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Notes

Improved performance of immobilized lipase from optimized biosupport material (polyvinyl alcohol/AlgNa) and its characterization

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Enzymes play an imminent role as biocatalyst for various biotechnological applications as well as production of biodiesel. Here, we focused on the preparation of biosupport materials (polyvinyl alcohol/AlgNa) and their use in immobilization of lipase from *Pseudomonas cepacia*. Lipase was successfully immobilized onto polyvinyl alcohol/AlgNa in the form of biosupport materials by entrapment method. The mechanical strength, swelling ratio, thermal properties, optimum temperature and pH, lipase loading, leaching, immobilization yields and activity, characterization of the support materials were performed. The optimized pH and temperature for free lipase were 8.0 and 40°C, respectively, while the best pH and temperature for polyvinyl alcohol/AlgNa immobilized lipase were 8.0 and 50°C. 73.12% of the initial activity was retained for the immobilized biosupport catalyst in 6 cycles. The biosupport catalyst beads showed a fascinating degree of immobilized lipase activity along with high immobilization yield. The highest immobilized lipase activity and loading efficiency found to be 87.28 (U/g) and 55.2%, respectively. SEM analysis confirms the development of macro-porous structure from the combination of alginate. No sharp chemical interaction was observed in the behaviour of the functional groups of polyvinyl alcohol and AlgNa in the polyvinyl alcohol/sodium alginate blends, which were confirmed from Fourier transform infrared (FTIR) spectra. The immobilized biosupport catalysts are easily separable, recyclable and could be frequently used for transesterification.

Keywords: Biosupport catalyst, Entrapment, Loading efficiency, *Pseudomonas cepacia*, Transesterification

The role of enzymes a biocatalyst for various biotechnological applications is well established. Lipase or triacylglycerol acyl ester hydrolases (EC 3.1.1.3)[1] is an industrially versatile enzyme which can be used for the manufacture of household detergents, dairy industries, food industry, pharmaceuticals, bio-oil production, etc[2]. It has the intrinsic capacity to break the carboxyl ester bonds in tri-, di-, and monoacylglycerols (the prime components of plant, microbial fats, oils and animal fats). The biocatalyst enzyme can be added to the reaction mixture a free enzyme in soluble form or can be immobilized on any support material. Generally, a higher conversion can be obtained from free lipase[3]. The separation and purification of the product increase the cost of production, further. Also, the catalytic activity of lipase an organic solvent is usually lower than those an aqueous medium[4]. Immobilization of lipase the appropriate solid support can address the above mentioned limitations of the free enzyme. Immobilization increases thermal and operational consistency, ease of handling, and avoids kind of aggregation and stabilizes the active form. Also, the immobilized enzyme can easily be separated from the reaction mixture. Immobilization can be done in numerous ways, adsorption, cross-linking or entrapment[2,5]. The selection of support material is a crucial parameter in immobilization of enzymes. The support material can be inorganic or polymeric. Inorganic support materials provide better mechanical strength and higher stability along with low cost in comparison to polymeric matrix material. However, due to their hydrophilic nature, the activity of the biocatalyst is affected. Therefore, polymeric support materials like polysaccharides, polyethylene, polyvinyl alcohol, polystyrene, and polymethyl methacrylate, are used which offer a hydrophobic environment for immobilizing and activating the enzyme. Materials like sodium alginate-silicate sol-gel matrix[6], gelatine hydrogel[7], polyacrylamide bead[8], chitosan beads[9] are also used on account of their elevated immobilized enzyme activity, high yield of immobilization, high consistency, and economics.

