Journal of Biotechnology 231 (2016) 32–39

Contents lists available at ScienceDirect

Journal of Biotechnology

journal homepage: www.elsevier.com/locate/jbiotec

Citric acid production from partly deproteinized whey under non-sterile culture conditions using immobilized cells of lactose—positive and cold-adapted *Yarrowia lipolytica* B9

Nazli Pinar Arslan [a], Mehmet Nuri Aydogan [a,*], Mesut Taskin [b,*]

[a] Department of Biology, Science Faculty, Ataturk University, 25240 Erzurum, Turkey
[b] Department of Molecular Biology and Genetics, Science Faculty, Ataturk University, 25240 Erzurum, Turkey

ARTICLE INFO

Article history:
Received 20 March 2016
Received in revised form 15 May 2016
Accepted 23 May 2016
Available online 24 May 2016

Keywords:
Yarrowia lipolytica B9
Citric acid
Immobilized cells
Non-sterile culture
Whey
Yeast

ABSTRACT

The present study was performed to produce citric acid (CA) from partly deproteinized cheese whey (DPCW) under non-sterile culture conditions using immobilized cells of the cold-adapted and lactose-positive yeast *Yarrowia lipolytica* B9. DPCW was prepared using the temperature treatment of 90 °C for 15 min. Sodium alginate was used as entrapping agent for cell immobilization. Optimum conditions for the maximum CA production (33.3 g/L) in non-sterile DPCW medium were the temperature of 20 °C, pH 5.5, additional lactose concentration of 20 g/L, sodium alginate concentration of 2%, number of 150 beads/100 mL and incubation time of 120 h. Similarly, maximum citric acid/isocitric acid (CA/ICA) ratio (6.79) could be reached under these optimal conditions. Additional nitrogen and phosphorus sources decreased CA concentration and CA/ICA ratio. Immobilized cells were reused in three continuous reaction cycles without any loss in the maximum CA concentration. The unique combination of low pH and temperature values as well as cell immobilization procedure could prevent undesired microbial contaminants during CA production. This is the first work on CA production by cold-adapted microorganisms under non-sterile culture conditions. Besides, CA production using a lactose-positive strain of the yeast *Y. lipolytica* was investigated for the first time in the present study.

© 2016 Elsevier B.V. All rights reserved.

1. Introduction

Citric acid (CA) is a commercially valuable microbial product used primarily in the foods/beverages, pharmaceutical/chemical, textile, and electroplating industries. This compound is mainly produced by microbial fermentation (Adeoye et al., 2015).

A large number of microorganisms are employed for CA production, but a few of them are reported to produce CA in industrial scale (Karasu-Yalcin et al., 2010a). For example, the yeast *Yarrowia lipolytica* and the mould *A. niger* are considered as potential microorganisms for fermentative production of CA (Kamzolova et al., 2011a; Rywinska et al., 2011; Moeller et al., 2011). On the other hand, a low-cost nutrient medium is usually preferred for the reduction of production cost in industrial biotechnology. In this means, low-cost agricultural wastes such as grape pomace, kiwi fruit peel, apple pomace, date seeds, molasses, carob pod and olive-mill waste water are used as substrates for CA production by *Y. lipolytica* and/or *A. niger* strains (Karasu-Yalcin et al., 2009; Adeoye et al., 2015).

Similarly, some studies have reported that cheese whey can be also utilized as substrate for fermentative CA production by *A. niger* strains (El-Samragy et al., 1996; El-Holi and Al-Delaimy, 2013). On the other hand, Karasu-Yalcin et al. (2009) have demonstrated that *Y. lipolytica* strains can produce CA using cheese whey as nitrogen, vitamins or mineral source. But, *Y. lipolytica* is reported to be lactose-negative (Kurtzman et al., 2011). Therefore, use of lactose-rich cheese whey as a carbon source for CA production by *Y. lipolytica* seems impossible. But, our previous study (Taskin et al., 2015) proposed a new strain of the yeast *Y. lipolytica* being capable of utilizing whey lactose as carbon source. Hence, this strain may be also used for CA production from whey.

Fermentative CA production is currently performed at the temperatures such as 28 and 30 °C using mesophilic microbial strains (Levinson et al., 2007; Papanikolaou et al., 2008; Adeoye et al., 2015). But, there is no report on CA production by cold-adapted strains of microorganisms including *Y. lipolytica* and *A. niger*. Cold-adapted microorganisms have important advantages such as high

* Corresponding authors.
E-mail addresses: mnaydogan@atauni.edu.tr (M.N. Aydogan), mesuttaskin61@hotmail.com (M. Taskin).

http://dx.doi.org/10.1016/j.jbiotec.2016.05.033
0168-1656/© 2016 Elsevier B.V. All rights reserved.

microbial growth rates as well as high enzymatic activities and catalytic efficiencies in the temperature range of 0–20 °C. Besides, the application of cold-adapted microorganisms in the temperature range of 0–20 °C decreases the risk of microbial contamination, thereby making the use of expensive heating/cooling systems unnecessary (Skowronek et al., 2003). As a result, it may be said that when the temperature of culture medium is adjusted to a low value that prevent the growth of mesophilic and thermophilic microorganisms, cold-adapted microorganisms may be cultivated for CA production under non-sterile culture conditions. Especially significant energy saving may be possible when the production media with large volume are directly used without sterilization.

Cell immobilization has some advantages when compared with free cell culture. For example, the reaction speed can be accelerated when immobilized cells are used. Immobilized cells are more resistant against the effect of inhibitory compounds and nutrient depletion. Immobilized cells can be easily separated from culture broth, and they can be reused. Furthermore, immobilization process reduces susceptibility of the cells to contamination and increases productivity and stability (Aydogan et al., 2014). Although the immobilized cells of mesophilic strains have been tested for CA production in some studies (Kim et al., 2002; Kosseva 2011), no attempt has been done on CA production by free and/or immobilized cells of cold-adapted microbial strains. Use of cell immobilization process well as low pH and temperature of the culture may help to prevent the growth of undesired mesophilic contaminants, thereby making the selective growth of the target microorganism possible under non-sterile culture conditions.

