LWT - Food Science and Technology 87 (2018) 553–561

Contents lists available at ScienceDirect

LWT - Food Science and Technology

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

Use of silicon dioxide nanoparticles for β-galactosidase immobilization and modulated ethanol production by co-immobilized *K. marxianus* and *S. cerevisiae* in deproteinized cheese whey

Arun Beniwal, Priyanka Saini, Anusha Kokkiligadda, Shilpa Vij*

Dairy Microbiology Division, National Dairy Research Institute, Karnal, 132001, India

ARTICLE INFO

Article history:
Received 13 June 2017
Received in revised form
14 September 2017
Accepted 18 September 2017
Available online 21 September 2017

Keywords:
Silicon dioxide
Co-immobilization
Ethanol
Whey
K. marxianus

ABSTRACT

β-galactosidase from dairy yeast was immobilized on silicon dioxide based nanoparticles for the hydrolysis of whey. The biocatalyst was checked for the optimal thermal and pH stability. Consequently, the immobilized β-galactosidase was recycled 15 times for the hydrolysis of lactose (37 °C and pH 7.0) without loss of a significant amount of catalytic activity. Almost 91% of lactose hydrolysis was obtained from using concentrated cheese whey. Considering the available approaches for efficient bioconversion of hydrolyzed whey into ethanol, co-immobilization strategy was used using *Saccharomyces cerevisiae* and *Kluyveromyces marxianus* for fermentation. Same vessel hydrolysis and fermentation process was employed using silicon dioxide nanoparticles based enzyme immobilization along with co-immobilized yeast cell and provided a maximum ethanol titer of 63.9 g/L on concentrated cheese whey (150 g/L). Hence, the nano-biocatalytic system along with immobilized *S.cerevisiae* could improve the viability of ethanol production from cheese whey.

1. Introduction

There is an increase in demand of the suitable enzyme in the energy sector because they offer economic and environmental sustainability. But due to their facile nature and shorter life span, it hampers their use from an economic point of view. Enzyme immobilization is, therefore a well-established technology that offers economic viability at industrial scale utilization (Jochems, Satyawali, Diels, & Dejonghe, 2011). Nano particle based enzyme mediated bioethanol production carry an advantage over other methods due to its greater specificity. But still, there is need of innovative and cost effective immobilization method possessing efficient and higher activity with stability.

The lactose present in Cheese whey, the major by-product of the dairy industry, imposes a major environmental concern regarding its disposal due to its high BOD and COD content (Saini, Beniwal, & Vij, 2017; Saini, Beniwal, Kokkiligadda, & Vij, 2017). Several species of yeast such as *Kluyveromyces lactis, K. marxianus,* and *Candida pseudotropicalis* carry an ability to metabolize lactose present in whey. There were reports of inhibitory effects and low ethanol tolerance of yeast during fermentation of concentrated cheese whey (Saini et al., 2017). Moreover, the ethanol yield is also low using these yeast strains as compared to model fermentative *S.cerevisiae* strains. But the traditional *S.cerevisiae* strain cannot be used for the whey fermentation as this strain is unable to utilize lactose directly due to the absence of lactose permease and β-galactosidase (Guimaraes, François, Parrou, Teixeira, & Domingues, 2008; Rubio-Texeira, 2006). Another alternative strategy may be the use of co-immobilization of *S.cerevisiae* and *K. marxianus* for the production of ethanol from hydrolyzed concentrated whey. This co-culturing offer advantage of both strains as *K. marxianus* carry higher consumption rate on galactose as compared to *S.cerevisiae* which offer better glucose utilization (Gabardo, Pereira, Rech, & Ayub, 2015). But the main challenge for using the co-immobilization technique is regarding the availability of the optimal environment for fermentation by two different strains (Chandrakant & Bisaria, 1998).

Co-immobilization of cells offer different niches and conditions helpful for utilization of sugar. Many methods for the immobilization has been reported in literature such as using entrapment, covalent binding and adsorption (Talebnia, Niklasson, & Taherzadeh, 2005). Calcium alginate as a matrix for immobilization of cells is the most commonly employed matrix for ethanol production as the

* Corresponding author.
E-mail address: shilpavijn@yahoo.co.in (S. Vij).

https://doi.org/10.1016/j.lwt.2017.09.028
0023-6438/

matrix is non-toxic and offer high entrapment (Kourkoutas, Bekatorou, Banat, Marchant, & Koutinas, 2004). So in the present study, we present a method for the immobilization of β-galactosidase (E.C.3.2.1.23) with co-immobilized *S. cerevisiae* and *K. marxianus* in a spatially controlled environment for hydrolysis of cheese whey with subsequent ethanol production. We further evaluated the fermentation parameters and efficiency of the system.

