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Sustainable production of bioethanol using lipid-extracted biomass from *Scenedesmus dimorphus*

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ABSTRACT

Bioconversion technologies of biomass to bioethanol require intensive energy process due to several pretreatment steps to break down the biomass in order to obtain fermentable sugar for subsequent fermentation. Hence, a feasible approach to the production of bioethanol from lipid-extracted biomass of *Scenedesmus dimorphus* will be presented in this study in a biorefinery concept. The lipid-extracted biomass was directly subjected to simultaneous saccharification and fermentation thereby avoiding the costly pretreatment, lowering the contamination risk and reducing the complication of high sugar content. The technological challenges for this fermentation process were investigated to identify optimum conditions for amyloglucosidase enzyme activity and *Sacchromyces cerevisiae* yeast ethanolic fermentation. As a result, the optimum key parameters for the fermentation were identified at an enzyme concentration of 60 units/ml, pH 5, temperature at 36 °C and yeast loading of 3 g/L. At the optimum condition, an overall conversion of more than 90% of the theoretical yield was achieved with maximum bioethanol yield of 0.26 g bioethanol/g lipid-extracted biomass. The direct usage of lipid-extracted biomass into simultaneous saccharification and fermentation with single enzyme ensures the feasibility of the biofuel produced.

1. Introduction

Algae biofuels have been considered as promising renewable energy but the feasibility and sustainability of the energy production is yet to be well established (Demirbas, 2010). To date, a lot of researches are still looking into solutions to improve the drawbacks of biofuels production in all aspects from upstream to downstream processing (González-Fernández and Ballesteros, 2012). Most of the studies focus on how to maximize carbohydrate productivity from microalgae as reviewed by Markou et al. (2012) and improvement on the conversion efficiency of biomass to bioethanol as in the works by Aikawa et al. (2013). The two most potential biofuels that are actively studied are bioethanol and biodiesel. The properties of two fuels are comparative with petroleum. In fact, they provide much cleaner fuel; particularly, transportation fuel compared to petroleum (Balat et al., 2008).

Scenedesmus dimorphus is simple, easily harvested green microalgae and the utmost important is its capability to accumulate high starch content. Lipid and starch content inside cell are valuable composition for biodiesel and bioethanol, respectively. Apart from typical characteristics such as high photosynthetic efficiency, simple structure of the *S. dimorphus* simplifies subsequent biofuels processing compared to other complex plant. Biofuels processing from biomass is crucial to determine the cost and energy sustainability of microalgae as resources to replace conventional agriculture resources, which have been discussed by Liew et al. (2014). Fuel versus food crisis soon reaches their critical point, which will urge the replacement of agriculture resources with non-edible microalgae, which also have been discussed by Singh and Gu (2010). Hence, the challenge here is to improve the bioethanol conversion technologies to its industrial level using microalgae as feedstock while limiting additional energy consumption as assessed by Medeiros et al. (2015).

Biorefinery concept has becomes one of the great ideas to produce multiple products from biomass especially microalgae as the biofuel feedstock according to Cuellar Bermudez et al. (2015) but information on this area is scarce (Cherubini, 2010). Prior to this, more insight into these conversions from biomass is essential to manage efficient operation like petroleum refinery (Pires et al., 2012). As such, lipid extracted from microalgae is potential to

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produce biodiesel through transesterification (Chen et al., 2012). While the extracted biomass containing carbohydrate and protein, could be valuable components to produce bioethanol and fertilizer, respectively.

Fermentation is a commercialized process to produce bioethanol especially in Brazil and the United State. Carbohydrate content of microalgae especially starch or cellulose can be targeted for bioethanol production through biochemical conversion, using yeast to carry out alcoholic fermentation (Brennan and Owende, 2010). Due to its high tolerance to bioethanol concentration, *Saccharomyces cerevisiae* is widely used as the fermentation microbe (Balat et al., 2008). However, this kind of yeast consumes only simple sugar which is glucose. Therefore, starchy materials need to be converted to simple sugar through acid, alkaline or enzymatic hydrolysis. Enzymatic hydrolysis is an efficient method since it does not produce any fermentation inhibitors and extreme conditions is not required (Choi et al., 2010). Current improvement in enzyme processing technologies could reduce the cost issue for enzymatic hydrolysis in near future.

The prospects of microalgae biomass as liquid biofuels feedstock in biorefinery concepts are not be restrained by ethanolic fermentation process alone. The fermentation process could be costly and requires high energy thus further contribute to the overall production cost. This concern can be addressed by applying the right fermentation configuration. There are few fermentation configurations employed in current bioethanol production and separate hydrolysis and fermentation (SHF) is the most common method where it involve hydrolysis step before the fermentation. The positive point of this configuration is the hydrolysis step and yeast fermentation operate at their optimum conditions respectively which maximize the fermentable sugar for the yeast to utilize. However, the fermentation process is always upset by high level of glucose content which further reduces the efficiency of yeast ethanolic production. Due to this, additional unit operation is required to further dilute the sugar content for subsequent process. In the other hand, simultaneous saccharification and fermentation (SSF) is able to solve the SHF problems where hydrolysis and fermentation work all together in one reactor. The challenge here is to be able to identify and provide the most conclusive working environment for both the enzyme and yeast. Information on this configuration using lipid-extracted microalgae biomass is scarce thus important in the establishment of microalgae biorefinery industry.

