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Bioethanol production from acidic and enzymatic hydrolysates of mixed microalgae culture

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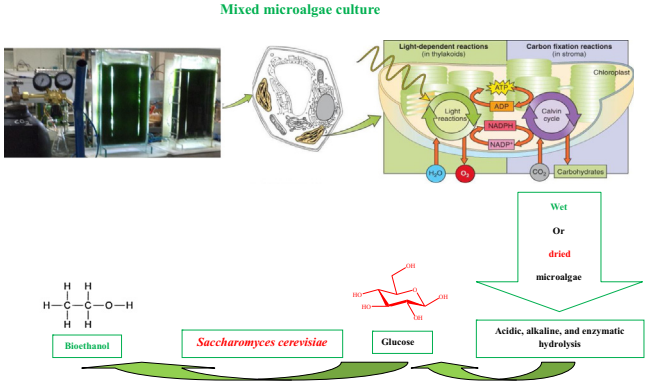
HIGHLIGHTS

- Reducing sugars extraction from mixed culture of microalgae via different pre-treatment strategies.
- The effects of $MgSO_4$ and $CaCl_2$ as lewis acid in acidic pretreatment on sugar yield.
- Comparison of the reducing sugar yield of dried and wet microalgae using enzymatic hydrolysis.
- Comparison of bioethanol yield of the fermentable sugars derived from different pre-treatment procedures.

GRAPHICAL ABSTRACT



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ABSTRACT

Mixed microalgae cultures are considered as an attractive research area compared to traditional pure culture to dominate cultivation contamination risk and enhance economic feasibility of large-scale biofuel production. However, pre-treatment and bioethanol production from mixed microalgae culture has not been reported yet. Therefore, this study was aimed to evaluate the effect of different pre-treatment strategies including acidic, alkaline, and enzymatic hydrolysis on the sugar extraction from mixed microalgae. Besides, the effects of $MgSO_4$ and $CaCl_2$ as lewis acids in acidic pre-treatment on reducing sugar yield were studied.

Results showed that the mixture of dilute sulfuric acid and $MgSO_4$ exhibited a higher sugar yield than dilute acid. Among all pre-treatments used, the enzymatic treatment with thermostable enzymes showed the highest recovery of 0.951 g extracted glucose/g total sugar. Moreover, the enzymatic pre-treatment of wet microalgae was compared with dried ones at identical operational conditions and dried biomass concentration of 50 g/l, similar sugar yields were achieved which would be advantageous to reduce the need for drying of the microalgae biomass. Fermentation of the acidic and enzymatic treated samples to ethanol using *Saccharomyces cerevisiae* showed yield of 0.38 and 0.46 g/g glucose, corresponding to 76% and 92% of the theoretical values, respectively. The obtained results revealed that bioethanol yield after enzymatic hydrolysis of mixed microalgae culture are higher than those of acid hydrolysis.

1. Introduction

The quick growth of the world population and fast expansion of economies have both led to sharp increase in universal energy

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consumption [1–3]. Therefore there is a strong incentive to reduce the CO_2 emissions and develop other energy sources as alternatives to fossil fuels [4]. Algae would be good candidates for renewable energy sources, receiving energy from the sunlight and building their biomass by eliminating CO_2 from atmosphere through photosynthesis [5].

Carbohydrates in microalgae biomass are mainly starch and cellulose (with the absence of lignin), thus they are more easily hydrolyzed to monosaccharide than other lignocellulosic materials [6]. These carbohydrates in microalgae are not readily fermentable to bioethanol, thus pre-treatment processes including chemical (acid and alkaline) or enzymatic hydrolysis are crucial [7,8]. The cost of pre-treatment contributes significantly to the total cost of biomass conversion process, up to 30% [9]. Consequently, pre-treatment has a great potential for improvement of converting biomass to fermentable sugars.

To date, studies of ethanol production from sugars derived from enzymatic hydrolysis of algae by thermostable enzymes are rare [10]. The enzymatic hydrolysis using thermostable enzymes has advantages in reducing the need for primary acid pre-treatment. Despite the fact that there are few researches on bioethanol production using pure culture micro- [6,11,12] and macroalgae [13–17], bioethanol production from mixed culture of algae has not been reported yet.

Cultivation of microalgae in pure culture is a main barrier for large-scale biofuel production due to its costly aseptic process. On the other hand, application of mixed culture of microalgae is a desirable solution to dominate cultivation contamination risk, operate in different conditions, and enhance economic feasibility. Thus, mixed cultures are considered as an attractive research area compared to traditional pure culture. Mooij et al. introduced the concept of "survival of the fattest", a strategy for enrichment of species with a high storage compound productivity in mixed microalgae culture [18]. Later on, Hassanpour et al. applied a gravimetric enrichment method for screening carbohydrate and lipid accumulating species in a mixed microalgae culture [19].

In this work, sugars extraction from mixed culture of microalgae via different pre-treatment strategies include acid, alkaline, enzymatic hydrolysis using thermostable enzymes were investigated and compared. Subsequently, the bioethanol yield of the fermentable sugars derived from different pre-treatment procedures was compared through cultivating *Saccharomyces cerevisiae* yeast.

