Immobilization of Lipase from *Geobacillus* sp. and Its Application in Synthesis of Methyl Salicylate

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Abstract: The present study showed unique properties of an alkaline, thermophilic lipase of *Geobacillus* sp. which was isolated from soil of hot spring. The study was aimed to investigate the optimum immobilization conditions of lipase onto silica gel matrix (100-200 mesh) by surface adsorption method and its application in the synthesis of methyl salicylate. Lipase immobilized by surface adsorption onto silica pretreated with 4% glutaraldehyde showed 74.67% binding of protein and the optimum binding time for glutaraldehyde was found to be 2 h. The enzyme showed maximum activity at temperature 55°C, incubation time of 10 min at pH 9.5 of Tris buffer (0.1 M). Free as well as immobilized lipase was more specific to *p*-NPP (20 mM). All the metal ions and detergents used had inhibitory effect on free as well as immobilized enzyme. The silica immobilized enzyme was reused for hydrolysis and it retained almost 40.78% of its original activity up to 4th cycle. On optimizing different parameters such as molar ratio, incubation time, temperature, amount of enzyme, amount of molecular sieve, the % yield of methyl salicylate was found to be 82.94.

Key words: *Geobacillus* sp., immobilization, surface adsorption, thermophilic lipase, methyl salicylate

1 INTRODUCTION

Lipase catalyses hydrolysis of triglycerides and produces esters by esterification reaction. Lipases are enzymes which belong to the group of the hydrolases, whose main biological function is to catalyze the hydrolysis of insoluble triacylglycerols to free fatty acids, mono- and diacylglycerols and glycerol1. Knowledge of the three-dimensional structure of lipases plays an important role in designing and engineering of lipases for specific purposes. Lipases are used in many sectors such as food, pharmaceutical, fine chemical, oil chemical, biodiesel and in detergent industries2-7. The main application is in the enantioselective synthesis of precursors of pharmaceutically active compounds and the conversion of natural fats and oils into high value products such as cocoa, butter and oil enriched omega-3 fatty acids. Lipases remain enzymatically active in organic solvents that enhance their potential and flexibility as biocatalysts against a wide range of unnatural hosts4-7.

Industrial strain improvement plays a central role in the commercial development of microbial fermentation processes8. Efforts have been directed to reduce the enzyme production costs through improving the yield, and the use of either cost-free or low-cost feed stocks or agricultural byproducts as substrate(s) for enzyme production8. The numerous industrial applications of lipases have stimulated interest in isolation of new lipases from novel sources and strong efforts have been concentrated on the engineering of enzymes with specific properties or better performance for industrial applications. Recently, it has been found that combination of rice straw and Mary olive cakes could be used as suitable and low-cost substrate for lipase production10. Because of inherent stability of thermostable lipase enzyme, they find various applications in industries and biotechnological sectors such as additives in detergents and food industries, environmental bioremediations and in molecular biology11,12. The ester(s) of salicylic acid are useful in preservation of food, pain control and fever control. To cater to the needs of these enzymes in industries, novel lipase genes have to be isolated and the existing lipases are to be engineered for desired properties.

Lipases are mostly inducible enzymes and inducers such as oils are important for lipase secretion13. Biocatalysis shows a distinct advantage over the chemical route in terms of process simplification, quality of product, and reduction in waste formation. Lipases are the most commonly used enzymes for enzyme-catalyzed synthesis because they have the ability to recognize a wide variety of substrates and catalyze a large number of reactions14. Various supports and several inorganic materials have been successfully used for the immobilization of enzymes15. The se-
lected substrate should have high surface area, be thermal-
ly stable, chemically durable, resistant to contamination and of reasonable cost\(^\text{16}\). Silica gel is an amorphous inor-
ganic polymer composed of siloxane groups (Si-O-Si) in the
inward region and silanol groups (Si-OH) distributed on its
surface\(^\text{17}\). Recently, *Candida antarctica* lipase B (CALB)
was immobilized on a modified Silica-based material by
physical adsorption and used for ring opening polymerization
of \(\varepsilon\)-Caprolactone\(^\text{18}\). Earlier, lipases have been used in
synthesis of various esters\(^\text{19-21}\). Among the esters, methyl
salicylate is a constituent of wintergreen and other plants
*Amblyomma herbarium* and *Amblyomma veriegatum*
salicylate is a constituent of wintergreen and other plants
either procured from Sigma Aldrich

\[ \text{2.1 Chemicals} \]

