Electronic Journal of Biotechnology 18 (2015) 428–432

HOSTED BY

Contents lists available at ScienceDirect

Electronic Journal of Biotechnology

Research Article

Biodegradation of deproteinized potato wastewater and glycerol during cultivation of *Rhodotorula glutinis* yeast

Anna Maria Kot *, Stanisław Błażejak, Agnieszka Kurcz, Iwona Gientka

Department of Biotechnology, Microbiology and Food Evaluation, Faculty of Food Sciences, Warsaw University of Life Sciences, Nowoursynowska 159C, 02-776 Warsaw, Poland

ARTICLE INFO

Article history:
Received 10 May 2015
Accepted 9 September 2015
Available online 28 October 2015

Keywords:
Biodegradation
Chemical oxygen demand
Industrial waste
Rhodotorula

ABSTRACT

Background: Deproteinized potato wastewater and glycerol are two by-products which are difficult to dispose. The objective of this study was to determine the ability of *Rhodotorula glutinis* to use glycerol and nitrogen compounds present in deproteinized potato wastewater and to evaluate the ability of simultaneous biodegradation of potato wastewater and glycerol via microbiological methods.
Results: It has been found that *R. glutinis* used glycerol and potato wastewater as a source of carbon and nitrogen, respectively. The highest degree of glycerol content (70.6%) reduction was found after cultivation of the investigated strain using a medium with 5% glycerol. In this medium, a significant reduction in the total protein content, estimated at 61%, was observed. The process of 72 h cultivation of yeast in a medium containing potato wastewater and 5% glycerol reduced the chemical oxygen demand (COD) more than 77%. Supplementation of media with high doses of glycerol (i.e. 20 and 25%) led to decreased metabolic activity in the yeast strain tested.
Conclusion: It has been found that there is a possibility of simultaneous biodegradation of potato wastewater and glycerol during the cultivation of *R. glutinis*.

1. Introduction

In many industrial units, production processes always generate by-products. For these by-products, no rational management concepts exist and their disposal under natural conditions may lead to a progressive degradation of the natural environment. Examples of such wastes are deproteinized potato wastewater generated during the production of potato starch and glycerol fraction occurring during the production of biodiesel. Problems arising during the disposal of these two by-products have led to the search for new biotechnological methods for their treatment.

Potato wastewater is the primary waste generated during the production of potato starch. It has been estimated that during the processing of 1000 tons of potatoes, about 600 m^3 of potato wastewater is produced [1]. Due to the high content of organic substances, potato wastewater from the production of potato starch creates waste disposal problems. To reduce the content of nitrogen compounds, potato-protein precipitation is performed during the thermal-acid coagulation process. Deproteinized potato wastewater contains 2.9–4.3% of dry matter on average, of which protein, sugar and fat (emulsified with water) contents are estimated at 0.93–1.57%, 0.5–0.8% and approximately 0.2%, respectively. It also contains significant amounts of minerals (about 1%), of which potassium (600 mg/L) and phosphorus (about 300 mg/L) are dominant. Moreover, this waste is characterized by a high value of chemical oxygen demand (COD), which is equal to at least 20,000 mg O_2/L [2]. Potato wastewater is mostly utilized during field sprinkling [3], which may lead to adverse eutrophication of water [2] and soil sealing [3] in the natural environment.

The main by-product generated during the production of biodiesel is a glycerol fraction. This consists mainly of pure glycerol (50–65%), and among the remaining components one can distinguish methanol, free fatty acids, mono- and diacylglycerols, phospholipids, tocopherols, colorants, soaps, water, and catalyst residues [4]. In 2012, biodiesel production was estimated at 23.40 million tons, of which about 40% was produced in the European Union, a leading manufacturer of this fuel for years. In comparison to 2009, the global production of biodiesel has increased by more than 30% [5]. It is expected that in the future, biodiesel production will increase, which in turn will generate higher amounts of waste glycerin. The process of refining crude glycerol to high purity glycerol is energy intensive and expensive; therefore, new methods for the disposal and management of this waste are emerging [6].

Biodegradation is an alternative method of decomposition of pollutants using living organisms [7]. The fundamental issue concerning biotechnological disposal is the selection of micro-organisms, which

* Corresponding author.
E-mail address: anna_kot@sggw.pl (A.M. Kot).
Peer review under responsibility of Pontificia Universidad Católica de Valparaíso.

http://dx.doi.org/10.1016/j.ejbt.2015.08.006

must decompose contaminants in a short time without producing toxic metabolites [8]. So far, molds have particularly been used for this purpose [9,10,11]. In this paper, an attempt was made to use *Rhodotorula glutinis* for simultaneous biodegradation of potato wastewater and glycerol.

R. glutinis (*syn. Rhodotorula gracilis*) yeasts are anamorphic stages of *Rhodosporidium toruloides* [12,13,14,15]. They are aerobes and their optimum growth temperature lies in the range of 20–40°C. They have the ability to use many substrates as a carbon source, e.g. glucose, galactose, sucrose, maltose, trehalose, ethanol, glycerol, or hexadecane [16]. *R. glutinis* yeasts are capable of the biosynthesis of various important compounds, including carotenoids [17], phenylalanine ammonia lyase (PAL) [18] and microbial lipids [19].

The objective of this study was to evaluate the ability of *R. glutinis* yeast to use nitrogen compounds present in deproteinized potato wastewater and glycerol, and to determine the possibility of simultaneous biodegradation of these two wastes via microbiological methods.

