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Research article

Bio-oil production and removal of organic load by microalga *Scenedesmus* sp. using culture medium contaminated with different sugars, cheese whey and whey permeate

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ABSTRACT

The objective of this study was to evaluate the bio-oil production and the organic load removal using the microalga *Scenedesmus* sp. The cultivation was carried out in reactors with a total volume of 3 L and 0.7 vvm aeration, with illumination in photoperiods of 12 h light/12 h dark for 12 days. The following sugar concentrations were tested: 2.5, 5.0 and 10 g/L of glucose, lactose, fructose and galactose with 10% inoculum volume. After experiments were performed with cheese whey in natura and cheese whey permeate with different lactose concentrations (1.5, 2.5, 3.5 and 5.0 g/L). In these experiments the inoculum concentrations were 10, 15, 20 and 30% (v/v). The results showed that this microalga was effective for the production of lipids when it was cultivated in medium with cheese whey in natura with 2.5 g/L of lactose and 20% inoculum (v/v). Using cheese whey in natura at the concentration of 3.5 g/L of lactose and 30% (v/v) of inoculum obtained 77.9% of TOC removal and 38.447 mg of TOC removed/mg oil produced. It was also observed that when there is increased production of bio-oil, there is less removal of organic matter. The addition of glucose, fructose or galactose in the medium did not enhance the production of bio-oil by *Scenedesmus* sp. when compared to lactose, but increased the organic matter removal.

1. Introduction

The search for renewable sources for biofuels production is growing every day in our society mainly due to environmental problems such as greenhouse gas emissions from the burning of fossil fuels. The limited supply of oil as fuel requires the discovery of new sources of alternative energy which are renewable and sustainable, such as the fatty acids extracted from microalgae (Ho et al., 2012).

Microalgae are a promising raw material for biodiesel production due to their high growth rate and high yields of lipids, and the ability to grow in different environments contaminated with different types of sugars. But the high production of lipids is often achieved only under certain stress conditions, which prevent their growth to provide an ideal production of lipids. According to Blatti et al. (2013), the use of microalgae for bio-oil extraction can be a potentially cost-effective alternative to other fuel sources (Martín and Grossmann, 2013).

According to Martinez et al. (2000), several microalgae can be used in bioremediation such as *Scenedesmus* sp. Microalgae use carbon, light energy and nutrients to produce the biomass, which can be used for the most diverse applications, such as the production of lipids, dyes, enzymes, antibiotics, carbohydrates, vitamins and biofuels. Through several studies on processes that involve the microalga metabolism, researchers are looking to identify new species of microalgae that are capable of generating more and more lipids and adding value to other co-products (Blatti et al., 2013).

Lipids of microalgae are made up of different saturated and unsaturated fatty acids (Huang et al., 2010). At the end of the lipid extraction process from microalgae, the residual biomass can also be used as biofertilizer due to its high nitrogen/phosphorus ratio (Singh and Gu, 2010).

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In the region where this study was developed there is a large production of whey. This effluent is considered a major contributor to the pollution caused by the dairy industry. In many dairies the whey is disposed of with other waste. This effluent is about a hundred times more polluting than domestic sewage. Cheese whey and milk acid, due to their nutritional values and high organic loads, should not be mixed with other industry effluents (Silva et al., 2006).

The application in areas of contaminated water or residues treatments is also a justification for studying the behavior of microalgae. According to Brennan and Owende (2010), biofuels production from microalgae together with residues treatment is a biotechnological area with potential commercial application. Moreover, the literature has no paper in which a microalga is evaluated at the same time in relation to both its ability to remove organic load and the production of bio-oil using various types of sugars, cheese whey (often discarded as effluent) and cheese whey permeate as a substrate. In this respect, the objective of this study is to evaluate the bio-oil production and the removal of organic load using the microalga *Scenedesmus* sp. in growth media with the sugars lactose, glucose, fructose, galactose, and cheese whey with and without supplementation of salts and cheese whey permeate.

2. Materials and methods

2.1. Microalga

The microalga *Scenedesmus* sp, collected at 'Cota Cota Pont' in La Paz (Bolivia) and isolated at the IIDEPROQ – UMSA Laboratory (Instituto de Investigación y Desarrollo de Procesos Chemicals) of the Universidad Mayor de San Andres was used in this study. The microalga was donated by the Department of Chemical Engineering, Federal University of Rio Grande do Sul in Porto Alegre – Rio Grande do Sul, Brazil, and kept in an incubator equipped with a photoperiod of 12 h light/12 h dark at 22.5 ± 0.5 °C. Recultivations to maintain the microalga were done every 15 days. The microalga was subsequently stored until used in the experiments.

