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Biomass production of yeast isolate from salad oil manufacturing wastewater

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Abstract

Conversion of oil-rich salad oil manufacturing wastewater (SOMW) into protein source for animal feed through biomass production of yeast isolate was investigated in this study. Five species of yeasts, including *Rhodotorula rubra*, *Candida tropicalis*, *C. utilis*, *C. boidinii*, *Trichosporon cutaneum*, were isolated from SOMW following enrichment culture. Of them, *C. utilis* was chosen as the sole biomass producer in the study due to its greatest oil uptake rate, 0.96 kg oil kg^{-1} biomass d^{-1}, and highest specific growth rate, 0.25 h^{-1}. The cells of *C. utilis* contained 26% protein, 9% crude lipid, 55% carbohydrate and balanced amino acid compositions. The initial N:C ratio in SOMW drastically influenced oil reduction efficiency, biomass production and protein content of *C. utilis*, and therefore a range between 1:6 and 1:8 was recommended in consideration of these three factors simultaneously.

Keywords: Oil-rich wastewater; Microbial biomass; Protein; Yeast; *Candida utilis*

1. Introduction

Since World War II, some high quality yeast species such as *Candida* and *Saccharomyces* spp. has been employed as a producer of microbial protein to convert agro-industrial wastes, e.g., effluents from paper mill and olive mill, into a valuable protein supplement for animal feeds (Ejiofor et al., 1996; Gharsallah, 1993; Nigam, 1998). The process is generally thought to be an attractive way to both enhance wastewater purification and increase resource utilization.

Recently, more and more yeast species are isolated from nature and proven superior to conventional protein producer in laboratory or field studies (Nigam, 2000; Arnold et al., 2000; Urano et al., 2002; Yang et al., 2003), which offers more choices for the microbial protein process. For example, a yeast species, *C. langeronii*, is isolated under selective conditions as an alternative microorganism of *C. utilis* because of the inability of the latter to utilize L-arabinose at temperatures above 42 °C in the absence of vitamins (Nigam, 2000). In comparison with a strain of *C. utilis*, the isolate, *Galactomyces geotrichum* T2B, gives consistently higher biomass yields from silage effluent along with excellent nutrient removal (Arnold et al., 2000). The co-culture of two yeast isolates, *C. halophila* and *Rhotorula glutinis*, could effectively remove organic pollutants, above 85%, from fermentative wastewater even when ammonium–nitrogen concentration reaches as high as about 19 $g l^{-1}$ (Yang et al., 2003).

In salad oil manufacturing, a considerable amount of fatty acid in raw vegetable oil is separated from glyceride by a water washing process in the refining plant, and discarded as high-strength salad oil manufacturing wastewater (SOMW) (Zheng et al., 2001). The effluent contains no harmful substance and is a potential source

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of cell mass production. This study sought to determine if it is possible to achieve effective resource reutilization from SOMW as well as high organic utilization efficiency through the use of yeast isolate. In the study, we evaluated the biomass production from SOMW using yeast isolate following enrichment, isolation and identification. Aiming to improve oil reduction, protein content and cell yield, the study thereafter discussed the effect of initial N:C ratio.

2. Methods

2.1. Enrichment, isolation and identification of yeast

Field soil was collected from several spots contaminated with SOMW and enriched in 500 ml Erlenmeyer flasks containing 150 ml of 10-fold diluted SOMW at 170 rpm on a reciprocal shaker at 25 °C. The pH of the solution was adjusted to 5.5%, and 0.25% sodium propionate was added to the solution, which minimized the propagation of bacteria and molds. The cultivation was conducted in a fill-and-draw mode for 20 days during which the solution was changed once a day. After being cultured in YPD medium (2% dextrose, 1% yeast extract, 1% casein peptone) for 2 days, the culture was transferred to YPD agar plates for isolation. The isolated yeast strains were identified based on morphological and biochemical characteristics described by Kreger-van Rij (1984).

2.2. Comparison of yeast species

The yeast isolates were individually inoculated into YPD medium and cultured for 48 h at 25 °C on a rotary shaker at 170 rpm under the aseptic conditions. The cells were centrifuged at 4000 rpm for 15 min and washed three times using physiological saline prior to storage in aseptic physiological saline at 4 °C. Subsequently, a loopful of the yeast cells was individually inoculated and cultured at pH 5.5 in 5 l of 10-fold diluted SOMW to which about 3 g NH_4Cl was added to give an initial N:C ratio of about 1:25. The liquids were aerated at 25 °C by air pump to maintain a dissolved oxygen level of 2 mg l^{-1}. Cell growth was monitored by dry weight of yeast biomass in 10 ml culture following centrifugation at 4000 rpm for 15 min. Finally, specific growth rate and oil reduction efficiency of individual yeast species were summarized over the experimental period.

2.3. Yeast cell composition analyses and effect of initial N:C ratio

C. utilis OZ993 cells, which was cultured in 5 l of 10-fold diluted SOMW with an initial N:C of 1:25, were collected to analyze its chemical composition and to use as consequent experimental inocula. The analytical items of chemical composition included crude protein, crude lipid, amino acid, and so on.

To investigate the effect of initial N:C on *C. utilis* OZ993, a series of experiments was conducted in 5 l of 10-fold diluted SOMW with N:C ratios ranging between 1:3 and 0. In the beginning, the inocula of *C. utilis* OZ993 were inoculated to achieve an initial cell concentration of approximately 1 g l^{-1}. Cell mass, protein content and oil reduction were respectively measured at the end of experimental period of 24 h.

2.4. Analytical methods

Analyses of the wastewater followed standard methods (CSEPA, 1998). Prior to chemical composition analysis of yeast cells as described by Xia and Zhu (1994), the resulting cells were harvested by centrifugation and desiccated at 103–105 °C for 6 h. Among them, crude protein was estimated by the method of Kjeldahl and crude lipid by the Soxhelt ether extraction method. The ash content was determined after combusting samples at 550–560 °C for 2 h. Amino acids were analyzed with a Hitachi Amino Acid Auto-Analyzer (model 825-10) after hydrolyzing the yeast cells in 6 N HCl for 22 h at 110 °C under vacuum.

3. Results and discussion

Table 1 listed the characteristics of SOMW sample. It contained a high amount of oil, typically 90 g l^{-1}, which consisted mainly of fatty acids. There was also plentiful phosphorus content in the SOMW that amounted to 6.5 g l^{-1}. In contrast, the total nitrogen concentration was extremely low, at 154 mg l^{-1}, which led to N:C ratios as low as 1:265.

A total of ten isolates capable of utilizing SOMW were obtained from an enriched culture of SOMW. According to their morphological and physiological characteristics, the isolates consisted of five species belonging to three genera and labeled as OZ991–OZ995 in Table 2. *Candida* spp., including *C. tropicalis*

Table 1
Characteristics of salad oil manufacturing wastewater used in the study

Component	Concentration
Chemical oxygen demand (g l^{-1})	134 ± 0.38
Total organic carbon (g l^{-1})	39 ± 0.45
Oil (g l^{-1})	90 ± 1.12
Carbohydrate (g l^{-1})	9.1 ± 0.41
Protein (g l^{-1})	0.44 ± 0.01
Total nitrogen (g l^{-1})	0.15 ± 0.01
Total phosphorus (g l^{-1})	6.5 ± 0.15
pH	8.5–9.0

Values are the means ± standard errors of three determinations.