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Comparative evaluation of chemical and enzymatic saccharification of mixotrophically grown de-oiled microalgal biomass for reducing sugar production

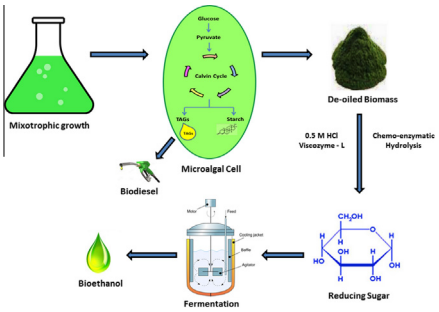
Imran Pancha [a], Kaumeel Chokshi [a,b], Rahulkumar Maurya [a,b], Sourish Bhattacharya [b,c], Pooja Bachani [a,b], Sandhya Mishra [a,b,*]

[a] Salt & Marine Chemicals, CSIR-Central Salt and Marine Chemicals Research Institute, Bhavnagar 364002, India
[b] Academy of Scientific & Innovative Research (AcSIR), CSIR-Central Salt and Marine Chemicals Research Institute, Bhavnagar 364002, India
[c] Process Design and Engineering Division, CSIR-Central Salt and Marine Chemicals Research Institute, Bhavnagar 364002, India

HIGHLIGHTS

- De-oiled biomass of mixotrophically grown microalgae is rich in carbohydrate.
- Chemical hydrolysis yield 37% saccharification based on residual biomass.
- Enzymatic hydrolysis using Viscozyme L results in 43.44% saccharification yield.
- Fed-batch enzymatic hydrolysis resulted in highest reducing sugar yield.
- Microalgal hydrolysate is efficiently utilized by yeast for ethanol fermentation.

GRAPHICAL ABSTRACT



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ABSTRACT

For the commercialization of microalgal based biofuels, utilization of de-oiled carbohydrate rich biomass is important. In the present study, chemo-enzymatic hydrolysis of mixotrophically grown *Scenedesmus* sp. CCNM 1077 de-oiled biomass is evaluated. Among the chemical hydrolysis, use of 0.5 M HCl for 45 min at 121 °C resulted in highest saccharification yield of 37.87% w/w of de-oiled biomass. However, enzymatic hydrolysis using Viscozyme L at loading rate of 20 FBGU/g of de-oiled biomass, pH 5.5 and temperature 45 °C for 72 h resulted in saccharification yield of 43.44% w/w of de-oiled biomass. Further, 78% ethanol production efficiency was achieved with enzymatically hydrolyzed de-oiled biomass using yeast *Saccharomyces cerevisiae* ATCC 6793. These findings of the present study show application of mixotrophically grown de-oiled biomass of *Scenedesmus* sp. CCNM 1077 as promising feedstock for bioethanol production.

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1. Introduction

High greenhouse gas (GHG) emissions as well as the rapid depletion of fossil fuel reserve have resulted in search of new renewable energy resource like biodiesel and bioethanol

* Corresponding author at: Salt & Marine Chemicals, CSIR-Central Salt and Marine Chemicals Research Institute, Bhavnagar 364002, India. Tel.: +91 278 256 5801/256 3805x6160; fax: +91 278 256 6970/256 7562.
E-mail address: smishra@csmcri.org (S. Mishra).

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(Chisti, 2007). Apart from terrestrial sugar and oil based crop plants, microalgae have gained much interest due to their higher lipid and carbohydrate contents, short harvesting time, high photosynthetic ability, etc. Microalgae do not compete with agriculture due to their ability to obtain nutrients from wastewater and seawater, which makes them ideal feedstock for biofuel generation (Pancha et al., 2015a).

Despite huge potential of microalgal based biofuels, its commercialization is still under development due to lower biomass and lipid productivity as well as high downstream processing cost (Wijffels and Barbosa, 2010). Many cultural conditions like nutritional starvation and salinity stress significantly enhance the biofuel production potential of various microalgae (Pancha et al., 2014, 2015b). Among all the cultivation methods, mixotrophic growth of microalgae utilizing organic substrate has been found to be the most promising approach due to its higher biomass, lipid and carbohydrate production with shorter cultivation period (Pancha et al., 2015c).

Large amount of biomass remains unutilized after biodiesel processing from microalgae. Utilization of this de-oiled microalgal biomass is important for the overall reduction in the cost of microalgal based biofuels. Utilization of such biomass for various applications has been studied by different researchers (Maurya et al., 2014; Lam et al., 2014). De-oiled microalgal biomass is mainly rich in carbohydrates, which can be utilized for the production of reducing sugars and subsequent bioethanol production. Microalgal carbohydrates have many advantages compared to terrestrial sugar and starch based crop plants like microalgae contain cellulose in their cell wall and starch in their cytoplasm, which can be easily converted into fermentable sugars; microalgae do not contain lignin as well as they have very low hemicelluloses content, which reduces requirement of harsh pre-treatments and overall bioethanol production cost (Markou et al., 2012; Chen et al., 2013). In order to obtain high amount of reducing sugars from microalgal biomass, various physical, chemical and biological pre-treatments are generally employed. Chemical pretreatments utilizing various types of acid and alkali have helped in the production of high amounts of reducing sugars from microalgae (Harun et al., 2011). Apart from the high sugar production, many inhibitory compounds like 5-hydroxymethylfurfural (HMF), formic acid, levulinic acid and phenols are also produced during chemical hydrolysis, which have a negative effects on the growth of fermentative organisms and results in lower production of desired end products (Miranda et al., 2012).