The blending of two polymers not only improves the mechanical strength but also improves the activity of the enzyme, thereby reducing the mass transfer resistance. The superiority of polyvinyl alcohol (PVA)/sodium alginate (AlgNa) based biosupport material in the essence of immobilized enzyme activity, reusability, and consistency makes it different from other immobilization material[10]. Commercial availability of AlgNa is found in the form of alginate

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sodium salt, also known as sodium alginate (AlgNa). AlgNa can capture multivalent cations, evolving the generation of covalent bonds and insoluble biosupport material. Polyvinyl alcohol is a biodegradable, non-toxic and synthetic polymer, which has high mechanical strength. It was estimated that polyvinyl alcohol might be able to enhance the longevity and the bead strength, while AlgNa could raise the bead surface characteristics, diminishing the ability to agglomerate[11]. Polyvinyl alcohol/AlgNa beads have the ability to contain immobilized cells and enzymes along with them[12-14], and hence have considerable applications in biotechnology[15-17]. In the field of enzyme immobilization, limited studies have been done on the biological applications of alginate gel along with polysaccharide & synthetic polymer blends[18-20].

The present work focuses on the feasibility of utilizing polyvinyl alcohol/AlgNa biosupport catalyst treated with boric acid and $CaCl_2$ solution for transesterification. The immobilization impacts of polyvinyl alcohol/AlgNa biosupport catalyst materials under various planning conditions, activity, temperature, pH, reuse, swelling ratio, protein loading, strength, etc. were observed, and the optimal conditions were picked.

Materials and Methods

Lipase protein from *Pseudomonas cepacia* was bought from Sigma-Aldrich. Polyvinyl alcohol (normal MW 1, 15000) and sodium alginate were acquired from Qualikems Fine Chem. Pvt. Ltd., India. Folin Ciocalteu reagent was acquired from Sigma Aldrich. Iso-propanol was acquired from Sisco Research Laboratories Pvt. Ltd., India. Potassium hydroxide pellets were bought from S.D. Fine Chem. Ltd. The Fourier Transform Infrared Spectroscopy (FTIR-Thermo-Nicolet mode 5700) was utilized for investigation of biosupport material, polyvinyl alcohol, AlgNa, Boric acid, $CaCl_2$ and lipase. p-NPP was obtained from Sigma-Aldrich. Different chemicals and solvents which were utilized as a part of these experiments were of analytical grade and acquired from the S.D. Fine Chemical Ltd. India.

Formation of the immobilized polyvinyl alcohol/AlgNa biosupport catalyst

Interfacial insolubilization reaction of polyvinyl alcohol/AlgNa made the desired biosupport catalyst materials[17]. AlgNa and polyvinyl alcohol were taken at different concentration of 1.5-2.5 wt% and 9.0-11.0 wt%, respectively. The powdered raw materials were put into 50 mM Tris HCl buffer under continuous blending through overhead stirrer (1500 RPM) till complete dissolution, at ambient temperature (30°C) for AlgNa and 70°C for polyvinyl alcohol. At that point, the lipase powder was added to the polyvinyl alcohol /AlgNa aliquot, and the aliquot was blended at 300 rpm at room temperature (30°C) for 30 min. Optically clear aliquot was obtained and no stage separation was observed. The aliquot was added drop wise into the 30 mL calcium borate (CaB_4O_7) solution of various proportion of (3.0-4.5% w/v) boric acid and totally different proportion of (1.5-2.5% w/v) $CaCl_2$. The biosupport catalyst materials had undergone curing process in the CaB_4O_7 solution for 30 min. The biosupport catalyst materials were washed properly for two times with 3 mL of the 50 mM Tris HCl buffer to excretory overloading of boric acid and the biosupport catalyst materials were freeze-dried for further studies and kept at 4°C10. The same method was used for preparing biosupport materials without lipase.

Estimation of polyvinyl alcohol /AlgNa biosupport material strength

The mechanical quality of the polyvinyl alcohol/ AlgNa biosupport materials was gained by a 4-cutting edge overhead stirrer and a tube-shaped glass measuring beaker with four baffles[21]. 75 polyvinyl alcohol/AlgNa biosupport materials were extruded into the tube-shaped measuring glass beaker with oil at 40°C to a height of 8 cm. The mixing speed was controlled from 500-3000 rpm[20]. The polyvinyl alcohol/AlgNa biosupport materials were mixed in the container for 5 min, and the surviving polyvinyl alcohol/AlgNa biosupport materials were checked properly, and every experiment was taken into consideration thrice.

