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Utilization of microalgae feedstock for concomitant production of bioethanol and biodiesel

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ABSTRACT

The present study focuses on the biorefinery approach of integrated production of bioethanol and biodiesel from microalgae feedstock. Various pretreatment methods were used to determine the maximum recovery of sugars from *Scenedesmus* sp. The total sugar yield of 93% was obtained when the biomass was pretreated by acid hydrolysis. The hydrolysate produced 86% of ethanol (theoretical yield) after the fermentation using *Saccharomyces cerevisiae*. Enzyme catalyzed direct transesterification of the biomass was performed using dimethyl carbonate as a solvent and the maximum yield of 92% methyl ester, 1.86% glycerol carbonate and 4.93% glycerol dicarbonate was achieved. The integrated process of bioethanol and biodiesel production was optimally achieved when direct transesterification was done first followed by ethanol fermentation yielding 92 and 93% of methyl ester and ethanol, respectively.

1. Introduction

Biofuels from microalgae have been considered as the promising alternative fuel. However, the feasibility of the biofuel production is yet to be well established [1]. Microalgae derived lipids are efficiently used for the biodiesel production [2]. In addition to lipids, microalgae also reserve the carbohydrate in the form of starch and cellulose which can be utilized for the bioethanol production [3]. Researchers are still looking for the solutions to fix the hurdles in upstream and downstream processing of the biofuel production. *Scenedesmus* sp. is the well-known microalga which has the ability to accumulate both lipid and starch. The lipid and starch present in the *Scenedesmus* sp. are the valuable sources for the production of bioethanol and biodiesel [4].

In recent days, biorefinery concept gets much attention and it describes how the microalgae biomass can be utilized to produce multiple products [5,6]. To utilize the constituents of microalgal feedstock to the maximum, researchers are focusing on novel biorefinery approaches to commercialize the method [7,8]. Microalgae derived products are very much satisfactory for biorefinery concepts. The microalgae biofuels are considered as the potential liquid biofuels. Moreover, microalgae store a considerable amount of carbohydrates composed mainly of starch and cellulose polysaccharides with the absence of lignin, which make them suitable for bioethanol production [3,9]. Microalgae also store a significant amount of neutral lipids suitable for biodiesel production. However, some non-transesterifiable lipids like chlorophyll and carotenoids etc., are also present in the microalgae [10]. Among the different biofuels present, biodiesel comprises the major alternative for petro-based fuels [11]. Biofuels derived from microalgae can be an alternative source for the depleting fossil fuel. Apart from the application for biofuels, microalgae can also be used for CO_2 sequestration, wastewater treatment and production of various commercially important bioproducts [12].

Scenedesmus sp. contains lipids suitable for efficient production of biodiesel through transesterification [13]. Glycerol carbonate synthesized during transesterification can be used as surfactant and other glycerol carbonate related industrial applications [14]. *Saccharomyces cerevisiae* is widely used for the production of bioethanol due to its high tolerance toward ethanol during fermentation [15]. However, *Saccharomyces cerevisiae* converts only simple sugars to ethanol. Thus, starchy materials of microalgae need to be hydrolysed through acid, alkali or enzyme hydrolysis [16].

Many studies successfully reported about the independent methyl ester and bioethanol production. So far, there have been few reports on integrated process of methyl ester, glycerol dicarbonate and bioethanol production. In this study, the *Scenedesmus* sp. was used for the independent methyl ester and bioethanol production and compared with the integrated production of methyl ester and bioethanol. The different parameters involved in the sugar extraction and direct transesterification were tested to achieve a maximum sugar recovery and methyl ester yield respectively. The integrated approach was done by either sugar extraction first followed by direct transesterification or direct transesterification first followed by sugar extraction.

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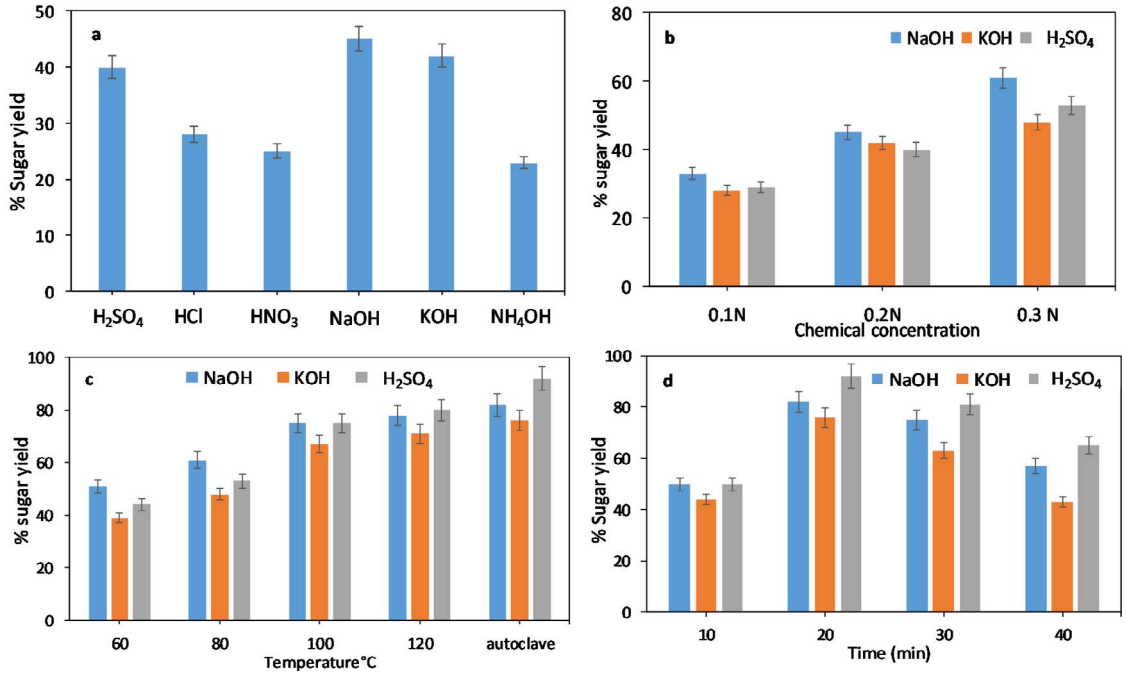