The focus of this research was the simultaneous saccharification and fermentation where *S. dimorphus* is able to accumulate large amount of starch compared to other biochemical composition. Lipid-extracted biomass is directly subjected to SSF without any energy-intensive pretreatment. In this regards, optimization on the reaction will be studied in order to determine the most suitable condition for single enzyme in saccharification to convert starch content to fermentable sugar while maintaining an optimum condition for the yeast fermentation process to occur.

2. Materials and methods

S. cerevisiae (YSC2, type II bakers yeast) used in fermentation and amyloglucosidase enzyme from *Aspergillus niger* (aqueous solution, ≥300 U/mL) were purchased from Sigma–Aldrich, Malaysia.

2.1. Production of microalgae biomass as fermentation substrate

Scenedemus dimorphus (UTEX 1237, Universiti of Texas) was cultured at 30 °C in bioreactor containing 2 L medium with the following composition: 0.25 g/L NaNO3, 0.075 g/L $MgSO_4.7H_2O$, 0.025 g/L NaCl, 0.075 g/L K_2HPO_4, 0.175 g/L KH_2PO_4, 0.114 g/L H_3BO_3, 8.82 mg/L $ZnSO_4.7H_2O$, 1.18 mg/L $MnCl_2 \cdot 2H_2O$, 1.193 mg/L $Na_2MoO_4 \cdot 2H_2O$, 1.0 mg/L $CuSO_4$, 0.401 mg/L $CoCl_2 \cdot 6H_2O$, 50 mg/L EDTA-Na_2, 4.48 mg/L $FeSO_4 \cdot 7H_2O$, 0.2 mg/L Thiamine hydrochloride B1 and 0.01 mg/L Cyanocabalamin B12. Cultivation was started with inoculums size of 79.2×10^6 cells. The culture was continuously aerated with 2.5% (v/v) CO_2-enriched air and illuminated with two fluorescent lamps located at one side of reactor (Philip TL-D 36W/865, light intensity of 60–70 μmol/m^2s).

Cultivation was stopped at the early stationary phase (days 16) and biomass was collected after 12 h of natural sedimentation. The biomass was oven dried at 60 °C for 24 h and dry weight was taken. The dried biomass was subjected to total lipid and starch content analysis and considered as raw biomass in this study.

Lipid-extracted biomass as fermentation substrate was obtained via solvent extraction as described for total lipid extraction on the fresh biomass. The filtrated biomass resulted from solvent extraction was oven dried at 60 °C until constant weight was achieved and then subjected to starch content analysis.

2.2. Acid-catalyzed transesterification

Transesterification was carried out in Teflon-lined screw cap tube. 5 mL of hexane was added to the tube containing 0.1 g of lipid and the solution was sonicated for 5 min. After that, 4.25 mL of methanol and 0.215 mL of 37% HCl were added into tube. The tube was stirred in preheated water bath for 2 h at constant temperature of 85 °C. The solution was centrifuge at 3000 rpm for 2 min and the upper layer of solution was evaporated for potential leftover solvent. Biodiesel contents were determined and quantified with gas-chromatography (GC).

Biodiesel composition was analyzed with gas chromatography (Perkin-Elmer Clarus 500) equipped with flame ionization detector and Nukol™ column. Helium was used as carrier gas and the system operating condition was set as follows: the oven has a temperature heating rate of 10 °C min^{-1} from 110 °C to 220 °C for 8 min, injector and detector temperature at 220 °C and 250 °C, respectively.

2.3. Simultaneous saccharification and fermentation (SSF)

In the SSF process, fermentation media was a 50 mM sodium acetate solution (at various pH) containing 5 g/L yeast extract and 10 g/L peptone. One gram of lipid-extracted biomass was added into a reaction bottle and reaction volume was maintained at 40 mL. *S. cerevisiae* in active dry granule was added into 39 °C autoclaved distilled water to make yeast solution at various concentrations (12.5–100 g/L). 1 mL of amyloglucosidase at different concentration (15–75 units/mL) and 2 mL of yeast solution were added simultaneously to start the reaction. At certain interval time, 0.5 mL of samples were taken and centrifuged at 10,000 *g* for 5 min. Supernatant of the samples were analysed for sugar and bioethanol content. In this regard, the reaction time of 360 min was taken as total observation time.

Sugar content measurements were carried out with high performance liquid chromatography (Agilent series 1200) system equipped with evaporative light scattering detector (HPLC-ELSD) and Hi-Plex Ca column. Deionized water was used as mobile phase and flow rate was set at 0.6 mL/min.

Bioethanol quantification was carried out with gas chromatography (Hewlett Packard 5890 series) system equipped with flame ionization detector (GC-FID) and carbopack B-DA/4% Carbowax 20M column (2 m length, 0.2 cm ID, 80/120 mesh, supelco, USA). Helium was used as carrier gas and the system was operating at detector temperature of 225 °C, injector temperature of 225 °C, and