Lipases, the present work was focused on purification of
Owing to applicability and importance of thermophilic
applications and is widely used to control muscular pain.

\[ \text{2.2 Bacterial isolate} \]

The lipase producing bacterium *Geobacillus* sp. was iso-
lated earlier from soil of hot spring and identified at MTCG,
IMTECH Chandigarh. The bacterial strain was maintained
in nutrient agar medium and incubated at 55°C for 48 h.

\[ \text{2.3 Production of lipase} \]

The lipase producing thermophilic isolate was grown in
the medium containing Yeast extract (2.0 g), peptone (5.0
g), beef extract (1.5 g), cottonseed oil (10 mL/L) emulsified
with 0.5% Gum acacia)/pH-8.5 in 1L distilled water. The
seed culture (10% v/v inoculum) was transferred to 50 mL
production medium (250 mL Erlenmeyer flask) and kept at
55°C for 48 h under shaking conditions at 120 rpm.

\[ \text{2.4 Enzyme assay} \]

Lipase assay was performed by a colorimetric method\(^\text{24}\).
The reaction mixture comprised of 60 \(\mu\)L of \(p\)-NPP stock
solution and 40 \(\mu\)L of free enzyme or 40 mg of immobilized
lipase enzyme. The final volume of this reaction mixture
was made to 3.0 mL with 0.1 M Tris buffer, (pH-8.5). The
samples were incubated for 10 min at 55°C under continu-
ous shaking in water-bath-incubator. The reaction was
stopped by chilling at \(-20°C\) for 3-4 min. Appropriate
control (without enzyme) was included with each assay.
The absorbance of \(p\)-nitrophenol released was measured at
410 nm (LAB INDIA UV/Visible spectrophotometer). The
unknown concentration of \(p\)-nitrophenol released was
determined from a reference curve of \(p\)-nitrophenol. Each
of the assay was performed in triplicates and mean values
were presented.

\[ \text{2.5 Unit of lipase activity} \]

One unit (U) of lipase activity was defined as the amount
of enzyme required to release one micromole of \(p\)-nitro-
phenol per minute from substrate under standard assay
conditions.

\[ \text{2.6 Immobilization procedure} \]

The purified lipase from *Geobacillus* sp. was immobi-
лизирован onto silica gel matrix (100-200 mesh) by surface ad-
sorption method. The silica gel matrix (5 g) (100-200
mesh) was dipped in 0.1 M Tris buffer (pH-9.5). It was cen-
trifuged at 10,000 rpm at 4°C for 10 min. The supernatant
was discarded and pellet was washed 4-5 times with Tris
buffer. The matrix was then kept at 4°C overnight in Tris
buffer. 1-5% glutaraldehyde (cross linking agent)solution
was added to the matrix and kept at 37°C under shaking
conditions for 1 h. The matrix was washed 3-4 times with
Tris buffer (pH 9.5) to remove unbound glutaraldehyde.
The lipase from *Geobacillus* sp. was incubated with the
matrix up to 5 h under shaking condition. The supernatant
was discarded. Enzyme activity and protein content of
matrix bound enzyme and supernatant was determined.

One unit (U) of lipase activity for immobilized enzyme was
defined as the amount of bound enzyme required to release
one micromole of 4-nitrophenol per minute under standard
assay conditions. Binding efficiency was calculated by the
ratio of the activity expressed by bound enzyme to the
total activity added to the matrix.

\[ \text{2.7 Purification of enzyme} \]

The enzyme was earlier purified by Hydrogen Interaction
Chromatography and by Gel Filtration Chromatography.
The column used were Octyl Sepharose column (size 12 \(\times\) 2
cm) (Sigma Chemicals, USA) and Sephacryl S-300 column
(size 12 \(\times\) 2 cm) (Sigma Chemicals, USA) respectively. The
equilution buffer used was 0.02 M Tris HCl buffer having pH
9.5. All eluted fractions were assayed both for lipase activity
as well as total protein (A280). And the fractions showing
highest lipase activity were pooled and assayed for protein
content\(^\text{25}\).

\[ \text{2.8 Protein estimation} \]

The protein concentration was determined using
Lowery’s method\(^\text{26}\).
2.9 Optimization of immobilization conditions

2.9.1 Optimization of glutaraldehyde concentration and cross-linking time

Different concentrations of glutaraldehyde solution (1, 2, 3, 4 and 5% w/v) were used for cross linking of matrix and enzyme. To study the optimal binding time, matrix and optimized concentration of glutaraldehyde was incubated in the water bath shaker for the time intervals of 1 h, 2 h, 3 h, 4 h and 5 h. The enzyme activity and protein concentration was examined by standard methods.

