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The effect of amino acids on lipid production and nutrient removal by *Rhodotorula glutinis* cultivation in starch wastewater

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HIGHLIGHTS

- 9 Kinds of amino acids in starch wastewater were determined.
- Amino acids had promoting effects on biomass, lipid production and COD removal.
- High lipid yield was obtained in starch wastewater without nitrogen supplement.
- Starch wastewater can be as a potential nitrogen source for lipid production.

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ABSTRACT

In this paper, the components of amino acids in mixed starch wastewater (corn steep water/corn gluten water = 1/3, v/v) were analyzed by GC–MS. Effects of amino acids on lipid production by *Rhodotorula glutinis* and COD removal were studied. The results showed that mixed starch wastewater contained 9 kinds of amino acids and these amino acids significantly improved the biomass (13.63 g/L), lipid yield (2.48 g/L) and COD removal compared to the basic medium (6.23 g/L and 1.56 g/L). In a 5 L fermentor containing mixed starch wastewater as substrate to culture *R. glutinis*, the maximum biomass, lipid content and lipid yield reached 26.38 g/L, 28.90% and 7.62 g/L, with the associated removal rates of COD, TN and TP reaching 77.41%, 69.12% and 73.85%, respectively. The results revealed a promising approach for lipid production with using amino acids present in starch wastewater as an alternative nitrogen source.

1. Introduction

With the worldwide constantly increasing energy consumption due to the population growth and economic development, conventional fossil fuel resources such as petroleum, coal and natural gas are not able to meet the growing demand for energy and have caused serious environmental pollution problems in the past decades (Ajanovic and Haas, 2010; Gui et al., 2008). Biodiesel, as one kind of green and renewable substitute for fossil fuels, has been gaining a considerable worldwide attention. The utilization of biodiesel as a non-toxic energy source has some favorable environmental benefits in reducing CO_2 and toxic emission (Atadashi et al., 2010; Rajagopal et al., 2007). Meanwhile, biodiesel has better effects on the biodegradability than fossil fuels, which can be degraded by 84% after 30 days when compared with 13.6% degradation of normal diesel (Silva et al., 2012). Microbial lipid belonging to the third generation biodiesel feedstock has significant advantages compared with the second generation biodiesel, including shorter fermentation time, higher per-acre productivity, and less limitation of location, seasons and climates, which support the development of microbial lipid (Nigam and Singh, 2011; Xue et al., 2006). Oleaginous yeasts, especially *Rhodotorula glutinis*, are considered as a promising biofuel feedstock due to its unicellular relatively high growth rate and rapid lipid accumulation ability compared with bacteria, moulds and algae. Besides, oleaginous yeasts are less affected by the culturing conditions and able of generating different lipids according to a variety of low-carbon sources (Saenge et al., 2011). It has been reported that *R. glutinis* as pigmented yeasts can not only accumulate lipids but also produce certain carotenoids, namely β-carotene. Carotene has a high economic value as colorant for food, feed, and cosmetic products, as nutritional supplement due to its vitamin A character and as pharmaceutical products for the anticancer ability (Malisorn and Suntornsuk, 2008; Xue et al., 2008; Zhang et al., 2014).

However, no commercial application of biodiesel production from microbial lipid has been reported due to its high cost of lipid production, extraction and conversion. Particularly, the main

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barrier of microbial cultivation is the cost of nutrient source which accounts for more than 80%. The reduction of the cost of the raw sources is hence crucial in promoting the development of a microbial biofuel industry (Fontanille et al., 2012; Schneider et al., 2013). Previous studies have been conducted using cheap and abundant carbon sources as substrates for microbial biofuel production, such as wastewater including food processing wastewater, municipal and domestic wastewater (Ling et al., 2014; Ren et al., 2015). In China, the starch production plans have increased to total over 600 companies, generating over 20 million tons of starch wastewater (Lu et al., 2009). Starch wastewater produced from starch production industries has a high chemical oxygen demand (COD) because of abundant organic matter such as simple sugars, organic acids, proteins and inorganic salts, which make it more difficult to treat than municipal effluents. If starch wastewater is discharged into the environment without sufficient treatments, it will cause severe environmental pollution. Meanwhile, the wasteful organic matters in starch wastewater could be utilized and converted into biofuel by oleaginous yeasts. Therefore, researchers have been looking for an economic and effective method to treat starch wastewater. Coupling the oleaginous yeast cultivation and wastewater treatment is a feasible alternative treatment process. Many studies have demonstrated that oleaginous yeasts are able to grow in corn starch wastewater by utilizing the abundant organic carbon sources, nitrogen and phosphorus and translating these organic matters to microbial oils (Xue et al., 2010; Yang et al., 2015). The oleaginous yeasts, particularly *R. glutinis*, not only present good growth but also exhibit high effective degradation ability for removing large amounts of nutrients in wastewater. A previous study has suggested that *R. glutinis* cultured in starch wastewater can produce 40 g/L biomass and 35% lipid content with about 80% COD degradation ratio (Xue et al., 2010). Starch wastewater, rich in organic nutrients, might be an alternative medium with satisfactory performance for culturing oleaginous yeasts.