2. Materials and methods

2.1. Microorganism

R. glutinis LOCK 0051 yeast strain derived from the Pure Culture Collection of the Department of Biotechnology and Food Microbiology, Warsaw University of Life Sciences, constituted the biological material used for the study. Yeasts were stored on slants of YPD medium at 4°C and were subcultured every month.

2.2. Potato wastewater

Deproteinized potato wastewater was collected from the technological line of potato starch production (PEPEES S.A., Łomża, Poland). The waste was sterilized (117°C for 10 min, HICLAVE HG-80, HMC Europe) and stored at room temperature. To remove residual solids (starch and cellulose fibers) before use, potato wastewater was centrifuged at 12,900 × g for 10 min (Centrifuge 5804R, Eppendorf). The dry matter content (g/L) was determined by the gravimetric method through drying a certain volume of the sample to constant weight at 105°C for 24 h (SML 32/250, Zatmet). Total protein content was determined by the Kjeldahl method. Samples were subjected to burning in concentrated sulfuric (VI) acid with the addition of a catalyst (Büchi Digestion Unit K-435), followed by alkalinization and distillation with steam water (Büchi Distillation Unit K-355). The resulting product (g/L) was calculated per total protein content using a factor equal to 6.25. The content of reducing sugars (calculated per glucose) in potato wastewater was determined spectrophotometrically ($\lambda = 550$ nm) using 3,5-dinitrosalicylic acid [20]. The result was given in g/L. The rate of chemical-oxygen demand (g O_2/L) in potato wastewater was determined by the dichromate method using the Hach Lange cuvette tests in the Water Center of the Warsaw University of Life Sciences. All the obtained results are listed in Table 1.

2.3. Preparation of inoculum

To prepare inoculum, YPD medium containing glucose (20 g/L), peptone (20 g/L), and yeast extract (10 g/L) was used [21]. The initial active acidity was determined at 5.0 ± 0.1. Cultivation was carried out in flat-bottomed flasks containing 100 mL of the medium at 28°C for 24 h at 200 rpm/min (SM-30 Control, Edmund Bühler).

Table 1
Characteristics of potato wastewater after the process of thermal-acid coagulation (PEPEES S. A., Łomża, Poland).

Dry matter (g/L)	Total protein (g/L)	Reducing sugars (g/L)	COD (g O_2/L)
35.8 ± 0.5	9.0 ± 0.66	2.96 ± 0.13	37.4 ± 2.8

2.4. Control and experimental cultures

For submerged yeast cultures, two culture media were used, YPD of an optimal composition for yeasts [21] and non-enriched potato wastewater (PW). For experimental cultures, five experimental media were used containing glycerol as a carbon source (POCH, Poland) and potato wastewater as a source of nitrogen. Glycerol was added to the medium at an amount of 50, 100, 150, 200, and 250 g/L. In later parts of the experiment, culture media with glycerol were denoted by the following abbreviations: PW + G5%, PW + G10%, PW + G15%, PW + G20% and PW + G25%. Inoculation was estimated at 10% (v/v). Cultivation of yeast was carried out on a reciprocating shaker (SM-30 Control, Edmund Bühler) for 72 h, at a speed of 200 cycles/min and temperature equal to 28°C. The experiment was carried out in triplicate.

2.5. Growth characteristics of yeast

Yield of dry cellular biomass was determined by the gravimetric method. A specified volume of culture was centrifuged for 10 min at 6000 × g (Centrifuge 5804R, Eppendorf), and rinsed with distilled water. The wet-cell biomass was dried at 85°C (SML 32/250, Zatmet) to a constant weight. The result was given in $g_{d.w.}$/L.

The optical density of the culture (OD) was determined using the spectrophotometry technique. Two milliliters of culture medium were collected and centrifuged at 5000 g for 5 min (MiniSpin Plus, Eppendorf). Post-culture medium was removed and 2 mL of deionized water was added to the biomass, followed by careful stirring and centrifugation of the solution using the same parameters. The supernatant was decanted and the biomass was suspended in 2 mL of deionized water, followed by the measurement of absorbance of the cell suspension at a wavelength of $\lambda = 600$ nm (UV1800 spectrophotometer, Rayleigh) against deionized water.

2.6. Determination of the potential of yeast for biodegradation of wastewater

To determine the ability to use the added glycerol and the protein present in the potato wastewater, their concentrations were determined in the latter hours of cultivation, followed by the determination of COD in selected culture media.

Glycerol content was determined via the chemical method by using oxidative properties of *meta*-periodate acid. As a result of its activity toward two adjacent hydroxyl groups in a glycerol molecule, decomposition of the main carbon chain is observed. As a result, two molecules of formaldehyde and a formic acid molecule are formed. The latter is titrated with 0.1 M sodium hydroxide in the presence of bromothymol blue. The result was given in g/100 mL [22].

Total protein content in post-culture media was determined by the Kjeldahl method, using a conversion factor equal to 6.25. The results were expressed in g/100 mL.

The rate of chemical-oxygen demand in post-culture media, based on the conducted experiments, was determined using Hach Lange cuvette tests in the Water Center of the WULS and the results were expressed in g O_2/L.

The experiment was carried out in triplicate and the values represent in the figures and tables are mean ± SD. Based on the obtained results, the degree of utilization of glycerol and nitrogen compounds per total protein, as well as the degree of reduction of COD, were determined.