Estimation of equilibrium biosupport materials swelling ratio

To measure the swelling behavior, polyvinyl alcohol/AlgNa biosupport materials samples were dried in vacuum oven at 50°C until a stable weight (M_i) was obtained. Dried polyvinyl alcohol/AlgNa biosupport materials were soaked in water at 40°Ctemperatures for 6 h. It was then taken out, soaked on a filter paper the weight (M_t) was determined. The same procedure was repeated for 24 h time. The same procedure was done in three set for precise estimation for swelling ratio. Swelling water uptake was calculated[22] as follows:

Polyvinyl alcohol/AlgNa biosupport materials water uptake (%) = $\frac{M_t}{M_i} \times 100$ ... (1)

where M_t = Weight (g) of swollen polyvinyl alcohol/AlgNa biosupport materials at time t; and M_i = Initial weight (g) of dried polyvinyl alcohol/AlgNa.

Thermal properties of the biosupport material

The thermal properties of polyvinyl alcohol, sodium alginate, and biosupport material were analyzed by thermo-gravimetric analysis (TGA) with the help of NETZSCH Jupiter STA 449F3 TGA analyzer. The analysis was performed by an alumina-coated crucible under nitrogen flow in the temperature range of 28-500°C at a heating rate of 10°C/min[23].

Para-nitrophenyl palmitate (p-NPP) substrate for activity

Para-nitrophenyl palmitate (p-NPP) substrate was taken and spectrophotometric method for measuring lipase activity[24,25]. Analytical grade Iso-propanol was used for the preparation of stock solution (10 mM) of p-NPP. A final volume of 3.0 mL was prepared from 75 µL of the p-NPP stock solution, 10-50 µL of the test sample (commercial lipase) and Tris HCl buffer (50 mM, pH 8.0). Prepared mixture was shaken at 40°C for 20 min in a temperature controlled water bath. Then the reaction was ceased by heating (5 min in boiling water bath) with lipase (in triplicate), and the same procedure was followed for with each assay. During the reaction, *p*-nitrophenol was released, and the absorbance (A_{410}) of corresponding material was measured by UV/visible spectrophotometer. P-nitrophenol (2-20 μg/mL in 50 mM Tris buffer, pH 8.0) was taken as the reference curve for the calculation of p-nitrophenol concentration. All the assays were performed for three times. One unit of lipase activity is considered to be one mL of lipase that releases one µmol *p*-nitrophenol by hydrolysis of p-NPP at 40°C under assay conditions.

Estimation of optimized temperature on the activity of lipase

A temperature ranges of 20-80°C was selected to perform experiment at pH 8.0 for 20 min and optimum temp was determined[26]. After pre-incubation of 25 µL (5 mg/50 mL lipase) of lipase solution for 20 min at 40°C in the water bath, 75 µL p-NPP 5.0 mM, in 2900 µL 50.0 mM Tris-HCl buffer (pH 8.0), activity of lipase was checked by spectrophotometric assay for determination of optimum temperature. The assay mixture was equilibrated at the range of 20, 30, 40, 50, 60, 70 and 80°C before adding lipase. p-NPP substrate was taken as reference for measurement of lipase activity. The reaction was terminated by heat addition process (5 min in boiling water bath). All experiments were carried out at least for thrice.

