanced kinetics of ethanol oxidation at the anode and oxygen reduction at the cathode

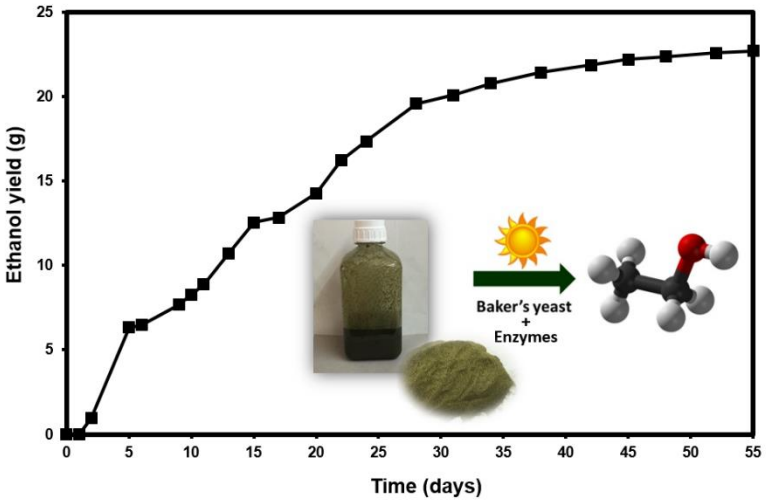
Gedanken et al., ChemSusChem, DOI: 10.1002/cssc.201500469

Preparation of the aqueous suspension of *Ulva rigida*



SSF of marine macroalgae Ulva rigida to bioethanol in a batch process

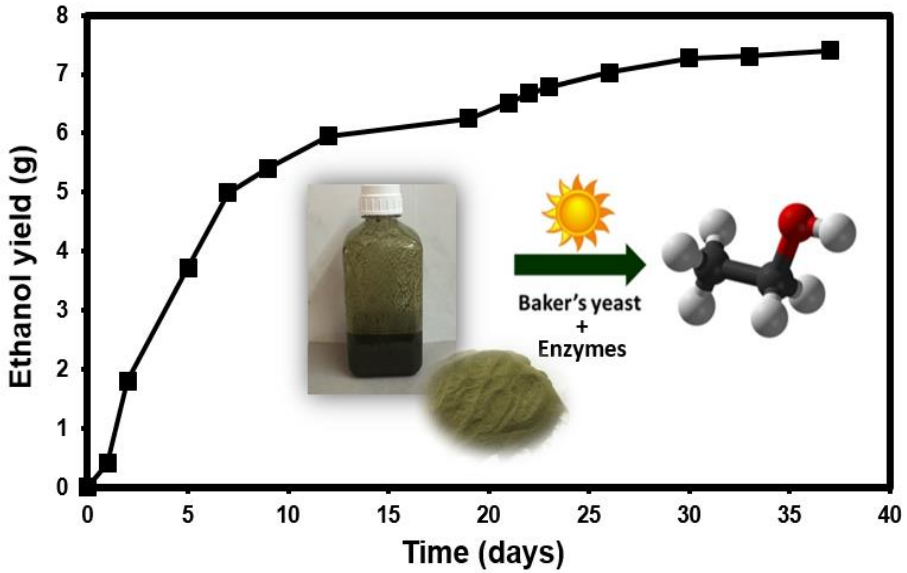
Initially, aqueous suspension of *Ulva rigida* (10 wt%) was fed to the solar reactor to test the solar-aided batch fermentation of marine macroalgae. In this solar-aided SSF process, a mixture of α-amylase, amyloglucosidase, endo-cellulase, exo-cellulase, and β-glucosidase enzymes was added to the feedstock solution which was fed to the reactor's first chamber over *S. cerevisiae* covered with activated carbon cloth.



The SSF process of 10 wt% *Ulva rigida* was monitored for 55 days (with the same enzymes and the yeast) at 28/20 °C average day/night temperature. Based on the fermentable sugar content of the biomass, 65% of the theoretical ethanol yield was collected at the end of the SSF process of 10 wt% *Ulva rigida* (0.41 g ethanol/day, 5.4 g ethanol/day/m^2).

Following the preliminary results with batch fermentation, the system was further developed into a solar-energy-driven continuous-flow fermentation process in order to increase the ethanol yield and to make the process industrially adoptable.

Time on stream studies of solar-energy-driven bioethanol production from continuous-flow SSF of *Ulva rigida*



The SSF process of 5 wt% *Ulva rigida* was monitored for 37 days in the solar reactor (with the same enzymes and the yeast) at 31/24 °C average day/night temperature. 84% of the theoretical ethanol yield was observed (7.4 g ethanol in 37 days, 2.64 g ethanol/day/m^2) based on the fermentable sugar content of the biomass