Considering the above mentioned facts, the present study was carried out to produce CA from partly deproteinized cheese whey under non-sterile culture conditions using immobilized cells of the cold-adapted (psychrotolerant) and lactose-positive yeast *Y. lipolytica* B9.

2. Materials and methods

2.1. Test microorganism and cheese whey

Cheese whey was obtained from Dairy Milk Processing System (Department of Food Engineering, Ataturk University, Erzurum). Cold-adapted and lactose-positive yeast *Yarrowia lipolytica* B9 (Taskin et al., 2015) whose genome was deposited into GenBank with access number KF486913, was used as test microorganism.

2.2. Preparation of partly deproteinized whey

Protein precipitation was performed by the heating of cheese whey at 90 °C for 15 min. Then, precipitated proteins were removed by filtration and centrifugation (5000 rpm for 5 min) (Roukas 1999; Taskin et al., 2015). The total sugar contents of partly deproteinized whey were measured by phenol–sulfuric acid method (Dubois et al., 1956). Nitrogen content was determined using a micro-Kjeldahl apparatus (Labconco Corporation, Kansas City, MO, USA), and total protein was estimated by multiplying the nitrogen content by 6.25.

2.3. Preparation of immobilized cells

Firstly, the yeast biomass was produced in the flasks containing malt extract broth (MEB). At the end of 48-h's growth, the yeast culture was centrifuged at 5000 rpm for 10 min. The obtained wet cells were removed and washed three times with sterile-saline water. Then, 6 g of wet cells were suspended in 40 mL of sterile-saline water. The total volume of cell suspension was adjusted to 50 mL with sterile-saline water (0.9% NaCl). Sodium alginate solution (0.5%–3% w/v) was prepared by dissolving sodium alginate in sterile-saline water at 70 °C. Then, sodium alginate solution was cooled at the room temperature. The cell suspension (50 mL) was mixed with an equal volume (1:1, v/v) of sodium alginate solution and stirred for 5 min. The cell density of the obtained final mixture (100 mL) was 2×10^8 cells/mL. This mixture was dropped into a well-stirred sterile $CaCl_2$ solution (2.5% w/v) from about 5–10 cm height using a sterile syringe. After the alginate drop contacted with $CaCl_2$ solution, beads containing the immobilized yeast cells formed. After hardening in $CaCl_2$ solution for 20 min, the beads (mean diameter of 3 mm) were washed with sterile buffer solution to remove excess calcium ions and un-encapsulated cells (Taskin 2013; Aydogan et al., 2014). In this way, about 82 g of immobilized beads were prepared. Based these results, it was calculated that per g of beads contained 0.073 g wet cells. Namely, the density of yeast cells in per g of beads was nearly 1.2×10^8. During all the optimization experiments, beads having the same cell density were used.

2.4. Optimization of culture conditions for citric acid production by immobilized cells

CA production using immobilized cells was performed in 250 mL flasks containing 100 mL of the production medium. This medium composed of only partly deproteinized whey during the initial optimization experiments. For the design of non-sterile culture conditions, the used media and apparatuses were not sterilized. The medium was prepared in a beaker and then transferred into the non-sterile flasks. Flasks were not sterilized and directly inoculated with the immobilized cells of the yeast. Furthermore, the flasks were not covered with cotton plugs during the cultivation. Consequently the media and growth flasks were open to the environment. To determine the degree of possible contamination in the medium, 0.1 mL sample taken from the culture medium was spread on a glass slide and then examined by using an Olympus BX51 microscope. Furthermore, samples diluted with sterile-saline water were spread on trypticase soy agar (TSA) medium (pH was between 5.0 and 8.0). The petri dishes were incubated at 15 °C for 120 h. The presence of bacterial colonies on TSA medium was accepted as a sign of contamination.

The initial experiments were carried out to determine the optimal culture temperature (5–35 °C) and initial pH (4.0–7.0), respectively. Then, different concentrations of lactose (0–25 g/L), ammonium sulfate (0–2.5 g/L) and potassium dihydrogen phosphate (0–2.5 g/L) as additional carbon, nitrogen and phosphorus sources were studied for maximum CA production, respectively. These experiments were performed in a shaking incubator at 150 rpm using average 100 beads/100 mL. Afterwards, different sodium alginate concentrations (0.5%–3%), beads numbers (50–200 beads/100 mL medium) and incubation times (with 12-h intervals up to 132 h) were tested, respectively. Finally, repeated use capability of beads was tested under optimized conditions.

2.5. Detection of citric acid and isocitric acid

First, 10 mL of the sample taken from culture broth was centrifuged at 5000 rpm for 5 min. One milliliter of the obtained clear supernatant was suitably diluted and then spectrophotometrically analyzed for the determination of CA concentration according to the modified method of Marier and Boulet (Marrier and Boulet, 1958). For this purpose, appropriately diluted 1 mL sample was transferred into a test tube and 1 mL pyridine was added. After the mixture was vortexed, 5 mL acetic anhydride was added to this mixture in the test tube. The final mixture was again vortexed and immediately placed in a water bath (30 °C) and incubated for 30 min. The optimal density was recorded at 410 nm with the blank set. Concentration of CA was determined by referring to a standard curve of pure CA and was expressed as grams per liter (g/L). Isoc-

Download English Version:

https://daneshyari.com/en/article/22684

Download Persian Version:

https://daneshyari.com/article/22684

Daneshyari.com