Estimation of optimized value of pH on the activity of lipase

Buffer solutions of pH ranging from 4.0 to 11.0[27] (50.0 mM concentration: citrate-phosphate buffer pH 4.0-6.0; 50.0 mM Tris-HCl buffer pH 7.0-8.0; 50.0 mM carbonate-bicarbonate buffer pH 9.0-10.0; 50.0 mM Glysin-NaOH pH 11.0) were taken at 40 & 50°C evaluate the optimum pH value of free and immobilized lipase, respectively. After pre-incubation of 25 µL (5 mg/50 mL lipase) of lipase solution for 20 min at 40°C in the water bath,75 µL p-NPP 5.0 mM, and in 2900 µL of the above-mentioned buffer solutions (pH 4.0-11.0) spectrophotometric assay was used to estimate the effect of pH on the activity of lipase. The activity of lipase was measured by taking p-NPP substrate as the reference. The reaction was terminated by heat addition process (5 min in boiling water bath). At least three attempts had been done for all the experiments.

Characterization of the morphology of polyvinyl alcohol /AlgNa biosupport materials

The morphology of the biosupport material was analyzed with the help of scanning electron microscopy (SEM; EVO/18 Research, ZEISS). The polyvinyl alcohol/AlgNa biosupport material was as dried and pierced by a knife to have a proper cross-sectional slice of the biosupport material[20]. Fourier transform infrared (FTIR) spectroscopy (FTIR-Thermo-Nicolet mode 5700) was used to identify the organic groups preset in polyvinyl alcohol/AlgNa biosupport material. The wave number was taken in the range of 500-4000 cm^{-1} to capture the absorbance of biosupport materials; with a resolution of 4 per cm[28]. Averages of 32 scans were recorded by using a multi-bounce ATR.

Estimation of protein loading

The content of protein of the lipase was estimated from the calibration curve provided by the Lowry standard procedure[29]. The surplus value among the quantity of protein taken into the biosupport materials and the quantity of protein present both in the filtrate and in the washing solutions after immobilization is the basis for the calculation of the amount of bound protein.

Reusability of lipase immobilized biosupport materials

About 0.5 g of lipase immobilized on biosupport materials were used to perform the reaction. The solution consisting of 3 mL of 50 mM Tris HCl buffer (pH 8) and 10 mM p-NPP at 50°C was shaken at

150 rpm[30]. Beads were washed rigorously with n-hexane in every 20 min, and the new reaction mixture was put to the biosupport materials, and then were used for the next batch as per the above method.

Results and Discussion

Mechanical strength properties

Strong mechanical strength and extremely high force are required to break the beads those had been treated for half an hour. The beads are stable even at higher agitation speed of 500-3000 rpm[20], when processed for five minutes (data not given).

The swelling ratio properties of biosupport materials

The influence of pH was avoided by immersing dried polyvinyl alcohol/AlgNa samples in distilled water. The effect of sodium alginate content and crosslink density on swelling ratio behavior of polyvinyl alcohol/AlgNa blend hydrogels were evaluated by calculating the swelling ratio of the samples[12]. Each sample has almost same swelling tendency; the size increased for the first 6 hours of conditioning & came into the level with the essence of time. The values of swelling ratio of the biosupport materials in this experiment were observed after for 24 h at every 6 h.

A higher swelling ratio was observed at the longer time of soaking due to the absorption of more water by biosupport materials. The properties of biosupport materials were fascinating due to its ability to absorb a lot of water and swell & insolubility in water. Instability of copolymer crosslinking was observed and a part of copolymer crosslinking broken down into polymer components as the swelling ratio reached the maximum. Among all the biosupport materials prepared, those having concentration of 10% polyvinyl alcohol and 2.0% of AlgNa have the swelling ratio of 40.25% (at a 6 h soaking time) and of 88.00% (at 24 h soaking time).

Thermal properties of the biosupport material

Fig. 1 shows three primary stages of degradation of the polymeric materials due to thermal effects (dehydration, degradation, decomposition)[28]. Initial weight losses of the samples were experienced between 28-235°C, which could be due to the breakage of bonds between water molecules resulting evaporation. Thermal degradation of the mixed sample is the reason behind the second weight loss at around 235-350°C. Third weight loss (350-500°C) is caused by the thermal break down of the C-C principal chain present in PVA-AlgNa blend.