Representative ^{1}H NMR spectrum of the SSF product of *Ulva rigida*

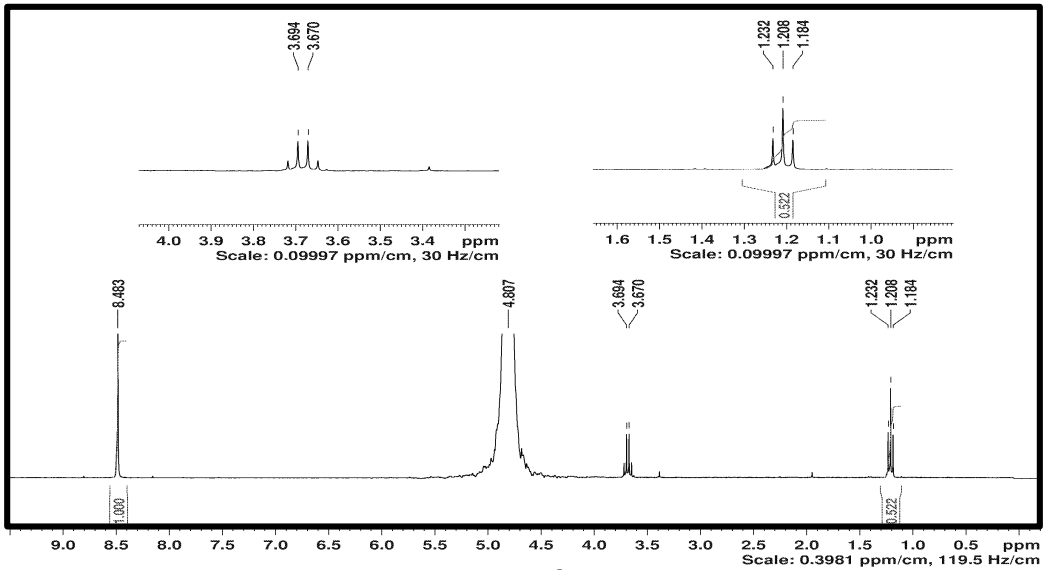
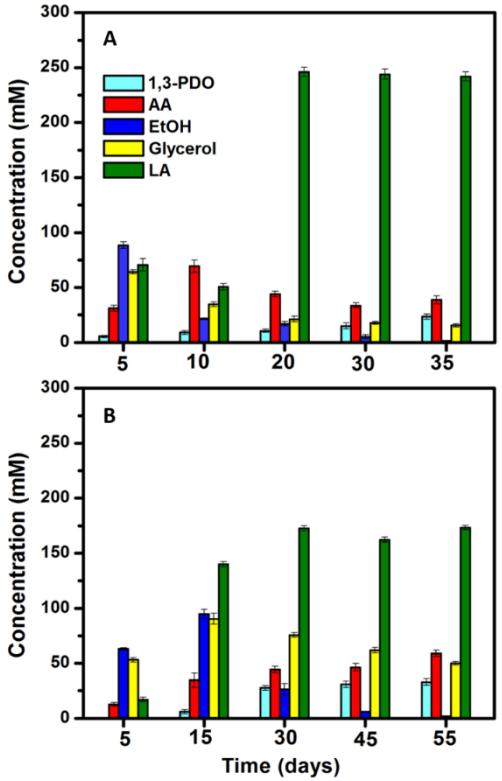


Fig. ^{1}H NMR spectrum of the solar-energy-driven continuous-flow SSF product of 5 wt% *Ulva rigida* on the 5th day (Inset shows the ethanol peaks, a 3H (t) centered at 1.20 ppm and a 2H (q) centered at 3.68 ppm)

The singlet at 8.48 ppm originates from the internal standard HCOONa, and the peak at 4.80 ppm corresponds to water. No reaction by-products were observed in the analytes, indicating the purity of the process (only aqueous ethanol).

Solar-energy-driven continuous-flow SSF of *Ulva rigida* – Secondary metabolites in the fermentation broth



In addition to ethanol, other metabolites such as 1,3-propanediol (1,3-PDO), glycerol, acetic acid, and lactic acid were also observed in the fermentation broth. These secondary metabolites could not be evaporated due to their low vapor pressures compared to bioethanol; therefore they remained in the fermentation broth and yet did not affect the catalytic activity of the microorganisms.

At the end of the continuous-flow SSF process of 5 wt% *Ulva rigida* (35^{th} day), 25 mM 1,3-PDO, 16 mM glycerol, 40 mM acetic acid, and 240 mM lactic acid was observed in the fermentation broth in addition to 1.5 mM ethanol.

At the end of the batch SSF process of 10 wt% *Ulva rigida* (55^{th} day), 33 mM 1,3-PDO, 50 mM glycerol, 59 mM acetic acid, 174 mM lactic acid, and 1.8 mM ethanol was observed in the fermentation broth.

The concentration distribution of the metabolites observed in the fermentation broth of *Ulva rigida* is quite different from the usual fermentations of glucose, starch, or cellulose where the major secondary metabolites are either glycerol or acetic acid. On the contrary, in the current study, the major secondary metabolite was observed to be lactic acid with smaller concentrations of acetic acid and glycerol.

Fig. Concentration of metabolites in the fermentation broth of solar-energy-driven bioethanol production from (A) 5 wt% (continuous flow) and (B) 10 wt% (batch) *Ulva rigida* suspension as a function of time

CONCLUSIONS

Energy-efficient sustainable batch and continuous-flow bioethanol production process was developed for the single step SSF of marine biomass (*Ulva rigida*) based on solar thermal energy

A novel ultrasound irradiation based method was developed for making a stable aqueous suspension of biomass that could be continuously flown to the solar reactor

High ethanol yield (82% of the theoretical yield) was achieved from the SSF of the marine algae *Ulva rigida* without electricity consumption

Ethanol was *in situ* separated from the fermentation broth by evaporation-condensation process (no external heating or additional energy input for separation)

No traces of reactant, catalyst, enzymes or by-products were observed in the product collected from the solar reactor (only aqueous ethanol)

The fermentation broth of solar-energy-driven SSF of *Ulva rigida* contained secondary metabolites such as acetic acid, glycerol, ethanol, 1,3-PDO, and lactic acid

Lactic acid was the major secondary metabolite of the SSF of marine algae *Ulva rigida*

No polluting effluent produced in the process

This economically-feasible and environment-friendly process produced the value-added chemical 1,3-PDO by *in situ* bioconversion of glycerol

Reusability of the biocatalyst (*S. cerevisiae*) and the enzymes (amylases and cellulases) was confirmed at least for a month without loss in the activity

Ulva rigida was used as a representative feedstock that contains both starch and cellulose

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