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Continuous Cultivations of the Oleaginous Yeast *Lipomyces starkeyi*

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ABSTRACT

Oleaginous yeasts are microbial factories capable of converting carbohydrates into lipids. The transesterification of these lipids results in the production of biodiesel. The oleaginous yeast Lipomyces starkeyi is an excellent lipid producer. To attain parameters for the understanding of the lipid production process, we performed continuous cultivations using glucose and xylose as carbon sources. The highest overall cell yield of 0.443 g/g and lipid yield of 0.236 g/g was observed at the dilution rate of 0.03 h^{-1} while the highest cell and lipid productivities were obtained at 0.06 h^{-1}. Gas chromatography analysis of the esterified lipids indicated that the major constituents of this complex are palmitic acid, stearic acid, oleic acid, and linoleic acid with an estimated cetane number (approximately 61) similar to that of palm biodiesel, which is important for biofuel production. These data should be very helpful to develop and design more efficient bioprocesses for microbial lipid production.

Palavras-chave: oleaginous yeast, dilution rate, biodiesel.

INTRODUCTION

Due to increasing recognition of the potential for environmental benefits and decreasing of petroleum reserve, there is a high interest in the development of biofuels (SHEN et al., 2013). Some yeast strains can accumulate intracellular lipids up to 70% of their cell dry weight (RATLEDGE and WYNN, 2002). Single Cell Oils (SCOs) are formed by triacylglycerols such as palmitic, stearic and oleic acids, which are feedstocks for biodiesel production. These metabolic capabilities of oleaginous yeast are indeed promising. However, to outcompete with the plant oil production, microbial lipid productivities need to be optimized (KOSA and RAGAUSKAS, 2011).

Lignocellulose can be converted into fermentable sugars, providing abundant, inexpensive carbon sources recovered from industrial byproducts. The sugar rich fraction in the lignocellulosic mixture containing mostly hexose and pentose sugars, can be utilized by microorganisms in the fermentation process for biofuel production (FEI et al., 2015). Some oleaginous yeasts can assimilate glucose and xylose simultaneously to accumulate intracellularly a considerable amount of lipid with a good lipid coefficient, in both artificial and real hydrolysates (HU et al., 2011; ANSCHAU et al., 2014; XAVIER and FRANCO, 2014; CORADINI et al., 2015). The fermentation of mixed sugars is not only significant for the improvement of the overall economics associated with microbial lipid production but also holds an interesting route for effective cell mass conversion (ZHAO et al., 2008).

A continuous cultivation (chemostat) has been described to control the growth rate of microorganism, thus enabling manipulation of process variables with good accuracy (NOVICK and SZILARD, 1950). However, few studies have investigated process analysis with the aim of evaluating different feeding strategies. Therefore, the present study focused on evaluating the effect of dilution rate using a mixture of glucose and xylose as carbon sources on cell mass and lipid production by continuous culture.

MATERIAL AND METHODS

Strain and media

The oleaginous yeast *L. starkeyi* DSM 70296 was used throughout this study. The composition of the inoculum and fermentation medium is described at Anschau et al. (2014).

Culture conditions

The inoculum was prepared through two successive cell propagations in liquid media at 28 ºC and 150 rpm in an orbital shaker. The first was incubated for 48 h, and the second was incubated for 30 h until a cell mass of 10 g/L (equivalent to 1x108 cells/ mL) was obtained. The experiments were conducted in a 3 L bioreactor (New Brunswick, USA) with a working volume of 1 L. The aeration rate, agitation, and temperature were set to 1 vvm, 400 rpm, and 28 ºC, respectively. The pH was maintained at 5.5 through the automatic addition of 2 M NaOH. Aliquots were collected at various intervals and stored at -20 ºC until their analysis for substrate concentrations, cell dry weight, and lipid content.

For the continuous cultures, a sugar solution (glucose:xylose) containing 60 g/L (30:70, w/w) was used and the C/N ratio was adjusted to 50. The dilution rate (D) was attained by varying the medium flow. According to Gill et al. (1977), maximum lipid accumulation requires the yeast to be grown at a dilution rate equal to one-third the value of the maximum growth rate. In this order, dilution rates of 0.03 h^{-1} and 0.06 h^{-1} were studied and the continuous culture started after 24 h of batch cultivation. Steady-state conditions were obtained after a continuous flow of at least four working volumes of the culture medium.

Analytical methods

The analytical methods used in this study are described at Tapia et al. (2012) and Anschau et al. (2014). All the analysis were performed in triplicate and error bars denote the standard deviation.