2.10 Effect of reaction condition on hydrolytic properties of free and immobilized lipase

2.10.1 Effect of incubation time on activity of free and immobilized enzyme

To work out the optimum incubation time, the enzyme assay of free as well as immobilized lipase was carried out at different time intervals ranging from 5, 10, 15, 20 and 25 min under standard assay conditions.

2.10.2 Effect of temperature on both free and immobilized enzyme

In order to optimize the incubation temperature, the reaction was carried out at different temperatures ranging from 40, 45, 50, 55 and 60°C under standard assay conditions. Enzyme activity of free as well as of immobilized enzyme was then calculated.

2.10.3 Effect of buffer pH on both free and immobilized enzyme

Tris buffer (0.1 M) with different pH values 8.0, 8.5, 9.0, 9.5, 10.0 and 10.5 was used to perform reaction of enzyme. For this 12.11 g Tris base (0.1 M) was dissolved in 800 mL distilled water then different pH was adjusted with the appropriate volume of concentrated HCl. Bring final volume to 1 liter with distilled water. Enzyme activity of free as well as immobilized enzyme was determined and compared.

2.11 Esterification process and optimization for the synthesis of methyl salicylate by silica-bound immobilized lipase

2.11.1 Analysis of methyl salicylate synthesis by GLC

The esterification studies were performed with silica-bound lipase in DMSO (5 mL reaction volume). The biocatalyst (20 mg/mL) was added to the reaction mixture containing an appropriate concentration of reactants (salicylic acid: methanol) incubated at 55°C under shaking (130 min⁻¹) for 12 h. The reaction mixture was assayed for the presence of methyl salicylate by gas liquid chromatography (GLC) using a sample of 2 μL. The GLC was equipped with a packed-column (10% SE-30 Chrom WHP, 2 m length, mesh size 80-100, internal diameter 0.32 cm, Netel Chromatographs, Thane, India). Nitrogen was used as a carrier gas (20 mL min⁻¹). GLC was programmed for oven temperature of 230°C, injector temperature of 240°C and FID temperature of 250°C for the analysis of sample.

2.12 Optimization of reaction conditions for the synthesis of ester

2.12.1 Effect of alcohol molarity on the synthesis of methyl salicylate

The effect of molar ratio of reactants was studied by reacting salicylic acid (200 mM) methanol (100-500 mM) and immobilized lipase (20 mg/mL) into DMSO to complete the reaction mixture volume 5 mL for 10 h at 55°C. The esterification was carried out under continuous shaking under standard conditions. The amount of ester synthesized was determined from the standard profile of methyl salicylate.

2.12.2 Effect of reaction time on synthesis of methyl salicylate

The reaction mixture comprised silica-immobilized lipase, salicylic acid and methanol in optimized molar ratio in solvent (DMSO) to complete the reaction mixture volume 5 mL. The glass vials were incubated in a Rotary Shaker for 6, 8, 10, 12 and 14 h at 55°C. The amount of ester synthesized was determined from the standard profile of methyl salicylate.

2.12.3 Effect of reaction temperature on synthesis of methyl salicylate

The effect of reaction temperature (40, 45, 50, 55 and 60°C) on the synthesis of methyl salicylate was studied under optimized molar concentration of reactants and reaction time. The amount of ester synthesized was determined from the standard profile of methyl salicylate.

2.12.4 Effect of amount of biocatalyst on synthesis of methyl salicylate

The synthesis of methyl salicylate was studied by taking different amounts of immobilized lipase (5, 10, 15, 20, 25 mg/mL) in reaction mixture (5 mL) under optimized molar concentration of reactants, time and temperature.

2.12.5 Effect of molecular sieve on synthesis of methyl salicylate

The synthesis of methyl salicylate was studied by taking different amounts of molecular sieve (20, 30, 40, 50, 60 mg/mL) in reaction mixture (5 mL) under optimized conditions.

