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Lipid production of *Chlorella vulgaris* from lipid-extracted microalgal biomass residues through two-step enzymatic hydrolysis

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ABSTRACT

Lipid-extracted microalgal biomass residues (LMBRs) were treated using cellulase, neutrase and alcalase in a two-step process and the resulting hydrolysates were used as a source of nutrients for the cultivation of *Chlorella vulgaris* under non-aerated and aerated conditions for lipid production. Aeration was favorable for cell growth and lipid accumulation and a biomass of approximately 3.28 g L^{-1}, lipid content of 35% and lipid productivity of 116 mg L^{-1} d^{-1} were obtained. Thus, the tested mode of LMBRs utilization was effective for nutrient recycling in microalgal biodiesel production.

1. Introduction

Lipid-extracted microalgal biomass residues (LMBRs) are the residual biomass from microalgal biodiesel production processes. These residues are rich in proteins and carbohydrates, and making use of these residues is necessary to increase the economic and environmental feasibility of microalgal biodiesel production (Chen et al., 2009). Using appropriate technologies, the primary components of microalgal biomass, proteins and carbohydrates, could be converted to products such as amino acids and sugars (Harun and Danquah, 2011; Mooibroek et al., 2007) which could be utilized as nutrient sources for a new crop of microalgae. Since nutrient supplies have a sizeable effect on cost, sustainability, and site selection for microalgal cultivation (Mata et al., 2010), the use of LMBRs could contribute to the economy of microbial biodiesel production.

Therefore, the purpose of the present study was to explore a microalgal biodiesel production mode based on two-step enzymatic hydrolysis of LMBRs and utilization of the hydrolysates (mainly amino acids and sugars) as nutrient sources for *Chlorella vulgaris* cultivation. Non-aerated and aerated cultures using the hydrolysates were investigated.

2. Methods

2.1. Materials

All chemicals were of analytical-reagent grade. The amino acids standards, buffer components and reagent grade salts were purchased from Sepax Technologies, Inc. (Jiangsu Province, China). The sugars standards (xylose, glucose and arabinose) were purchased from Sigma Chemical Company (Shanghai, China).

Cellulase derived from *Trichoderma* with an enzymatic activity of 80 U/mg, neutrase derived from *Bacillus subtilis* and alcalase derived from *Bacillus lincheniformis* with enzymatic activities of 100 U/mg were provided by Nanjing Genetime Biotechnology Co. Ltd., Jiangsu Province, China.

2.2. Microalgal strain and cultivation conditions

The microalga *C. vulgaris* (strain CCTCC M 209256) was obtained from the China Center for Type Culture Collection, Wuhan, China. The strain was preserved in 20% (v/v) glycerol at −80 °C. The culture medium was composed of instant ocean synthetic sea salt (Aquarium Systems, Inc., USA), 34 g L^{-1}; 200 mL L^{-1} hydrolysates of LMBRs. The hydrolysates were sterilized using a sterile 0.45 μm membrane filter (Millipore Corporation, USA). A 1.25 L bubble column photobioreactor (25.0 cm in height, 8.0 cm in diameter, a closed system) was used with a working volume of 1 L. In all cases, *C. vulgaris* was inoculated at 1:10 (v/v) ratio into the photobioreactor. The initial biomass concentration of 0.10 g L^{-1} was used

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