ORIGINAL RESEARCH PAPER

Biotechnology

OPTIMIZATION OF CHITOOLIGOSACCHARIDES PRODUCTION USING IMMOBILIZED CHITOSANASE

KEY WORDS: chitosanase, immobilization, calcium alginate, chitooligosaccharides

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ABSTRACT

Chitooligosaccharides (COS) are the pharmaceutically important product derived from chitosan using chitosanase. Enzyme immobilization helps to reuse the enzyme to certain extent without compromising the quality of product. In this study, COS production was carried out using chitosanase immobilized on to 2% calcium alginate. Immobilized chitosanase shown maximum COS production at pH 9 and temperature 50ş C. COS production was maximum at 10 h incubation time. Immobilized chitosanase beads were reused up to 3 cycles. Hence, COS could be produced using this immobilized chitosanase without affecting the quality of product.

Introduction

Enzymes are proteinacious macromolecules which helps in the catalysis of biological reaction. Interestingly, enzymes have numerous industrial and medical applications. These enzymes may be of plant, animal or microbial origin. However, enzymes are very expensive due to their production, extraction and purification cost. Enzyme immobilization provided an alternate solution in order to reuse enzyme and thereby prevent its loss during usage of free enzyme. Immobilization can be done by incorporating enzyme solution in inert polymers, organic and inorganic materials like agar, alginate, collagen, gelatin, chitin, chitosan and recently nanoparticles. An ideal carrier matrix should possess inertness, physical strength, stability, regenerability, ability to increase enzyme specificity/ activity, and reduce product inhibition, non specific adsorption and microbial contamination[8]. Enzyme immobilization can be achieved by adsorption, covalent binding, affinity immobilization and covalent binding. Alginate derived from brown seaweed was extensively used in immobilization of various enzymes. Several reports are available demonstrating the alginate crosslinking with divalent ions like (Ca^{2+}) enhancing the stability of enzymes[2].

Chitosanase are the enzymes which is prominently used in the conversion of chitosan in to COS. Novel and alkalophilic chitosanase production by Bacillus sp of marine origin has been reported recently[1]. Chitosanase immobilized on amylose coated nanoparticles was applied to produced chitosan oligosaccharides[6]. Agar gel support was successfully employed to immobilize chitosanase by multipoint attachment method[5]. Glutaraldehyde mediated immobilization of Penicillium sp ZDZ1 chitosanase on to chitin was characterized[10]. Chitosanase from Aspergillus sp was immobilized on electrospun PAN nanofibres for the production of COS[9]. Comparitive studies on whole cell immobilization of Gongronella sp JG was studied for economic and enhanced production of chitosanase[11]. Chitosanase from Capsicum annuum was immobilized on to chitin and stability was analyzed[3]. In this study, alginate is used as a carrier matrix to immobilize chitosanase enzyme obtained from Bacillus sp. of marine origin.

Materials and methods

Production of chitosanase

Culture of Bacillus sp strain kamtuty10 (GenBank: MF409014.1) was inoculated in to nutrient broth containing 1ml of 1% chitosan and incubated at 37 °C for 3 days. After incubation, supernatant was isolated by centrifugation at 8000 g for 15 minutes. The supernatant was used as crude enzyme source.

Immobilization of chitosanase

20 ml of crude chitosanse enzyme solution was mixed with 20 ml of 2% sodium alginate (1:1 ratio). The chitosanase alginate mixture was added dropwise into 0.2 M calcium chloride with continuous shaking at 4 °C. The beads thus formed were washed 3-4 times with deionized water and finally with Tris- Hcl buffer of pH 7.5. The beads were dried and weighed for further studies.

Effect of pH

About 0.5 g of beads were incubated with 3 ml of 1% chitosan and 2ml of different pH (6,7,8,9,10) buffer for 60 minutes followed by estimation of reducing sugars by DNSA method[7].

Effect of temperature

1% chitosan solution containing enzyme beads were kept at different temperatures 30-70 °C for 60 minutes followed by estimation of reducing sugars by DNSA method[7].