2. Materials and methods

2.1. Microalgae and growth medium

The original mixed culture of microalgae was obtained from a freshwater area in Osku located in northwest of Iran (latitude is 37.91523 and longitude is 46.119901). The culture has been enriched using the gravimetric method in sequence batch reactor in our pervious study for starch storage [19]. This stock culture sample was used to prepare pre-culture for 12 L photo-bioreactor. Under the pre-culture condition, microalgae strains were grown in a 1000 ml-Erlenmeyer flask with 500 ml working volume at ambient temperature 25 1 °C, pH 8.9, constant light intensity 60 μmol m^{-2} s^{-1}, and constant agitation rate of 150 rpm. The composition of medium was as follows (g/l): $NaHCO_3$ (1.25); $NaNO_3$ (0.8); KH_2PO_4 (0.2); $MgSO_4$ (0.1); $CaCl_2$ (0.1); KCl (0.1) and 2 ml/l trace element solution containing (concentrations in mg/l): EDTA (100); $MnCl_2{\cdot}4H_2O$ (10.12); $FeSO_4{\cdot}7H_2O$ (10); $ZnSO_4{\cdot}7H_2O$ (4.4); $(NH_4)_6Mo_7O_{24}{\cdot}4H_2O$ (3); $CoCl_2{\cdot}6H_2O$ (3.22); $CuSO_4{\cdot}5H_2O$ (3.14).

When optical density at 688 nm using UV/vis spectrophotometer (Pharo 300, MERCK, Germany) reached ~1.5, the grown algae were transferred to the main photo-bioreactor.

2.2. Design and operation of photo-bioreactor

The photo-bioreactor (12 L) with working volume of 10 L was inoculated with medium, which was pre-cultured in a 1000 ml-Erlenmeyer flask. This photo-bioreactor was illuminated with an external light source mounted on two sides of the photo-bioreactor at a light intensity of approximately 260 μmol m^{-2} s^{-1} and worked at pH 8.9, 25 ± 1 °C and an aeration rate of 8 vvm. The medium described above was used as nutrients in the photo-bioreactor. The main photo-bioreactor was always inoculated with the stock pre-culture in which the microbial contamination was negligible. The regular microscopic observation using cell counting by Neubauer counting chamber indicated that bacterial contamination was always less than 2%, and no protozoa were observed. The contamination risk was minimized through periodical renewing the culture in the reactor with the stock pre-culture.

When the nitrogen source in the medium was completely consumed, carbon dioxide was continuously fed with a rate of 0.1 vvm into the microalgae culture to enhance intercellular carbohydrate storage compounds, mainly as starch. End of the batch cultivation (37 d), algae were harvested and used for enzymatic hydrolysis of wet biomass. In addition, for the chemical and enzymatic hydrolysis experiments using dried biomass, the harvested algae were dried at 80 °C for 24 h and ground into a powder.

2.3. Chemical hydrolysis

In chemical hydrolysis tests, the microalgae powders were mixed separately with H_2SO_4 (0.5, 1, 2 M), HCl (0.5, 1, 2 M), H_3PO_3 (0.5, 1, 2 M) and NaOH (0.5, 1, 2 M). In addition, the effects of $MgSO_4$ and $CaCl_2$ as lewis acids in acidic pre-treatment on reducing sugar yield were studied. The resulting slurries were then autoclaved at 121 °C for 10, 20, 30, and 40 min. After hydrolysis, the samples were allowed to reach room temperature. Then, suspension was centrifuged at 4000g for 5 min and the supernatant was taken for sugar content analysis. The chemical materials used were based on the following references.

In the study conducted by Miranda et al. [20], the extraction of sugars from the microalgae *S. obliquus* was investigated with H_2SO_4, HCl and NaOH in autoclave at 121 °C for 30 min. Ho et al. [6] pretreated microalgae *C. vulgaris FSP-E* with H_2SO_4 in autoclave for 20 min. Nguyen et al. [21] used the hydrothermal acid pre-treatment of microalgae *Chlamydomonas reinhardtii* with H_2SO_4 in an autoclave vessel at different temperatures (100, 110, and 120 °C) from 15 to 120 min. Zhou et al. investigated the hydrolysis of algae *chlorella* for fermentable sugars in the presence of HCl and $MgCl_2$ at 180 °C from 10 min and 120 °C from 60 min [22].

2.4. Enzymatic hydrolysis

The enzymes used to hydrolyze carbohydrate components (cellulose and starch) in the microalgae were purchased from alpha enzyme company (Mashhad, Iran). They included thermostable β-glucosidase/cellulase from *Talaromyces emersonii*, thermostable α-amylase of *Bacillus licheniformis* (EC 3.2.1.1) and amyloglucosidase from *Aspergillus niger* with activities of 1000 U/g, 145,000 TSAU/ml and 600 AGU/ml, respectively. A summary of the optimal conditions for the enzymes is presented in Table 1.

For the enzymatic hydrolysis experiments, a sample with a concentration of 50 g/l dried microalgae powder was hydrolyzed in a fixed volume of 20 ml containing citrate buffer with a pH value of 5.5. Considering the optimum temperature for the enzymes (table 1), the biomass was hydrolyzed for 3 h by β-glucosidase/cellulase (denoted as enzyme 1), and α-amylase (denoted as enzyme 2) at 65 and 95 °C, respectively. For the subsequent saccharification, temperature was reduced to 55 °C and amyloglucosi-