2.2. Experimental module system and culture medium

The microalga was cultivated as described in literature Muñoz et al., 2004) in a 3 L acrylic vertical reactor (Fig. 1). In all experiments the operational conditions were: 23 ± 1 °C, photoperiod of 12 h and aeration rate of 0.7 vvm. These experiments were carried out in triplicate, over a period of 12 days of cultivation.

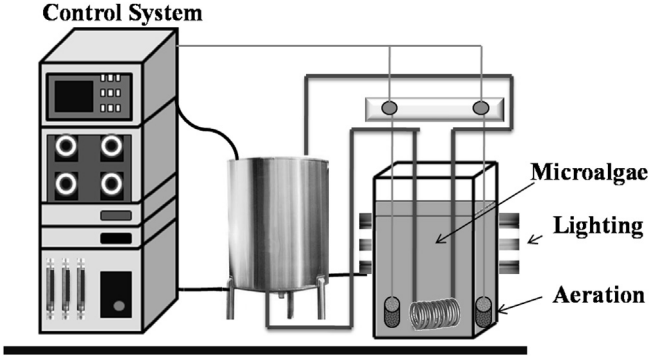

Fig. 1. Scheme of the experimental apparatus used in the studies of bio-oil production by microalga.

2.3. Evaluation of growth media and inoculum size

2.3.1. Study of sugar addition in the growth medium for microalga

The growth of *Scenedesmus* sp. and the bio-oil production were evaluated using glucose, fructose, galactose and lactose at concentrations of 2.5, 5.0 and 10.0 g/L with inoculum of 10% of the working volume of the reactor, which corresponds to 0.102 ± 0.013 g/L. These carbohydrates (carbon font) were added in the mineral-modified Guillard medium with the following composition of macronutrients (mg/L): $CaCl_2 \cdot 2H_2O$ (441.12), $MgSO_4$ (180.4), $NaHCO_3$ (151.2), $K_2HPO_4 \cdot 3H_2O$ (136.9), $NaNO_3$ (102.0), and micronutrients (mg/L): Na_2EDTA (52.32), $FeCl_3 \cdot H_2O$ (37.8), $CuSO_4 \cdot 5H_2O$ (0.144), $CoCl_2 \cdot H_2O$ (0.144).

The concentrations of micronutrients $ZnSO_4 \cdot 7H_2O$ and $MnCl_2 \cdot 4H_2O$ present in the modified Guillard (1975) medium were optimized by Borges (2014) through the implementation of a Central Composite Design. Thus, the optimal concentrations in the modified Guillard medium were 0.06 g/L for $ZnSO_4 \cdot 7H_2O$ and 0.5 g/L for $MnCl_2 \cdot 4H_2O$. In the culture conditions the final concentrations for these previously optimized micronutrients were $ZnSO_4 \cdot 7H_2O$ (0.036 mg/L) and $MnCl_2 \cdot 4H_2O$ (0.56 mg/L). After the sugar selection, cultivation experiments were carried out on *Scenedesmus* sp. in cheese whey in natura and cheese whey permeate.

2.3.2. Growing the microalga Scenedesmus sp. in medium supplemented with cheese whey and cheese whey permeate

The cultivation of *Scenedesmus* sp. was carried out with cheese whey in natura and with cheese whey supplemented with the salts (modified Guillard medium) in order to verify the behavior of the production of lipids and the TOC removal using the microalga *Scenedesmus* sp. The cheese whey in natura used in the experiments was provided by a dairy in the Uberlândia region of Brazil. The lactose concentration in this cheese whey was 40 ± 2 mg/L. Commercial cheese whey permeate was purchased from the SOORO Company and contained 92.97% lactose in its composition. The cheese whey and cheese whey permeate were diluted to the best lactose concentration found in tests described earlier (item 2.3.1). These experiments were performed with and without addition of salts (modified Guillard medium) and with 10% inoculum.

After checking the production of lipids and TOC removal with cheese whey and cheese whey permeate, experiments were conducted with different concentrations of lactose in the cheese whey and inoculum.

2.3.3. Variation of lactose concentration in the cheese whey and of inoculum concentration

Firstly, microalga cultivations with inoculum of 10% (0.223 ± 0.010 g/L) of the working volume of the fermentation medium in lactose concentrations in cheese whey in natura of 1.5, 2.5, 3.5 and 5.0 g/L were carried out. These experiments were supplemented with the salts of the modified Guillard medium.

Later, to verify the results obtained in experiments carried out with various lactose concentrations of cheese whey, the inoculum concentration was varied. Three experiments with a lactose concentration of cheese whey at 2.5 g/L were made with inoculum of 10, 15 and 20% (v/v). Finally, four experiments were carried out with the lactose concentration of cheese whey of 3.5 g/L and inoculum concentrations of 10, 15, 20 and 30% (v/v). These seven experiments were all supplemented with the salts of the modified Guillard medium.