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Lipid extracted algae as a source for protein and reduced sugar: A step closer to the biorefinery

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HIGHLIGHTS

- Lipid extracted algae (LEA) was used as a source for protein and reduced sugars.
- Comparable yields of these products were obtained from total algae and LEA.
- Microwave assisted extraction from oven dried samples provided highest lipid yield.
- Effective cell disruption for lipid extraction increased loss of other products.
- Maximizing the yields of all products requires proper process selection.

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ABSTRACT

The objective of this study was to investigate the feasibility of using lipid extracted algae (LEA) as a source for protein and reduced sugar, and the effects of various procedural treatments on their yields. LEA provided comparable yields of protein and reduced sugars to those from total algae. Oven drying provided highest yields of all products followed by freeze drying, while sun drying significantly lowered their yields. Effective cell disruption by microwave and autoclave increased the lipid yields from algae, but resulted in increased loss of other compounds with lipid extracting solvents lowering their yields during sequential extraction. Relatively inefficient cell disruption by ultrasonication and osmotic shock lowered the amount of cell protein lost to the lipid extracting solvents. These results highlight the complexity of concurrent extraction of all value added products from algae, and the need for proper selection of the processes to achieve the objectives of integrated biorefinery.

1. Introduction

Microalgae have drawn much attention in past few decades due to their nutrient removal potential from wastewater (Di Termini et al., 2011; Sawayama et al., 1998). In addition, microalgae are considered to be important resources for biofuels and a viable alternative to limited fossil fuels due to their lipid accumulation potential (Morweiser et al., 2010; Tsukahara and Sawayama, 2005). Recent efforts have focussed on achieving the dual objectives of wastewater treatment and biofuels production from microalgae (Park et al., 2011; Wu et al., 2012). However, algal biofuels are yet to achieve economical sustainability (Lundquist et al., 2010).

In addition to lipids, microalgae also produce other compounds of great economic value (Olguín, 2012). Algal proteins are an acceptable alternative of conventional food supplements due to their nutritional value and amino acid profiles (Becker, 2007). Similarly, algal polysaccharides can be hydrolyzed to reduced sugars which have great application in the production of bioethanol (Fu et al., 2010; Sun and Cheng, 2002). The use of residual algal biomass after lipid extraction for other applications can reduce the cost of algal cultivation and biofuel production (Rashid et al., 2013). The concurrent extraction of other valuable products in addition to lipids from algal biomass may result in optimal value extraction and economically beneficial algal technology. This is an important objective of integrated algal biorefinery approach for algal biofuels technology (Subhadra, 2010).

The application of residual algal biomass after lipid extraction has been investigated by several researchers for different objectives. These objectives can broadly be divided in two categories: first for energy production by utilizing the remaining carbon and hydrogen, and secondly for extracting products for their nutritional and economical values. Zhu et al. (2013) studied the hydrothermal liquefaction potential of lipid extracted algae (LEA) for their

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conversion to liquid fuels. Similarly, Yang et al. (2011) developed a two-stage process to produce hydrogen and methane gases from LEA. The potential of using LEA as a protein source in animal feeds was also established (Ju et al., 2012; Lodge-Ivey et al., 2014). Geun Goo et al. (2013) investigated the application of LEA for bioethanol production. The extraction of valuable compounds from LEA was found to lower the cost of biofuel production in comparison to the utilization of carbon and hydrogen in residual mass for energy production (Gao et al., 2012).

A major consideration in the concurrent extraction of all valuable products from algae is the effect of various treatments on the individual yields of such products. The algal biomass undergoes many processes during lipid extraction which invariably affects these coproducts. Theoretically, lipid extraction should not result in any loss of other cellular compounds resulting in their fractions in algal mass to increase in the LEA in comparison to whole cell algae, and thus increasing their yield (% w/w) in LEA. However, under realistic conditions, some loss of these compounds is unavoidable as cell disruption would unbind them from cell mass to some degree and the unbound fraction could be lost to the applied solvent during lipid extraction. For example, Lam et al. (2014) found reduction in the carbohydrate content by 7.2% in the algal biomass after lipid extraction in comparison to whole cell algae. The additional processes used for extracting individual products also have their process limitations, for example hydrolysis of polysaccharides to reduced sugars requires sufficient reaction time for achieving good yields (Fu et al., 2010). It is therefore important to investigate the effects of various treatments during lipid extraction and additional processes for individual product extraction on the final yields of these products. Comparison of yields from whole cell algae in order to establish the feasibility of using LEA as a source of these compounds is also beneficial. Studies investigating the effects of various treatments on product yields from LEA are not available in the literature to the best of our knowledge. This study, investigates the effects of various procedural treatments, i.e. drying and cell disruption, during extraction of lipids, and subsequently protein and reduced sugars from LEA on the product yields and comparison to yields from whole cell algae to identify the optimal treatments and establish the feasibility of LEA as a source for these products. Cell disruption by four methods: microwave, ultrasonication, autoclaving, and osmotic shock with 10% NaCl were investigated. In addition, main drying processes applied in literature include sun drying, oven drying, freeze drying, drum drying, spray drying, fluidized bed drying etc. However, not all of these are economically sustainable (Brennan and Owende, 2010; Guldhe et al., 2014). Hence in this study, focus has been on investigating the effects of sun drying, oven drying, and freeze drying processes, which are more economic in nature.