2.12.6 Reusability of immobilized lipase

The formation of methyl salicylate from salicylic acid and methanol catalyzed by immobilized lipase in DMSO was used to check the reusability of immobilized enzyme. The optimized amount of molecular sieve was used for the esterification reaction. The immobilized lipase (20 mg/mL) was used for 5 cycles of 12 h each, for methyl salicylate synthesis. After each cycle of esterification, the immobilized enzyme was washed in DMSO at room temperature. Thereafter DMSO was decanted and matrix was reused for fresh cycle of ester synthesis under similar conditions.
3 RESULTS AND DISCUSSION

3.1 Immobilization of lipase from *Geobacillus* sp. onto silica

The enzyme was earlier purified by 2-step chromatography (Hydrogen Interaction Chromatography (Octyl Sepharose Column) and Gel Filtration Chromatography (Sepharyl S-300 Column))\(^{121}\).

3.1.1 Immobilization of lipase obtained from *Geobacillus* sp. on silica gel matrix (100-200 mesh)

A gradual increase in binding efficiency was observed when glutaraldehyde concentration was increased from 1 to 4%, and then it decreased. Maximum binding efficiency (74.67%) was found at 4% glutaraldehyde concentration with 1.18 U/mg enzyme activity. Previously, lipase from *Bacillus coagulans* BTS-3 showed binding efficiency of 70% at 2.5% of glutaraldehyde concentration\(^{26}\). The binding efficiency of 99% in case of lipase from *Yarrowia lipolytica* and of 44% in *Candida rugosa* lipase has been reported earlier\(^{27}\). In another study, the binding efficiency of 68.48% was observed on immobilizing lipase from *Bacillus coagulans* on to molecular sieve\(^{28}\).

3.1.2 Effect of cross-linking time on lipase immobilization

The maximum activity of lipase (1.21 U/mg) was observed when cross-linking time was increased from one to two hours. After two hours there was a decrease in the activity of the enzyme as the relative activity of 61.86% was observed after five hours (Fig. 1). Previously, lipase from *Bacillus coagulans* BTS-3 immobilized on glutaraldehyde activated polymer nylon-6 showed the cross linking time of 1 h to be optimum\(^{29}\).

3.1.3 Effect of incubation time on the activity of free and immobilized enzyme

Free and immobilized enzyme showed maximum activity of 1.5 U/mL and 1.21 U/mg respectively after 10 minutes of incubation (data not shown). Further increase in incubation time led to decrease in the activity of both free and immobilized lipase. Previously, maximum lipase activity of *Bacillus coagulans* BTS-3 was obtained after 10 min of incubation at 55°C\(^{30}\).

3.1.4 Effect of incubation temperature on the activity of free and immobilized enzyme

An increase in the temperature led to increase in the relative activity of free and immobilized enzyme. An incubation temperature of 55°C was found to be the most suitable temperature for the optimal activity of free (1.52 U/mL) as well as immobilized enzyme (1.23 U/mg). A sharp decline in the activity was observed afterwards (Fig. 2). A rise in temperature increased the activity of the enzyme because at higher temperature both substrate and enzyme had more kinetic energy. Substrate with higher energy combined with enzyme to overcome its activation energy. But at very high temperature, enzyme molecules probably got denatured so activity decreased. In a study, the optimum temperature for the free and immobilized lipase from *Candida rugosa* was found to be 40°C and 45°C respectively\(^{31}\). The optimum temperature of 55°C for both free and immobilized lipase from *Bacillus coagulans* BTS-3 has been reported earlier\(^{27,32}\). Recently, the optimum temperature for production of lipase has been found to be 37°C for both *Bacillus* sp. and *Pseudomonas* sp.\(^{33}\).

3.1.5 Effect of buffer pH on the activity of free and immobilized enzyme

pH has an effect on state of ionization of amino acids so pH not only affects the shape of the enzyme but also affects charge on the enzyme. Thus pH can increase or decrease the activity of enzymes. As the pH of buffer increased from 8.0 to 9.5, a gradual increase in the activity of immobilized and free enzyme was observed (Fig. 3). The optimum activity of free (1.57 U/mL) as well as immobilized (1.28 U/mg) enzyme was found to be 9.5. A sharp decline in the activity was observed afterwards. In a previous study, poly(AAc-co-HPMA-co-EGDMAi) hydrogel-bound lipase of *Pseudomonas aeruginosa* MTCC-4713 showed maximum residual hydrolytic activity at pH 8.0\(^{34}\). The optimum pH of 8.5 for both free and immobilized lipase from *Bacillus coagulans* BTS-3 has been reported.

![Fig. 1](image1.png)

**Fig. 1** Effect of glutaraldehyde cross-linking time on immobilization of lipase from *Geobacillus* sp. onto silica matrix.