2. Materials and methods

2.1. Microorganism and materials

K.marxianus MTCC 4136 and *S.cerevisiae* MTCC 170, used for fermentation of cheese whey was procured from Microbial Type culture collection Chandigarh, India. Both MTCC strain were cultivated on YPD medium containing (w/v) 1% yeast extract, 2% peptone, and 2% glucose. Both the cultures were incubated at 30 °C for 48 h and maintained on agar slants also. The *K. lactis* β-galactosidase was procured from Sigma (G3665). Nanoparticle silicon dioxide (Aldrich St. Louis, USA) (10–20 nm), glutaraldehyde, *o*-nitrophenyl β-D-galactopyranoside (ONPG), were procured from Sigma. All other reagents were of analytical grade. The cheese whey used for the production of ethanol was kindly provided by the Experimental Dairy Plant of National Dairy Research Institute, Karnal, India. The ultrafiltration of the whey was carried out for removal of proteins followed by reverse osmosis for concentrating the lactose. The obtained whey was further treated with lactic acid to remove the residual proteins by deproteinization followed by autoclaving the whey.

2.2. Immobilization of enzyme on activated nanoparticles

2.2.1. Activation of silicon dioxide nanoparticles

The enzyme immobilization was carried using the silicon dioxide as supporting nanomaterial using the procedure described by Verma, Barrow, Kennedy, and Puri (2012) with slight modifications. The nanoparticles of the silicon dioxide were activated by treating with 5% glutaraldehyde. The silicon dioxide nanoparticles were washed with chilled deionized water. The nanoparticles were then separated by centrifuging at 4000 rpm for 5 min at 18 °C. The nanoparticles based matrix was then activated using 5% glutaraldehyde by incubating the matrix at 28 °C with 150 rpm for a period of 6 h. The activated support of nanoparticles was then recovered by centrifuging at 4000 rpm. The residual traces of glutaraldehyde were then removed by washing the nanoparticles 5 times with chilled deionized water and 2 times with assay Z-buffer(60 mM $Na_2HPO_4 \cdot 7H_2O$, 40 mM $NaH_2PO_4 \cdot H_2O$, 10 mM KCl, 1 mM $MgSO_4 \cdot 7H_2O$, 50 mM 2-mercaptoethanol).

2.2.2. Immobilization of β-Galactosidase on activated support

The covalent linkage was provided for immobilization of β-galactosidase on glutaraldehyde activated silicon dioxide nanoparticles. The purified β-galactosidase was mixed with the activated nanoparticles in 0.1 M Z-buffer, pH 7.0. (100 mg of protein per gram of support).The immobilization was carried out in a shaking incubator (SHELLAB) with 150 rpm for 30 h at 25 °C. The unbounded β-galactosidase was removed after washing 3 times with deionized water followed by Z buffer. The carrier nanoparticles obtained were used for further experiments of lactose hydrolysis. The supernatant was used for calculating the residual β-galactosidase activity.

2.3. Enzyme immobilization yield

The initial and the final β galactosidase activity of the immobilized suspension was carried out along with a reference being conducted in parallel. The immobilization yield was calculated using the method described by Verma et al. (2012) using the equation:

$$IY = [\{Ei-(Ew + Es)\}/Ei] \times 100, \text{ where}$$

Ei is the total protein content of the crude enzyme.
Es is protein concentration of supernatant after immobilization.
Ew is the protein concentration of wash solution after immobilization.

2.4. Optimum pH and temperature of free and immobilized β-Galactosidase

The optimum pH of the immobilized biocatalyst was measured by observing the β-galactosidase activity by keeping both free and immobilized biocatalyst in 100 mM potassium phosphate buffer with 1.5 mM $MgCl_2$ and 0.1 mM $MnCl_2$ with different pH 5.0–8.0 at temperature of 37 °C. The optimum temperature of the free and immobilized β-galactosidase was monitored at pH found to be optimum for enzyme and changing the temperature between 25 °C and 50 °C.

2.5. Thermal stability of free and immobilized β-Galactosidase

The thermal stability of the free and immobilized biocatalyst was evaluated by incubating the nanoparticles in a temperature controlled water bath at 50 °C. The stability of the immobilized nanoparticles was measured in Z buffer and also using a different concentration of lactose (50–100 g/L). The samples were removed at a different time interval and immediately kept on ice for stopping the thermal inactivation. The activity was then determined as described in above section.

2.6. Recycling of the immobilized β-Galactosidase

The recycling of the immobilized biocatalyst was carried out by hydrolyzing the standard ONPG solution. The recycling of the biocatalyst was carried out by the addition of nanoparticles in 2 ml of ONPG solution suspended in Z buffer at 37 °C for a period of 5 min. After each cycle of hydrolysis, the immobilized nanoparticles were removed by centrifuging at 4000 × g for 5 min. The immobilized biocatalyst was collected at the bottom and washed with cold water followed by Z buffer. The same procedure was repeated for successive 10 cycles with hydrolysis of ONPG. The activity of the first cycle was treated as the 100% activity and activity of the remaining cycles was carried out by comparing the activity of the starting cycle.

2.7. Co-immobilization of S.cerevisiae and K. marxianus on calcium alginate

The co-immobilization was carried out using pre-cultured cells of *K. marxianus* and *S.cerevisiae* grown in 1000 ml medium (YPD) for 16 h. Cells were harvested by centrifugation and the pellet was washed with sterilized double distilled water and centrifuged again. The pellet was dissolved in sterile saline such that the density of cells was adjusted of each yeast cells. The cells were then mixed with 100 ml of 2.5% (w/v) sodium alginate solution. The obtained mixture was then extruded through a 5 ml syringe and slowly dropped on 0.1 M $CaCl_2$ solution for getting uniform sized