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Experimental investigations on bioethanol production from halophilic microalgal biomass

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ABSTRACT

Although the microalgae biomass is a significant feedstock for bioethanol production, studies on "third generation" of algal bioethanol is at its early stages of investigation. In this study halophilic microalgae *Dunaliella* sp. was used for producing bioethanol. Physical and physicochemical methods of pretreatment procedures were used. Some important parameters such as KNO_3 (0–1000 mg/L), KH_2PO_4 (0–35 mg/L), $MgSO_4$ (0–500 mg/L) nutrient amendments and NaCl (15%–30%) concentrations for *Dunaliella* sp. cultivation were optimized. Changing fermentation pH within 4–7 range, and yeast inoculum amount by inoculation of 0.5–3 ml volumes were used for optimization of the bioethanol production processes. Dilute acidic hydrolysis with 1% sulfuric acid in autoclave was found to be very effective in saccharifying algal biomass and higher bioethanol production values were obtained under nitrogen-depletion conditions. The highest bioethanol production by *Saccharomyces cerevisiae* was obtained at 72 h of incubation at substrate concentration of 30 g/L microalgal biomass and 3 ml inoculums at pH 6. The results demonstrated that at the optimized saccharification and fermentation conditions the bioethanol production could be increased up to 7.26 g/L, which was 10.7-fold higher than the value obtained by using the microalgal biomass was not exposed to any pretreatment. In the present study we showed that bioethanol production from biomass of a halophilic microalgae, which is known as a promising feedstock for bioethanol production, can be increased by appropriate pretreatments.

1. Introduction

In today's world, there is an urgent need for alternative energy sources due to rapid depletion of fossil fuels and environmental concerns. The development of techniques providing opportunities for exploitation of renewable energy sources, including biodiesel and bioethanol (Gu et al., 2013). Since bioethanol is the most widely used liquid biofuel among these alternative fuels at present, considerable work has been done on production of bioethanol using various kinds of feedstocks such as starch rich agricultural products and cellulosic biomass (Tiquia-Arashiro and Mormile, 2013). Although the usage of microalgal biomass as a feedstock for biodiesel production has been extensively studied (Ji et al., 2013; Wu et al., 2013; Malla et al., 2015), this material also has the potential of being used for production of other biofuels such as bioethanol, because of its carbohydrate and fermentable sugar contents (Yoza and Masutani, 2013).

Exploitation of the potential of microalgae and macroalgae as renewable energy source as the third generation biomass has attracted much attention, considering the advantage of their mass culture production at an acceptable cost and their lignin free structure, allowing development of a cost-effective pretreatment process (Lee et al., 2013). Several species with high starch content, such as *Chlorella*, *Chlamydomonas*, *Dunaliella*, *Scenedesmus*, and *Tetraselmis* are the most studied ones to produce ethanol from their high carbohydrate contents (Goo et al., 2013; Ho et al., 2013a,b; Miranda et al., 2012)

As seawater is the most feasible and sustainable environment for marine or saline microalgal biomass, the advantages of seawater must be assessed in production of microalgal biomass for biofuel (Borowitzka, 2008). Therefore, a halophilic microalgal strain namely *Dunaliella* sp. was used as a feedstock for bioethanol production in the current study. The effects of various hydrolysis methods on the microalgal biomass production were investigated. The yeast *Saccharomyces cerevisiae* was used for the fermentation process. Some important parameters for bioethanol production, such as pretreatment procedures, nutrient limitations, pH of the medium and fermentation pH were optimized to find the highest

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rate of bioethanol production by the fermentation of *S. cerevisiae* yeasts.

2. Materials and methods

2.1. Microorganism and cultivation conditions

Dunaliella sp. strain was provided by Ankara University, Faculty of Science Laboratories' from the current culture collection. The microalgae cells were cultured in 250 ml Erlenmeyer flasks containing 100 ml Johnson fermentation medium with 15% NaCl (Johnson et al., 1968). The microalgal biomass was harvested and centrifugated at 10 000 rpm, at 4 °C for 15 min after 20 days of incubation.

To determine the effect of increasing amounts of NaCl concentrations from 15 to 30% NaCl were added to the Johnson medium.

2.2. Optimization of cell disruption methods

In a series of experiments cell disruption of *Dunaliella* sp. were performed with 30 g/L microalgal biomass in distilled water directly, and following different physical treatments such as sonication, temperature with pressure, and physicochemical ones including acid hydrolysis with and without autoclaving in order to investigate the most appropriate pre-treatment procedure for weakening the cell walls. Sonication experiments were performed by using Sonics Vibra Cell with 60 W net power input and 20 kHz frequency for 5 min. To evaluate the combined effect of temperature and pressure, the microalgal cells were autoclaved at 121 °C for 15 min. In physicochemical pre-treatment methods, the biomass was exposed to 70 °C for 15 min in the presence of 1% H_2SO_4 and also 121 °C for 15 min. The aliquots of biomass were exposed to 0.5%, 1% and 2% H_2SO_4 at 121 °C for 15 min to see the effects of different H_2SO_4 concentrations.