![Fig. 2](image2.png)

**Fig. 2** Effect of incubation temperature on the activity of free and silica-immobilized lipase from *Geobacillus* sp.
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Fig. 3 Effect of buffer pH on the activity of free and silica-immobilized enzyme from Geobacillus sp.

3.2 Optimization of reaction conditions for synthesis of methyl salicylate using silica-immobilized lipase

3.2.1 Effect of alcohol molarity on the synthesis of methyl salicylate

The molar ratio of 2:4 (salicylic acid:methanol) was found to be optimum for the synthesis of methyl salicylate which gave 70.25% yield (Fig. 4). However, any further increase in the concentration of the alcohol had an inhibiting effect on methyl salicylate synthesis, with only 52.82% conversion at molar ratio of 2.5 of salicylic acid:methanol. The difference in alcohol molarity towards the yield of methyl salicylate may be attributed to either steric hindrance or electronic effect of substrate on the immobilized lipase or specificity of immobilized lipase towards the substrate. Moreover, it appeared that such a decrease in the ester synthesis might be because of change brought about by excessive concentration of either component at the catalytic site that comprised of the triad of serine, aspartic acid (or glutamic acid) and histidine; serine being highly conserved residue in various lipases including that from Geobacillus sp. The optimum molar ratio of 1:3 (propionic acid:ethanol) for the synthesis of ethyl propionate in hexane using silica immobilized lipase from Bacillus coagulans has been reported earlier. It was reported that optimal synthesis of butyl acetate by lipase from Bacillus coagulans, immobilized on nylon-6 was achieved when acid and alcohol were used in equimolar ratio (100 mM) in the reaction mixture. In a previous study, the molar ratio of 1:1 was found to be optimum for the synthesis of isoamyl butyrate using “Lipozyme TL IM”.

3.2.2 Effect of reaction time on synthesis of methyl salicylate

The maximum yield of methyl salicylate i.e. 73.07% was recorded at 12 h under optimized reaction conditions, after which there was a decline in conversion rate. Thus in the subsequent esterification reactions, a reaction time of 12 h was considered optimum for the synthesis of ester (Table 1). The optimum incubation time of 12 h for the synthesis of ethyl propionate using silica bound lipase from Bacillus coagulans BTS-3 has been reported earlier. A reaction time of 3 h for immobilized lipase from Bacillus coagulans BTS-3 was considered optimum for synthesis of p-Nitrophenyl acetate. In a previous study, immobilization of Pseudomonas aeruginosa lipase onto a synthetic poly(AAc-co-HPMA-cl-EGDMA) hydrogel catalysed the esterification of methanol and acetic acid into methyl acrylate in a short period of 6 h at 55°C.

3.2.3 Effect of reaction temperature on the synthesis of methyl salicylate

The maximum yield of methyl salicylate i.e. 73.64% (Table 2) was recorded at 55°C under the above reaction conditions, after which there was a decline in conversion rate with only 51.98% at 60°C. This suggested that at higher temperature, the conversion rate is controlled by reaction temperature. In contrast, at lower temperature, the reaction rate is limited by mass transport phenomena. Increase or decrease in temperature of reaction mixture might interfere with the porosity, hydrophobic character and diffusion of the reactants and/or products at the catalytic site of the enzyme. In an earlier study, a reaction temperature of 55°C was found to be optimum for the synthesis of ethyl propionate using silica bound lipase from Bacillus coagulans BTS-3. A reaction temperature of 65°C for immobilized lipase of Bacillus coagulans was considered optimum for synthesis of p-nitrophenyl acetate. In the previous study, the optimal temperature of methyl salicylate synthesis might be because of change brought about by excessive concentration of either component at the catalytic site that comprised of the triad of serine, aspartic acid (or glutamic acid) and histidine; serine being highly conserved residue in various lipases including that from Geobacillus sp.

Table 1 Effect of incubation time on synthesis of methyl salicylate using silica immobilized lipase from Geobacillus sp.

| Incubation time (h) | Ester Yield (%) |
|--------------------|-----------------|
| 6                  | 31.73           |
| 8                  | 53.78           |
| 10                 | 65.45           |
| 12                 | 73.07           |
| 14                 | 62.03           |

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for the synthesis of ethyl ferulate using celite-bound commercial lipase, Steapsin was found to be 45°C.