RESULTS AND DISCUSSION

The chemostat culture of *L. starkeyi* at a D of 0.03 h^{-1} resulted in cell mass and lipid concentrations of 13.3 and 7.1 g/L (48.4%), respectively. The concentration of unconsumed sugars was approximately 30 g/L (Figure 1A), which is equal to 50% of the initial concentration. The cell mass yield was 0.443 g/g, whereas the lipid yield reached 0.236 g/g. Cell mass, lipid content and lipid yield increase with the decreasing of the growth rate. At a D value of 0.06 h^{-1}, the cell mass reached 10 g/L (Figure 1B) with a lipid content of 43.3% (4.33 g/L). The yields of cell mass and lipids were also lower than those obtained with a chemostat culture at 0.03 h^{-1} (0.333 and 0.161 g/g, respectively). Shen et al. (2013) studied chemostat cultures ranged from 0.02 to 0.20 h^{-1} by *Rhodosporidium toruloides*. At the lowest D, 77% of glucose was consumed resulting in a cell growth of 8.67 g/L with 61.8% of lipids. At D of 0.20 h^{-1}, only 4% of glucose was consumed reaching a cell mass of 1.63 g/L and 13.2% of lipids.

At a D of 0.06 h^{-1}, xylose and glucose were directed to non-lipid cell mass production because this condition favored cell growth using consumed nitrogen. A D value lower than 0.06 h^{-1} is normally required for optimum conversions (HUANG et al., 2009) because the microbial cells need to remain within the chemostat for at least 12–24 h to consume the available nitrogen and convert the remaining sugars to oil.

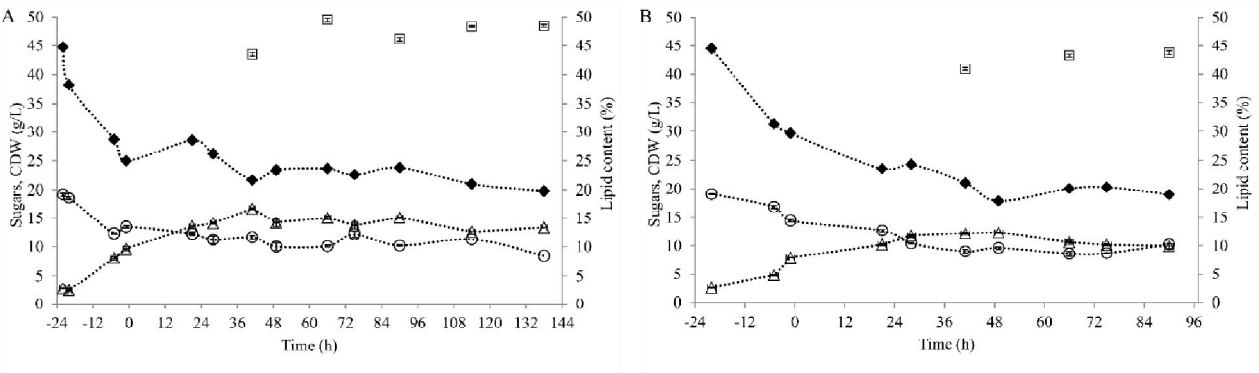

Figure 1. Cultivations at (A) 0.03 h^{-1} and (B) 0.06 h^{-1}. The results show CDW (△), lipid content (□), xylose (♦) and glucose (○) concentrations. Continuous cultivations started at 0 h after a batch stage (negative scale).

Papanikolaou and Aggelis (2002) described that higher concentrations of sugars are detected in the culture fluid at high dilution rates, which results in an increased cell mass yield but a decreased lipid fraction. At both dilution rates investigated in our study, the residual sugar remained at the same concentration (approximately 30 g/L), and the cell mass yield and lipid fractions were slightly higher at the lower dilution rate. Lipid-free material and lipid yields are influenced by the value of D. At higher D, sugars are consumed mainly for non-lipid material synthesis.

The fatty acid profile showed slight changes between the dilution rates. The lipids produced mainly include palmitic acid and oleic acid. The microbial lipids chains from *L. starkeyi* contain 14 to 18 carbons and exhibit low degrees of unsaturation, which is desirable for their application in biodiesel production. The fatty acid composition profile was quite similar to that of palm oil (LIU et al., 2010). Soap, hand and body lotions, fatty acid methyl esters, and epoxidized palm oil are the main industrial products that are produced from palm oil. It is also an excellent frying oil due to its non-sticky and non-foaming characteristics (NORHAIZAN et al., 2013), indicating that the lipids produced by *L. starkeyi* have great potential as a feedstock for biodiesel production and other oleochemical applications.

CONCLUSIONS

This work demonstrates the possibility of using *L. starkeyi* DSM 70296 for the production of cell mass rich in lipids from xylose and glucose (30:70, w/w), which simulates the hemicellulose hydrolysate of sugarcane bagasse, at continuous cultivations. The identification of microorganisms that can utilize both glucose and xylose simultaneously and efficiently appears to be a key aspect of the utilization of a lignocellulosic feedstock, and the elucidation of the

mechanism underlying the assimilation of these sugars is crucial for the further development of this industrial process.

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