Effect of incubation time

Test tubes containing 1% chitosan and enzyme beads were incubated at 50 °C. Samples were withdrawn after every 1 hour and reducing sugars was estimated by DNSA method[7].

Reusability studies of immobilized chitosanase beads

Immobilized chitosanase beads were reused in the chitosan converting reaction for several times by washing the beads with deionized water until no reducing sugars detected.

Results

Immobilization of chitosanase

Marine originated Bacillus sp strain kamtuty10 alkalophilic chitosanase was successfully immobilized in to calcium alginate (Figure 1). Beads were white in colour, round in shape and 0.5 cm in diameter with polished outer surface.

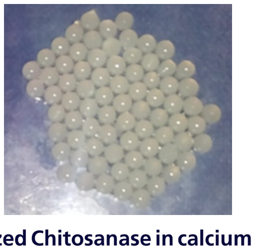

Figure 1. Immobilized Chitosanase in calcium alginate beads

Effect of pH

Reducing sugars(COS) production was moderate at pH 6 with gradual increase at pH 7,8 and attain maximum at pH 9 followed by slight drop at pH 10. (Figure 2)

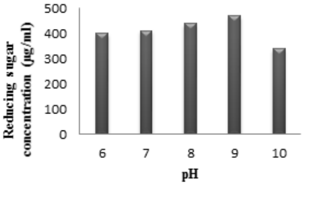

Figure 2. Effect of pH on COS production

Effect of Temperature

COS production was low at 30 °C followed by slight increase at 40 °C and attain maximum at 50 °C and thereafter decreases slightly up to 70 °C and completely stopped beyond it (Figure 3).

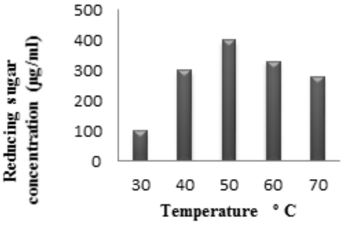

Figure 3. Effect of Temperature on COS production

Effect of incubation time

COS started producing after 1h incubation and shown continuous increase up to 7h followed by gradual increase and reach maximum concentration in 10 h (Figure 4).

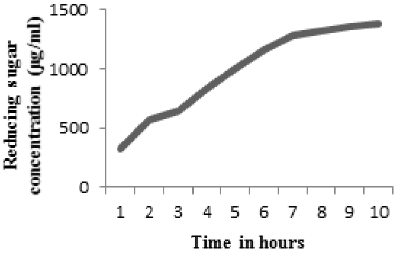

Figure 4. Effect of incubation time on COS production

Effect of reusuability

COS production was 400 µg/ml during first cycle followed by 390 µg/ml during second cycle. However, COS production was reduced to 230 µg/ml during third cycle during which crack started appearing in beads and completely deformed during fourth cycle (Figure 5).

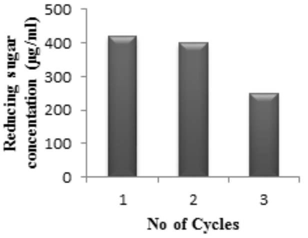

Figure 5. Reusability of immobilized chitosanase for COS production

Discussion

Chitosanase are pharmaceutically and commercially important enzymes involved in the production of COS. Enzyme immobilization technique offers an advantage of reusing the enzyme with minimal loss and yields products of better quality. Several work has been done on chitosanase immobilization from Penicillium sp ZDZ1 chitosanase on to chitin support10, Aspergillus sp chitosanase immobilized on electrospun PAN nanofibres for enhanced production of COS9. Plant derived chitosanase immobilization was also reported3. However, only few bacterial chitosanase immobilization work has been done. The enzyme mentioned in this study has ability to produce COS at various pH 6 to 10 with minimal variation and maximum COS of 1380 µg/ml in 10 h. The beads were successfully reused for up to 3 cycles, after which the beads deformed. Further studies on characterization of COS is required to understand the behavior of immobilized chitosanase on COS production.

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