2.3. Fermentation conditions

Saccharomyces cerevisiae yeast, which was obtained from Faculty of Science Laboratories of Ankara University from the current culture collection, was used for the fermentation studies. *S. cerevisiae* cells were precultured in YPG medium and 10 (v/v)% yeast suspension was aseptically transferred to anaerobic fermentation medium at pH 6. The fermentation medium was prepared with distilled water containing microalgae sugar which was obtained after pretreatment of cells. All the experiments were carried out in three replicates.

In order to find the maximum bioethanol production changing fermentation pH within 4–7 range, and inoculum amount by inoculation of 0.5–3 ml volumes were used for optimization of the production processes. Furthermore, to obtain the highest yeast growth increasing NaCl (15%–30%) concentrations for *Dunaliella* sp. cultivation was optimized.

2.4. Analytical methods

The bioethanol concentration was analyzed by a Shimadzu, Model GC-14B gas chromatograph equipped with a flame ionization detector. One microlitre of sample was injected into a glass column (2 mm i.d., 2 m long) packed with chromosorb 101 (80/100 mesh). Isothermal separation with nitrogen carrier gas was performed at 220° injection port, 160 oven and 250 °C detector temperatures (Avcı and Dönmez, 2006).

The growth of yeast cells was determined by measuring optical density of the samples at 600 nm during the incubation period, by constructing a standard calibration curve for yeast concentration which was based on the dry weight and OD600 measurements (Harun and Danquah, 2011).

Absorbance measurements and centrifugation were performed using a Shimadzu UV 2001 model spectrophotometer and Hettich EBA12 model centrifuge, respectively (Xiong et al., 2008).

Table 1
The effect of different pretreatment methods on bioethanol production from *Dunaliella* sp. biomass cultured in the 15% NaCl Jonhsons medium (microalgae loading: 30 g/L, fermentation pH: 6, fermentation time: 72 h).

Pretreatment method	Bioethanol (g/L)
autoclave	0.78 ± 0.1
1% H_2SO_4	0.70 ± 0.06
sonication	0.70 ± 0.13
autoclave + 1% H_2SO_4	0.91 ± 0.05
without any pretreatment	0.68 ± 0.04

3. Results and discussion

In the current study, the biomass of *Dunaliella* sp. was used as a feedstock for bioethanol production. The parameters such as pretreatment procedures, nutrient limitations in the culture medium, effects of acid concentrations used for pretreatment, pH of the fermentation medium and yeast inoculum quantities were optimized to find the highest bioethanol production from the fermentation activity of *S. cerevisiae* yeasts.

3.1. Disruption of microalgae cells

The most important step in biofuel production from biomass is to optimize the pretreatment processes (Gu et al., 2013). Pretreatment methods are either physical or chemical or some of incorporate both of them. The pretreatment of biomass is the first step in the bioethanol production process. Because of this step has a crucial role, there are many studies on discovering more efficient pretreatment methods of microalgal biomass, aiming to destroy the crystalline structures, microfibrils in the cell walls in order to enhance the substrate digestibility (Harun et al., 2011). Since the cell walls contain different types of carbohydrates as the necessary carbon sources for the fermentation process, we exposed them by disruption of *Dunaliella* sp. cells after photoautotrophic culturing, harvesting and concentrating them. As mentioned above, in Section 2.2. different physical and physicochemical methods were tested to investigate the most appropriate pre-treatment procedure for these microalgal cells.

The bioethanol production values of *S. cerevisiae* fermentation in the presence of 30 g/L microalgae load at the end of 72 h incubation at pH 6, which were obtained by different pretreatment methods tried, are shown in Table 1. In the experiments for disruption of *Dunaliella* sp. cells, the biomass, which was harvested from the medium with pH 6, was washed two times with water to expose sugars in the cells without any physical or physicochemical method. The extract was used directly for fermentation studies and 0.68 g/L bioethanol was obtained. The data in Table 1 depicts that, another lower ethanol concentrations were obtained as 0.70 g/L when the biomass treated with 1% H_2SO_4 and sonication. When the microalgal biomass was autoclaved for pretreatment, the bioethanol concentration was started to increase in our study.

In the literature pretreatment of biomass in autoclave is a common method for bioethanol production. For example in a study that is performed for bioethanol production from sugarcane trash, pretreatment was carried out in a laboratory autoclave at 121 °C for 60 min (Raghavi et al., 2016). In another study that is about usage of grape marc as a source for bioethanol production, the researchers reported that the acid/autoclave treatment liberated the highest proportion of monosaccharides for both red and white marc and the