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Short Communication

Biotransformation of vegetable and fruit processing wastes into yeast biomass enriched with selenium

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Abstract

Water extracts of cabbage, watermelon, a mixture of residual biomass of green salads and tropical fruits were used for yeast cultivation. These extracts contained from 1420 to 8900 mg/l of dissolved organic matter, and from 600 to 1800 mg/l of nitrogen. pH of the extracts was in the range from 4.1 to 6.4. Biomass concentration of yeast, *Saccharomyces cerevisiae* CEE 12 grown at 30 °C for 96 h in the sterilized extracts without any nutrient supplements was from 6.4 to 8.2 g/l; content of protein was from 40% to 45% of dry biomass. The yield was comparable with the yield of yeast biomass grown in potato dextrose broth. The biomass can be considered as the protein source. Its feed value was enhanced by incorporation of selenium in biomass to the level of 150 μg/g of dry biomass. Therefore, it was recommended to transform the extracts from vegetable and fruit processing wastes into the yeast biomass enriched with selenium.

Keywords: Vegetable and fruit processing wastes; Yeast; Cultivation; Feed protein; Selenium

1. Introduction

Approximately 1,000,000 tonnes of food waste in Singapore produced annually, but only 1% of this volume is recycled (NEA Web Site, http://www.nea.gov.sg). The nutrients of food waste may be re-used in agriculture by composting or by biotransformation of food waste into animal feed. Vegetable and fruit processing wastes contain mainly starch, cellulose, soluble sugars and organic acids. It is known that some microorganisms, mainly yeast, can utilize soluble sugars and organic acids, producing biomass with high protein content. Yeasts are also common microorganisms, which grow in vegetable and fruit processing wastes. For example, citrus processing waste usually was predominantly colonised by mesophilic yeast (van Heerden et al., 2002). Cultivation of yeast or filamentous fungi have been proposed for decreasing the content of organic acids in orange processing waste before its anaerobic treatment (Srilatha et al., 1995). The yeast, *Candida utilis* was selected for cultivation in concentrated effluents of the food industry after its anaerobic acidogenic treatment (Elmaleh et al., 1999). Recently published papers have described the utilization of different vegetable processing waste, for example, Chinese cabbage juice (Choi et al., 2002), waste brine generated from kimchi production (Choi and Park, 1999), deproteinized leaf juices (Chanda and Chakrabatri, 1996), corn silage juice (Hang et al., 2003), as a nutrient source for yeast growth.

Therefore, easily extracted organic matter from vegetable and fruit processing wastes can be used as a medium for yeast cultivation. In this case, the solid residue of food waste after extraction can be converted by aerobic treatment/composting into fertilizer or by

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anaerobic digestion into biogas (Srilatha et al., 1995; Yun et al., 2000).

Another interesting approach is to increase the nutritional value of yeast biomass by enriching with selenium. Selenium (Se) has been identified as an antioxidant of importance in the diet. The need for selenium in human and animal nutrition is well recognized (Schrauzer, 2001). Yeast, enriched with selenium, are appropriate supplements of Se for humans (Alfthan et al., 2000; Schrauzer, 2001) and animals (Pehrson et al., 1999). Under appropriate conditions, yeasts are capable of accumulating large amounts of selenium, and incorporating them into organic selenium-containing compounds, mainly selenomethionine, which is the best source of selenium for organisms (Demirci et al., 1999; Suhajda et al., 2000).

The technologies of food waste aerobic bioconversion into fertilizer (Wang et al., 2003) and food waste anaerobic bioconversion into biogas and fertilizer (Wang et al., 2002) were developed. Production of yeast biomass using extracts of easily dissolved organic matter from vegetable and food processing wastes was proposed as a first step of the technology for food waste bioconversion into value-added by-product followed by aerobic or anaerobic treatment of solid residue. The aim of this research was biotransformation of the part of selected vegetable and fruit processing wastes into yeast biomass enriched with selenium.