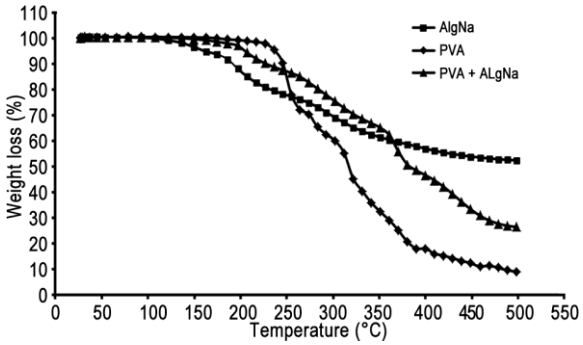

Fig. 1 — Thermal degradation, of AlgNa, PVA and PVA+AlgNa

Effect of pH on lipase activity

The ionization state of the amino acids is affected by due to the presence of protein in lipases. This helps in dictating the structure (primary & secondary) of the lipase as well as controlling the overall activity. Any change in pH affects the built in property of protein & lipase activity[27]. Bacterial lipases do not show any change for pH value from 4 to 11. A detailed study of all bacterial lipase demonstrates that optimum activity of lipases has been found at pH greater than 7[31]. Bacterial lipases offer neutral or alkaline optimal pH. Interestingly, *Pseudomonas* lipases known as alkaline, e.g., *P. fluorescens* HU380[32], *P. mendocina* PK-12CS[33], and *P. aeruginosa* MCCB 123[34] have less pH value of 8.5, 8.0, and 9.0, respectively. Alkaline proteases have broad pH value in between 8 and 12[30].

In this work, alkaline lipase (*Pseudomonas cepacia)* was the most active between pH 7.0 and 9.0. Fig. 2A shows the impact of pH on the activity of immobilized biosupport and free enzyme. Optimal lipase activity was observed to be at pH 8.0 when Tris HCl buffer was applied for free and immobilized biosupport catalyst. Superior activity of the immobilized lipase was found in the range of pH 7.0-9.0. At pH 7 & 10, the activity was increased and reduced, respectively in a drastic way.

Optimum temperature for lipase activity on free and immobilized biosupport materials

Fig. 2B shows that the optimal temperature for the immobilized biosupport catalyst (50°C) was approximately 10°C higher than that for free lipase (40°C). At 40°C, free lipase showed optimum activity, but the immobilized biosupport catalyst had an optimal temperature of 50°C. This elevates the capability of the immobilized biosupport catalyst as a real catalyst in an industrial level, because elevated temperatures cause the viscosity to decrease in the reaction phase diminishing the mechanical energy during agitation and other industrial applications. For

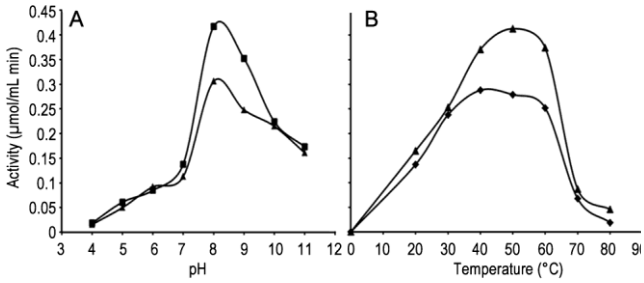

Fig. 2 — Impact of (A) pH; and (B) temperature on --▲---Free lipase activity and--♦--- Immobilized biosupport catalyst activity

phospholipase A1 in gelatin hydrogel, similar outputs were obtained[30] and other lipases immobilized in matrices[35,26]. The optimal temperature of the lipase from *P. aeruginosa EF2* was 50°C[36].