2. Methods

2.1. Algae culture

Scenedesmus obliquus (Genbank accession number: FR751179.1) used in this study was isolated from Durban region, KwaZulu Natal, South Africa (Misra et al., 2014). A raceway pond of 300,000 L was operated with BG11 medium for algal cultivation under natural sun light (400–1200 μmol m^{-2} s^{-1}) and temperature (18–27 °C). The grown biomass was harvested by gravitational settling and then centrifuged to obtain thick algal slurry.

2.2. Drying of the harvested algae

The thickened biomass slurry was dried by three different methods: (a) sun drying, (b) oven drying, and (c) freeze drying. Sun dried biomass was obtained by placing the thickened slurry on a drying bed lined with 1500 μm white plastic for three days under natural sunlight and ambient conditions (400–1200 μmol m^{-2} s^{-1}, 18–27 °C). Similarly algal slurry was placed in a hot air oven at 60 °C for 24 h for oven drying. A freeze dryer (Mini Lyotrap, LTE Scientific Ltd., United Kingdom) was used to lyophilize the samples after overnight freezing at −84 °C in a biofreezer (Glaciar NU9668E, Nuaire, Japan). Dried biomass was pulverized with mortar and pestle and stored in a desiccator.

2.3. Lipid extraction assisted by cell disruption

Dried biomass of *S. obliquus* was further subjected to various cell disruption procedures to increase the efficiency of solvent based lipid extraction. Four cell disruption methods were investigated: (a) microwave, (b) ultrasonication, (c) autoclave, and (d) osmotic shock with 10% NaCl. Total lipids were extracted by the method of Folch et al. (1957) using 2:1 (v/v) mixture of chloroform and methanol.

For microwave assisted lipid extraction, 1 g of dried biomass was mixed with 20 mL of solvent mixture (chloroform and methanol in 2:1 ratio) and heated at 100 °C for 10 min at 1000 W in a microwave digester (Milestone S.R.L., Italy; 1200 W of output power). Solvent containing lipids was separated by centrifugation and then vacuum filtered. After such separation from cell debris, solvent was evaporated in oven at 60 °C. The remaining total lipids were quantified gravimetrically. Similarly during ultrasonication, 20 mL of solvent mixture was added to the 2 g of dried biomass in a 50 mL centrifuge tube, after which the entire mixture was sonicated for 2 min at 15 kHz (Misonix XL-2000-010; 100 W of output power, 22.5 kHz of output frequency). The mixture was centrifuged and supernatant transferred to a separate tube. A further 20 mL of the solvent mixture was added to the centrifuged cell debris, sonicated and supernatant separated by centrifugation and mixed with previously recovered solvent. The pooled solvent was vacuum filtered and evaporated in oven at 60 °C to recover the extracted lipids. Autoclaving was also investigated as a cell disruption procedure in which 500 mg of dried biomass was added with 50 mL of ultrapure water and autoclaved at 121 °C and 15 lbs for 5 min (Prabakaran and Ravindran, 2011). The autoclaved sample was mixed with extracting solvent mixture in 1:1 ratio and transferred to a separatory funnel after mixing for 5 min, where two layers formed. The lipid containing layer was carefully removed and lipids were recovered after drying in oven at 60 °C. Osmotic shock with 10% NaCl also results in cell disruption and accessibility to intracellular content due to changing osmotic pressures on both sides of cell walls. 500 mg of dried biomass was mixed with 50 mL of 10% NaCl solution and vortexed (VM-300, Gemmy, Taiwan) for 1 min, after which the biomass was left suspended in NaCl solution for two days. After two days, this suspension was added to lipid extracting solvent mixture in 1:1 ratio and transferred to a separatory funnel after 5 min of mixing. The distinct layer containing lipids was carefully removed and evaporated at 60 °C in oven to recover lipids. After each extraction, the lipid mass was quantified gravimetrically and lipid yields (% w/w) of the processes were calculated.

The algal biomass remaining after lipid extraction was vacuum filtered and air dried at room temperature to obtain lipid extracted algae (LEA) from which proteins and reduced sugars were extracted. This recovered biomass was quantified and utilized in calculating the subsequent yields from LEA.

2.4. Protein extraction from LEA

Dried whole cell algae or LEA biomass was subjected to protein extraction as per Lowry method (López et al., 2010; Lowry et al.,