Fig. 1. Effect of different parameters on sugar extraction (a) Chemical pretreatments, conditions: chemical concentration at 0.2 N, 80 °C and 20 min (b) Chemical concentration, conditions: 80 °C and 20 min (c) Temperature, conditions: chemical concentration at 0.3 N and 20 min (d) Time, conditions: chemical concentration at 0.3 N and autoclave (121 °C at 15 psi). Data points were mean of triplicate values with the error bar showing standard deviations.

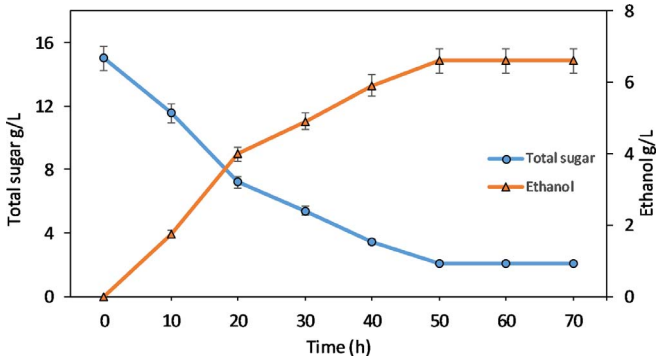

Fig. 2. Effect of fermentation time on ethanol production and sugar depletion. (conditions: hydrolysate in YPD medium, 5% (v/v) of inoculum, 30 °C, 180 rpm for 72 h). Data points were mean of triplicate values with the error bar showing standard deviations.

2. Materials and methods

2.1. Organism and culture conditions

Scenedesmus sp. obtained from the previous study [4] was maintained and grown in BG11 medium under continuous illumination of 50 µmol photons/m^2/s with shaking at 100 rpm at 27 ± 1 °C. To assure the purity of the culture, regular microscopic analysis was carried out. The organism was isolated from the stone quarry pond water and its accession number was KR025877 (GenBank). The 12 days grown cell suspension was treated with ultrasound (Bandelin, Heinrichstrasse, Berlin) for 5 min using ultrasonic probe (24 kHz). The treated biomass was then dried in freeze dryer. The total lipid, carbohydrate and protein contents were determined as 40, 22.2 and 19% (g/g of biomass) respectively. The microscopic (Seek, light microscope, Melbourne, Australia) image of *Scenedesmus* sp. is shown in Supplementary file (Fig. S.1).

2.2. Pretreatment

The ultrasound pretreated dried biomass was used in acid hydrolysis for the sugar extraction. 1 g of pretreated biomass was taken in a 125 mL flask with 20 mL liquid. The amount of sugars extracted by different concentrations of acid (H_2SO_4, HCl and HNO_3) and base (NaOH, KOH and NH_4OH) ranging from 0.1 to 0.3 N was analyzed. The effect of temperature was determined in the range of 60–120 °C and 120 °C in an autoclave (15 psi), whereas the reaction time was varied in the range of 10–40 min. After hydrolysis, the samples were centrifuged at 5000*g* for 10 min and the supernatant containing reducing sugars was collected. After the hydrolysate was obtained, the solution was neutralized with acid or base till the pH 6 was reached. The amount of sugars was determined by dinitrosalicylic acid (DNS) [17] method. The sugar yield (%) was calculated according to Eq. (1).

$$\%\text{sugar yield} = \text{sugar extracted/total sugar in biomass} \times 100 \quad (1)$$

2.3. Fermentation

The hydrolysate 20 mL, 5 g/L yeast extract, 10 g/L peptone and distilled water (up to 47.5 mL) was taken in a 125 mL flask and autoclaved. The fermentation was initiated by adding 2.5 mL of *S. cerevisiae* solution (grown aerobically for 24–48 h OD_{600} ~3) and grown for 72 h with 180 rpm at 30 °C in a shaker and the overall working volume of fermentation was 50 mL. The 0.5 mL of samples was taken from the fermentation broth at the certain time intervals. The samples were centrifuged at 10,000*g* for 5 min and the supernatant was used for the ethanol and sugar content analysis in GC-FID.

2.4. Direct transesterification

The *Scenedesmus* sp. used in this study contained 40% of lipids which is determined by solvent extraction method and explained in the Supplementary data (Section S.1). The dried microalgal powder was mixed with dimethyl carbonate (DMC), lipase (Lipozyme – Novozyme CAL-B from *Candida antarctica*, Novozymes Denmark) immobilized on