International Journal of Pharmacy and Biological Sciences
ISSN: 2321-3272 (Print), ISSN: 2230-7605 (Online)
IJPBS | Volume 8 | Issue 2 | APR-JUN | 2018 | 647-650
Research Article | Biological Sciences | Open Access | MCI Approved| ज्ञान-विज्ञान विमुक्तये
|UGC Approved Journal |

PRODUCTION OF BIOFLAVOR USING IMMOBILIZED LIPASE FROM *BACILLUS MEGATERIUM*

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ABSTRACT

Lipases are enzymes that catalyze the hydrolysis of triacylglycerol to free fatty acids and glycerol. In addition, lipases catalyze the hydrolysis and transesterification of other esters as well as the synthesis of esters and exhibit enantioselective properties. Extracellular lipase producing Bacillus megaterium was isolated from soil sample. Encapsulation of the extracellular crude lipase enzyme was carried out using calcium alginate beads. The reaction mixture was run for 2 cycles onto which the maximum production of Butyl acetate was found to be 48.71%. Further the production of the Butyl acetate was stable over 2 cycles.

KEY WORDS

Bacillus megaterium, Encapsulation, Butyl acetate

INTRODUCTION

Lipases (triacylglycerol acylhydrolases) are a class of hydrolase which catalyze the hydrolysis of triglycerides to glycerol and free fatty acids over an oil–water interface **(1-2)**. Lipases catalyze the hydrolysis and transesterification of other esters as well as the synthesis of esters and exhibit enantioselective properties. However, microbial sources of lipase were explored when the industrial potential of lipases boosted and when demand for the lipase could not be met by the supply from the animal sources. The broad substrate specificity makes lipases operational in a wide range of applications and their market is still budding **(3)**. Flexibility of lipase leads to multiple industrial applications in food and flavour making, pharmaceuticals, synthesis of carbohydrate esters, amines and amides, biodetergents, cosmetics and perfumery. The use of free lipases is restricted by their substantially unsteady nature and the resulting requirement of rigorous conditions, such as particular pH and temperature. **(4-6)**

In order to use them more economically and competently in aqueous as well as in non-aqueous solvents, their activity, selectivity and operative stability can be modified by immobilization **(7)**. Immobilization of enzymes is the significant strategy to magnify the applications of these natural catalysts by aiding easy separation and purification of products from reaction mixtures and efficient recovery of enzyme proteins. The enzyme immobilization is carried out to entangle the enzymes in a semi-permeable support, which prevents the enzyme from leaving while allowing substrates and products to pass through **(8)**. With immobilized enzymes, enhanced stability, reuse, continuous-operation, probability of improved control of reaction, product yields and hence more enzymatic activity can be obtained. **(9)**

Entrapment of enzyme in calcium alginate is one of the significant methods of immobilization. Alginates are commercially available as water-soluble sodium alginates and they have been used for more than 65 years in the food and pharmaceutical industries as

solidifying, emulsifying and film forming agent. Entrapment within insoluble calcium alginate gel is a rapid, non-hazardous, reasonable and easy method for immobilization of enzymes as well as cells **(10)**.

Literature survey

Solvent free lipase catalyzed synthesis of butyl caprylate, **Meera T Sose et al**, 2017

Meera T Sose *et al* studied the effect of various reaction parameters and optimization study for the synthesis of butyl caprylate in the presence of a bio-catalyst. To achieve maximum change the optimum parameters thus established include; mole ratio of caprylic acid and butanol as 1:2, lipase loading 2% (w/v), 250 rpm speed of agitation, temperature 60°C and 4g of molecular sieves. The immobilized enzyme was also recycled and reused for 7 cycles that resulted into 30% loss from its initial activity. The esterification was directed effectively with 92% conversion in 5 h in a stirred batch reactor under solvent free system and in presence of molecular sieves that was used to adsorb water formed in reaction. **(11)**

Green Synthesis of Butyl Acetate, A Pineapple Flavour via Lipase- Catalyzed reaction, **S. Mat Radzi et al**, 2011

S.MatRadzi *et al* in the research work includes a synthetic method to produce chloramphenicol esters by taking benefit of the high enantio- and regio-selectivity of lipases. A series of chloramphenicol esters were synthesized using chloramphenicol, acyl donors of different carbon chain length and lipase LipBA (lipase cloned from *Bacillus amyloliquefaciens*). Among acyl donors with different carbon chain lengths, vinyl propionate was found to be the finest. The synthesis of chloramphenicol propionate (0.25 M) with 4.0 g L−1 of LipBA charging gave a conversion of ~98% and a purity of ~99% within 8 h at 50 °C in 1,4-dioxane as solvent. The optimal mole ratio of vinyl propionate to chloramphenicol was increased to 5:1. **(12)**

MATERIALS AND METHODS

In previous studies screening, selection, optimization and production of Butyl acetate (bioflavor) was carried out. The maximum bioflavor production was shown by*Bacillus megaterium*. **(13)**

- **Immobilization of Lipase enzyme from *Bacillus megaterium***

Crude enzyme from *Bacillus megaterium*was extracted by centrifuging the Sterile Tributyrin broth containing enzyme at 10,000 rpm for 10 minutes at 28°C.The crude lipase enzyme from*Bacillus megaterium* was immobilized by entrapment in polymer matrix of Ca-alginate.