Fourier Transform Infrared (FTIR) analysis

Fourier transform infra red peaks of prepared samples have shown in earlier published paper by the author Kumar et al., 2018[10]. For pure AlgNa the absorption peaks at 3420, 3281, 2612, 1801, 1033 and 615 cm^{-1} were recognized to be hydroxyl, -OH, broad peak –OH, C=O, C-O, and C-H groups respectively[10]. FTIR values of pure PVA gives absorption peaks at 3419, 3289, 2545, 1929, 1015, and 619 cm^{-1} were recognized to hydroxyl, -OH, broad peak –OH, C=O, C-O, and C-H groups, correspondingly. It is clear from the FTIR spectrum, that there was no peak within the spectrum of the combined material from individual components indicating no proof of a robust chemical interplay changing the character of the functional groups inside the sodium alginate/ polyvinyl alcohol blends and sodium alginate + polyvinyl alcohol + lipase (biosupport catalysts).

Reusability performance of lipase immobilized biosupport materials

One of the benefits of using immobilized enzyme is its re-use which enables us to save cost on industrial production. Immobilized lipase activity after ten cycles of operation is given in Fig. 3. After six cycles, the immobilized biosupport catalyst could retain 73.12% of its initial activity; while after 9 cycles, the immobilized lipase had lost half of the initial activity. The results recommend that the method of PVA-boric acid could be used favourably for immobilization of lipase. Operational stability shows that 36% of the initial activity was reserved for PVA immobilization of naringinase after 6 reuses[26].

Scanning electron microscopy (SEM)

The SEM of polyvinyl alcohol/AlgNa biosupport material indicates the solubilization produced by the

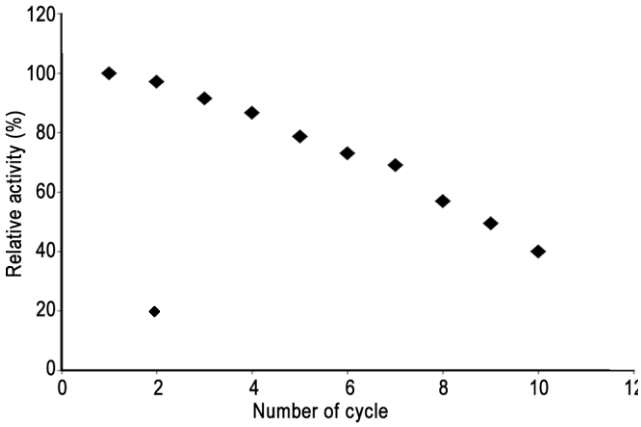

Fig. 3 — Recycling of immobilized lipase in biosupport materials

method of interfacial. Biosupport materials having a better spherical geometry were observed. The SEM microscopy demonstrates score–shell morphology after cross linking with CaB_4O_7. Kumar *et al*.[10] have reported the size of the lipase to be greater than substrate molecule, which is the reason behind the application of the lipase entrapment method[37]. The specific surface area of the biosupport materials and diffusion of substrates is increased due to the coarse and uneven pores on the biosupport materials which formed the little pores generated on the surface of the biosupport materials.

Kumar *et al*. [10] has reported formation of few layers near the vicinity of the surface of biosupport material along with an evenly distributed micro structural layer on the surface of biosupport materials. The biosupport material holds a huge internal cavity close to the surface and a compact shape in the center. Clear layers of cross linked polymers inside the biosupport materials can be seen from the cross-sectional view that showed that the lipase was present mainly in the sub-marginal parts of the immobilized biosupport materials making contact with substrates and raise the activity of the immobilized lipases. In case of PVA/AlgNa beads with lipase, the extraction of CaB_4O_7 was moderately higher than the PVA/ AlgNa beads without lipase resulting in the generation of higher micro porous structure in the former case, which can be confirmed from the SEM characteristics.