Starch wastewater, a mixture generated from different stages of the corn starch production processing, mainly includes corn steep water and corn gluten water (Wang et al., 2015). Corn steep water, generated in the process of corn steeping and wet-grinding, contains abundant soluble nutrients, such as carbohydrates, proteins and amino acids which are not only used as organic carbon and nitrogen source but also essential factors for promoting growth and metabolism. Corn gluten water, produced from the separation process of corn starch milk, is rich in proteins. Numerous studies focused on taking advantage of various kinds of sacchariferous organic wastewater as carbon source to produce biofuel (Chu et al., 2015; Peng et al., 2013; Wang et al., 2015). However, few studies have been done to explore the potential of wastewater as alternative nitrogen source for lipid production and the effect of the nitrogen ingredient in wastewater on microorganism growth. In addition, a few papers reported the analysis of wastewater compositions through detailed methods due to the complex ingredients in wastewater.

The present study aimed of establishing a qualitative and quantitative method to analyze the compositions of amino acids in starch wastewater and investigate the effects of amino acids on *R. glutinis* growth and nutrients removal. Furthermore, the lipid production and starch wastewater treatment performance by using amino acids as nitrogen source were evaluated in a 5 L fermentor containing mixed starch wastewater as substrate.

2. Material and methods

2.1. Microorganism, culture conditions, and wastewater

Yeast strain *R. glutinis* CGMCC No. 2258 used in this study was obtained from the China National Research Institute of Food and Fermentation Industries and kept on yeast extract, peptone and dextrose (YPD) agar slant at 4 °C.

The seed/basic medium contained (g/L) glucose 40, $(NH_4)_2SO_4$ 2, KH_2PO_4 7, Na_2SO_4 2, $MgSO_4$ 1.5, Yeast extract 1.5. The amino acids in starch wastewater were analyzed by GC–MS. The basic medium, containing the same amount of amino acids as starch wastewater, was regarded as the amino acid medium. The starch wastewater medium, used in a 5 L fermentor, contained corn steep water and corn gluten water (1:3, v/v), with 40 g/L glucose supplement.

The strain kept on the YPD agar slant was activated for 24 h at 30 °C before cultivation in the seed culture medium. The inoculums were cultured in a 250 mL flash containing 50 mL seed culture medium for 24 h at 30 °C with rotation speed of 220 rpm, and then, inoculated into the fermentation medium with 10% (v/v), which was cultured at 30 °C with rotation speed of 220 rpm. The initial pH value of all mediums was adjusted to 5.5 and all mediums were sterilized at 121 °C for 20 min for subsequent culturing and analysis.

Starch wastewater was obtained from the Rui Xing Biological and Chemical Company of Shan Dong, China. The wastewater samples were stored at −20 °C for subsequent culturing and analysis.

2.2. Analytical methods

2.2.1. Measurement of glucose, biomass and lipid

The glucose concentration was analyzed by a glucose biosensor (SBA 40C, Shandong Academy of Sciences, China). Biomass was detected by the dry cell weight method (Yen and Zhang, 2011; Zhang et al., 2014). After the culture sample was centrifuged at 8000 rpm for 15 min at ambient temperature, cell pellets were washed three times with distilled water. Due to a certain relationship between biomass dry weight and turbidity, the washed cell pellets were diluted with distilled water and the absorbance was detected at 600 nm (OD600), using a standard curve of absorbance against dry cell mass concentration. According to different culture mediums, this study used two kinds of standard curves. The biomass in the basic medium and the amino acid medium was determined using Eq. (1) and the biomass in mixed wastewater was determined using Eq. (2):

$$\text{Biomass (g/L)} = 0.0216 * \text{OD600} * \text{dilution factor} + 0.0009 \quad (1)$$

$$\text{Biomass (g/L)} = 38.167 * \text{OD600} * \text{dilution factor} + 1.3926 \quad (2)$$

Lipid was extracted as described in the previous study (Xue et al., 2008). The lipid content and the total lipid were determined by the gravimetric method. The lipid components were analyzed according to a previous report (Zhang et al., 2014).

2.2.2. Measurement of TN, TP, NH_4^+-N and COD

The concentrations of TN, TP and NH_4^+-N were measured using reagents purchased from HACH Company with reactor DRB200 and detector DRB3900 according to the Standard Methods (APHA/AWAA/WEF, 1995). The concentration of COD was measured by the $K_2Cr_2O_7$ method, using a standard curve of absorbance against COD concentration (COD concentration (mg/L) = 2110.3 * OD605 * dilution factor − 30.52) (Xue et al., 2006). The COD concentration of amino acids in wastewater was calculated according to the analysis result of amino acids.

2.2.3. Measurement of amino acids in wastewater

GC-2010 gas-chromatography (Shimadzu, Japan), coupling mass spectroscopy, was used for amino acids analysis. In order to remove proteins and inorganic salts, the sample was centrifuged at 10000 rpm for 5 min with adding the same volume of acetonitrile solvent and the supernatant was filtered through a filter membrane (0.45 μm). After 20 μm of filtrate was dried at 40 °C until