2. Methods

The strain of yeast *Saccharomyces cerevisiae* CEE 12 was isolated from enrichment culture on malt extract agar (MEA) (Difco Laboratories, USA). The shredded tropical fruits were placed in a 0.5 l flask filled with 0.5 l of tap water. Ethanol was added as a source of carbon to create the final concentration of 1% to promote selection of yeast. The flask was placed on a shaker at 30 °C and 150 rpm for four days. Microbiological isolation was carried out by a spread-plate method from serial ten-fold dilutions of the enrichment culture in phosphate-buffered saline solution (PBS) with a pH of 7.2. The plates were prepared with malt extract agar and were incubated at 30 °C for two days under aerobic culture conditions. The cells from individual colonies were transferred to further set of plates with MEA. The strain of yeast *Saccharomyces cerevisiae* CEE 12 was selected for the cultivation in the extract from vegetable and fruit processing wastes.

Fresh vegetable and fruit processing wastes were collected from a canteen of the university, shredded to particles with the size approximately $6 \times 6 \times 6$ mm^3 in Robot-Coupe (CL50 Ultra, France) and placed in flasks. Tap water was added in a ratio of 1:1 by weight. The flasks were placed on the shaker. The extraction was performed for 24 h under shaking at room temperature. The solid residue was separated from liquid fraction by centrifugation at 4000 g for 5 min; the supernatant was used for yeast cultivation after sterilization in autoclave at 121 °C for 15 min. To check the influence of nitrogen and phosphorus addition on yeast growth, 5 g $NH_4H_2PO_4$ was added to 1 l of extract before sterilization. Potato dextrose broth (PDB) (Difco Laboratories, USA) was used for starter culture growth. One litre of PDB contains potato infusion from 200 g of potato and 20 g of bacto dextrose. Solid residue of food waste was used for the aerobic bioconversion into fertilizer or by anaerobic bioconversion into biogas and fertilizer.

Selenium was added to the sterile medium before the start of yeast cultivation as a solution of sodium hydroselenite ($NaHSeO_3$), at a selenium concentration of 100 μg/ml. Initial selenium concentrations in the medium were 0 μg/ml in control and 5 μg/ml in experimental treatments.

Starter culture was prepared by yeast cultivation in PDB at 30 °C on rotary shaker at 150 rpm for 24 h. An aliquot of 10 ml of the culture was used to inoculate 200 ml of extract from vegetable and fruit processing wastes. Yeasts were cultivated at 30 °C on rotary shaker at 150 rpm for 96 h.

Cell growth was measured as an optical density at 540 nm using a spectrophotometer UV-1201V (Shimadzu Corporation, Japan). Dry weight of yeast biomass was determined after drying at 105 °C to a constant weight.

The enumeration of yeast and bacterial cells was carried out using spread-plate from a serial ten-fold dilution of the suspension produced by the vortexing of 1 ml of microbial suspension in 9 ml of phosphate-buffered saline solution. Tryptic soy agar (DIFCO Laboratories, USA) was used for the bacterial growth and malt extract agar (Difco Laboratories, USA) was used for the yeast growth. The enumeration of colony forming units (c.f.u.) was provided after incubation of Petri dishes at 30 °C for one and two days for bacteria and yeast, respectively.

The identification of bacteria, grown in enrichment culture, was performed by the partial sequences of 16S rDNA as described (Wang et al., 2003) by using forward primer 530F (5′-GTGCCAGCMGCCGCGG-3′) and reverse primer 907R (5′-CCGTCAATTCMTTTRAGTTT-3′). PCR amplicons were purified with a Qiagen PCR purification kit (Qiagen GmbH, Hilden, Germany). The nucleotide sequences of representative clones were determined using the dideoxy chain termination chemistry and the ABI model 310A sequencer (Applied Biosystems, Perkin-Elmer). The ABI PRISM® BigDyeTM Terminator Cycle Sequencing ready-reaction kit (version 2.0) (Applied Biosystems, Perkin-Elmer) was used as specified by the manufacturer. Partial 550 bp sequences were produced from experimentally determined sequences using BioEdit program (Hal, 1999). The Basic Local Alignment Search Tool (BLAST) search program