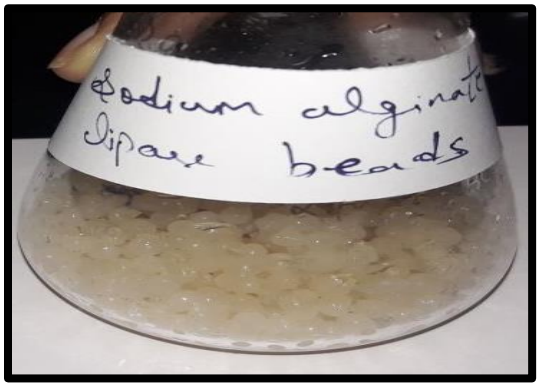


Fig-1 Crude Lipase enzyme beads of *Bacillus megaterium*

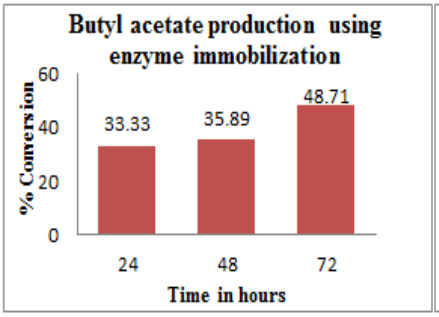
4% (w/v) slurry of Na-alginate was prepared in D/Wand sterilized.After cooling 10ml of the centrifuged crude lipase enzyme was added. Further the alginate enzymeslurry was addedinto chilled 6% $CaCl_2.2H_2O$ for polymerization and beadswere prepared using a pipette. The resultant beads were 3mm in diameter. The lipase enzyme beads were refrigerated at 4°C overnight for hardening.

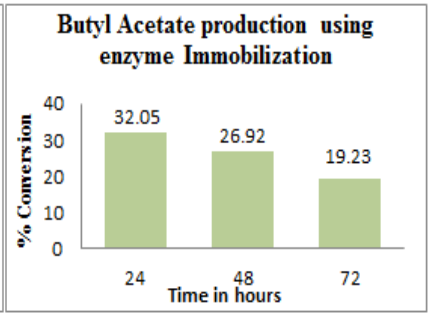
- **Running a Reaction mixture using beads for Butyl acetate production**

Reaction mixture consisted of 50mmol of n-Butanol and 50mmol of Acetic acid. 60 encapsulated crude enzyme beads from *Bacillus megaterium* were added to the reaction mixture. Quantitative estimation of butyl acetate from the reaction mixture was done after every 24 hours for 3 days. Thereafter beads were washed 2-3 times with D/W and stored at 4°C in sterile D/W for 3 days. Further these beads were reused for the second cycle of butyl acetate production. The product formed was estimated using titrimetric method.

RESULT

- **Running a reaction mixture**





Freshly prepared beads used for Butyl Acetate production(First Cycle)

Reused beads after 3 days for Butyl Acetate production(Second Cycle)

The reaction mixture was run using 60 beads and the production of butyl acetate was analyzed after every 24 hours for 3 days, the beads were washed after first cycle with D/W and reused after 3 days for the second cycle. First cycle of reaction mixture showed the product conversion upto 48.71% and on running the second cycle the product conversion was decreased which was found to be 19.23%.

DISCUSSION

Similar results were observed in **Vijay Kumar Garlapati*et al,* (2013) (14)** in which synthesis of methyl butyrate and octyl acetate through immobilized *Rhizopusoryzae* NRRL 3562 lipase mediated transesterification was studied under solvent-free conditions. A molar conversion of 70.42% and 92.35% was obtained using 80 and 60 U of immobilized lipase for methyl butyrate and octyl acetate, respectively. Reusability studies of the immobilized lipase wascarried out by using the recovered beads entrapping the enzyme for subsequent cycles. The immobilized enzyme reserved good relative activity (more than 95%) up to five and six recycles for methyl butyrate and octyl acetate, respectively. Another study included High performance enzymatic synthesis of oleyloleate, a liquid wax ester was carried out by lipase-catalyzed esterification of oleic acid and oleyl alcohol. The reaction system consisted of 2 mmol of oleic acid, 4 mmol of oleyl alcohol, 2.0 ml of hexane and 0.3 g of Novozym 435. After each cycle, the reaction mixture was removed and the Novozym 435 was rinsed with excess hexane, filtered and the solvent was evaporated under a stream of nitrogen before being used with fresh substrates. Novozym 435 retained high activity even after 9 cycles and product yield was found to be 91.9%. **(Mat Radz*iet al,*2011) (15).** In another study the esterification reaction of acetic acid and n-butanol using immobilized lipase encapsulated in calcium alginate beads (Lipase – CAB) and in chitosan coated calcium alginate beads (Lipase-CCAB) in n-hexane under mild reaction conditions were studied. It was observed that repeated use of Lipase - CAB and Lipase - CCAB brought about activity loss after the fifth cycle of reaction with 3-day reactions for each cycle. **(MohdZulkhair*iet al,* 2008) (16)**

CONCLUSION

This study employed production of lipase enzyme using microbial source which is easily accessible and reproducible. On encapsulating crude lipase enzyme from *Bacillus megatrium* using Ca-alginate beads, 48% conversion of n-butanol and acetic acid to Butyl acetate was achieved. Further the enzyme encapsulated beads were stable for 2 cycles. Further the purified enzyme must be tried out to check its activity under Immobilization conditions.

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