3.2.4 Effect of amount of biocatalyst on synthesis of methyl salicylate

The maximum synthesis of methyl salicylate i.e. 74.03% was obtained with 20 mg/mL of immobilized lipase, after which there was decline in conversion rate (data not shown). As reported in the synthesis of methyl acrylate 12.5 mg/mL of poly(AAc-co-HPMA-cl-EGDMA) hydrogel-bound immobilized lipase from Pseudomonas aeruginosa was used to get maximum yield of ester. In another study, it was found that 12.5 mg/mL of poly(methacrylic acid-co-dodecyl methacrylate-cl-N,N-methylenebisacrylamide) hydrogel immobilized Bacillus cereus MTCC 8372 was found to be optimum for the synthesis of geranyl acetate.

3.2.5 Effect of molecular sieve on the synthesis of methyl salicylate

Esterification is generally a water-limited reaction because the equilibrium catalyzed by hydrolytic enzymes is in favor of hydrolysis. In the reaction, possibly high water activity due to stoichiometric water released reversed the synthesis reaction to hydrolysis. Generally, molecular sieve is used to absorb water in the reaction medium to favour the equilibrium in forward direction for the esterification reaction to occur. GLC analysis of the reaction mixtures showed that the maximum synthesis of methyl salicylate (82.94%) occurred when 40 mg/mL of molecular sieve was present in the reaction mixture, after which there was a decline in conversion rate with only 52.1% at 60 mg/mL molecular sieve (Fig. 5). In a previous study, silica bound commercial lipase, Steapsin showed a moderate increase in the synthesis of isopropyl ferulate with 100 mg/mL of molecular sieves in a 2 mL of reaction mixture. It appears that an excess concentration of molecular sieves completely removed even traces of water molecules from the reaction mixture, thus rendering the bound-lipase relatively ineffective to perform the esterification reaction which resulted in a gradual decline in the ester yield.

3.2.6 Reusability of immobilized enzyme

The silica-bound lipase (20 mg/mL) was used for the synthesis of methyl salicylate, in DMSO up to five cycles (each cycle of 12 h) at 55°C. Immobilized enzyme gave 49.56% of ester yield after the 4th cycle (Fig. 6). With repeated use of immobilized enzyme, probably leaching of enzyme occurred due to the weakening of binding strength between the support and the immobilized enzyme. Frequent encountering of substrate into the active site might have caused its distortion, thus reducing its catalytic efficiency either partially or fully. In previous studies, the synthesis of isopropyl ferulate and butyl ferulate by silica bound commercial lipase, Steapsin was achieved in short periods of 3 h, and 6 h, respectively. In these studies, a good yield of the respective ester(s) was noted in the 1st and 2nd cycles of esterification/reuse but, ultimately, the product yield continued to decline with successive cycles of reuse.

Table 2 Effect of reaction temperature on the synthesis of methyl salicylate using silica immobilized lipase from Geobacillus sp.

| Incubation temperature (°C) | Ester yield (%) |
|-----------------------------|----------------|
| 40                          | 38.01          |
| 45                          | 45.04          |
| 50                          | 67.91          |
| 55                          | 73.64          |
| 60                          | 51.98          |

Fig. 5 Effect of molecular sieve on the synthesis of methyl salicylate using silica immobilized lipase.

Fig. 6 Reusability of silica-immobilized lipase in the synthesis of methyl salicylate.
3.2.7 Percent yield of methyl salicylate formed

The formation of ester methyl salicylate was analysed through GLC by comparing the retention time (RT 1.85) of the test sample to the retention time of the standard run for methyl salicylate (RT 1.83) as shown in Fig. 7. On optimizing different parameters such as molar ratio, incubation time, temperature, lipase amount, amount of molecular sieve, the % yield of methyl salicylate was found to be 82.94% which was calculated from the area covered under the peak to the area under the standard run of methyl salicylate in mM.

4 CONCLUSION

In this work, the use of a simple approach to employ a cheap silica support to achieve stable binding of a lipase from Geobacillus sp. by physical adsorption followed by glutaraldehyde cross-linking was successfully demonstrated. It appears that saturation of the available surface of the silica particle(s) allowed esterification of the alcohol with the acidic moiety (salicylic acid), which otherwise is regarded as less amenable to esterification. Moreover, immobilized lipase provided important advantages such as easy separation from the product conferring a high potential for reuse which would clearly render the whole process fairly economical. This approach could afford an economical option for producing esters/products of commercial value within a reasonably short period of 12 h with maximum yield of 82.94%.

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Conflicts of interests

The author(s) declare(s) that there is no conflict of interests regarding the publication of this article.

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