On the other hand, utilization of various biomass hydrolyzing enzymes is a green approach to produce high titer reducing sugars. Enzymatic hydrolysis have many advantages over chemical and physical pretreatments like it run at lower temperatures (40–50 °C) compared to high temperature required during liquefaction of biomass; enzymatic hydrolysis do not produce any non-sugar or inhibitory compounds during hydrolysis (Juturu and Wu, 2014). Apart from its advantages, enzymatic hydrolysis suffers from the high cost of enzymes as well as inactivation of enzymes in the presence of solvents and other physical parameters (Choi et al., 2010).

Most of the reports on chemo-enzymatic saccharification of microalgal biomass are available on photoautotrophically grown microalgae, which require more incubation time and yields low carbohydrate content. To best of our knowledge, no report is available on systematic study of chemo-enzymatic saccharification of mixotrophically grown de-oiled microalgal biomass. To bridge this gap, the present study was designed to optimize chemo-enzymatic saccharification of mixotrophically grown de-oiled biomass of *Scenedesmus* sp. CCNM 1077 for the reducing sugar production. Further, bioethanol production was also carried out using the algal hydrolysate.

2. Methods

2.1. Microalgae and cultural conditions

The microalgae used in this study was *Scenedesmus* sp. CCNM 1077. Microalgae was grown in BG-11 medium supplemented with 4 g/L glucose and incubated under 150 μmol m^{-2} s^{-1} light intensity for 12:12 h light dark cycle at 25 ± 2 °C for 8 days. The flasks were manually shaken thrice a day to avoid adherence of the cells to culture flask. After 8 days of growth, microalgal cells were centrifuged at 10,000 rpm for 10 min, supernatant was discarded and pellet was dried in oven at 80 °C. Lipid content from mixotrophically grown biomass was extracted as described in Pancha et al. (2014). In detail, approximately 200 mg of biomass was vortexed thoroughly for 30 s, ultrasonicated for 30 min at ambient temperature in 30 ml chloroform:methanol (1:2, v/v), centrifuged (5 min at 3000 g) thrice and measured gravimetrically (Bligh and Dyer, 1959).

2.2. Determination of cellular content of de-oiled Scenedesmus sp. CCNM 1077 biomass

The moisture and ash content of de-oiled biomass were determined according to ASTM D 3173 and ASTM D 3174, respectively, The elements C, H and N were analyzed through the CHNS analyzer (Perkin–Elmer Model 2 400, USA). For the determination of carbohydrate content in microalgae, 50 mg of de-oiled biomass was first hydrolyzed with 500 μl of 72% (w/v) H_2SO_4 for 1 h. Concentration of H_2SO_4 was reduced to 4% by the addition of distilled water and autoclaved at 121 °C for 1 h. After cooling down to room temperature, total volume was made up to 50 ml with distilled water (Van Wychen and Laurens, 2013). The resulting solution was centrifuged at 10,000 rpm for 10 min and the supernatant was used for sugar estimation by phenol sulfuric acid method (Dubois et al., 1956) using D-glucose as a standard.

2.3. Chemo-enzymatic saccharification of de-oiled microalgal biomass

For chemical hydrolysis, 2% (w/v) of the de-oiled microalgal biomass was mixed with different hydrolytic agents *viz.* HCl, H_2SO_4, HNO_3, H_3PO_4, NaOH and KOH at 1 M concentrations and autoclaved at 121 °C for 15 min.

After selection of best hydrolytic agent (i.e. HCl), its concentration was further optimized, i.e., 0.1, 0.3, 0.5, 0.7, 1 and 3 M using 2% (w/v) de-oiled microalgal biomass.

The chemical hydrolysis time was also optimized by autoclaving at 121 °C for different time, i.e., 15, 30, 45 and 60 min. Finally, biomass loading rate was also standardized for higher reducing sugar production by applying 2%, 4%, 6%, 8% and 10% w/v biomass.

For enzymatic hydrolysis, first, different biomass hydrolyzing enzymes, *viz.* amylase from *Bacillus licheniformis* (Sigma A4551), cellulase from *Aspergillus niger* (Sigma 22178) and Viscozyme-L (Novozymes Denmark) were screened. For that, initially 100 mg biomass was added to 5 ml of sodium citrate buffer (100 mM, pH 5.5) and pre incubated for 30 min (45 °C; 120 rpm on orbital shaker). After this pre incubation, hydrolysis was initiated by addition of respective enzymes at initial 10 U/g of de-oiled biomass loading rate. Samples were collected periodically and reducing sugar was measured spectrophotometrically using 3,5-dinitrosalisylic acid (DNS) method (Miller, 1959). After finding the best enzyme, effects of hydrolysis temperature (30–55 °C) as well as pH (4.5–6.5) on biomass saccharification was also determined. Finally, enzyme loading rate (5–50 FBGU/g biomass) and biomass loading rate (2–15% w/v) were also optimized.

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