Immobilization lipase activity and loading efficiency

Immobilization lipase activity can be defined as the proportion of lipase activity of the beads to the quantity of the beads[38].

$$\text{Immobilized lipase activity } \left(\frac{\text{U}}{\text{g}}\right) = \frac{\text{Lipase activity of the bio-support catalyst}}{\text{The quantity of the bio-support catalyst}} \quad \ldots (2)$$

Lipase from *Pseudomonas cepacia* was immobilized by polyvinyl alcohol/AlgNa H_3BO_3 and $CaCl_2$ method. The lipase entrapped in the polyvinyl alcohol/AlgNa, boric acid, and $CaCl_2$ beads. Lipase-polymer mixture was poured in the treatment mixture (4% H_3BO_3 and 2% $CaCl_2$ solution). Then spherical shaped biosupport materials (2.5 mm in average dia.) were observed without any agglomeration, which showed elastic behaviour equivalent to rubber. Strength and durability of the biosupport materials is the contribution of PVA, while calcium alginate enhanced their surface properties, lowering the extent of agglomeration. The percentage of polyvinyl alcohol in prepared beads was maintained between 10 and 12.5% (w/v), which is favourable for maximum strength of bead[39].

The beads showed strong and high elastic nature with spherical shape. The highest immobilized lipase activities of phospholipase A1 were obtained at the 10% polyvinyl alcohol concentration[20]. The immobilized lipase activity was increased by increasing polyvinyl alcohol concentration from 8% to 10%. The results evoke that the pore walls thickness was increased with the decrease in the amount and size of the macrospores[40]. The tiny pores inside the beads might be obstructing the substrate diffusivity. Low cross-linking rate of polyvinyl alcohol could justify the results. The immobilized lipase activity was decreased by increasing the concentration of AlgNa from 2 to 4%. The results show that the amount of hydrogen bonds in polymer chains were increased, which helped in elevating density of physical cross-linking sites[11]. The maximum immobilized lipase activities and loading efficiency of lipase were observed at 2% concentration of AlgNa and polyvinyl alcohol of 10%, 133.37 (U/g) and 57%, respectively (Table 1).

To modulate the efficiency of lipase immobilization, the synthesis condition such as the matrix concentration (polyvinyl alcohol and AlgNa) and cross-linkage (H_3BO_3 and $CaCl_2$) solution should be manifested. This is to be done because immobilized lipase activity is highly affected by synthesis conditions[11,40]. When treated with high concentration of boric acid, lipase immobilized in biosupport catalyst showed low activity. While in low concentration of boric acid activity of immobilized lipase is enhanced. An acid micro-environment could be created inside the beads by excess borate ions thus decrease in activity of immobilizes lipase. When boric acid concentration is high, biosupport materials become dense due to formation of smaller pores thus causing resistance to diffusivity of the substrate and along with reduction in immobilized lipase activity[11]. The mechanical strength of AlgNa gel is decreased with the decrease of calcium ion concentration. It results in reduction of calcium chloride concentration below 2%, thus causing lowering in immobilized lipase activity[41]. It is best to take 4% H_3BO_3 and 2% $CaCl_2$ concentration to prepare biosupport material; it results in equilibrium between immobilized lipase activity and enzyme loading. High immobilization lipase activity and loading efficiency of 87.28 (U/g) and 55.2%, respectively, was obtained at 2% calcium chloride and 4% boric acid solution (Table 1).

Table 1 — Loading efficiency and immobilized lipase activity with different concentration of PVA, AlgNa, H_3BO_3 and $CaCl_2$

PVA (%)	AlgNa (%)	Sources of lipase losses (mg)				Loading efficiency (%)	Immobilization lipase activity (U/g)
		Beads medium	First wash	Second wash	Total loss		
9.0	1.5	0.51	1.00	0.81	2.32	53.6	112.96
9.0	2.0	0.69	1.03	1.02	2.74	45.2	93.25
9.0	2.5	0.85	1.12	0.70	2.67	46.6	105.31
10.0	1.5	0.72	0.83	0.89	2.44	51.2	130.20
10.0	2.0	0.65	0.79	0.71	2.15	57.0	133.37
10.0	2.5	0.76	1.05	0.95	2.76	44.8	81.62
11.0	1.5	0.68	0.93	0.87	2.48	50.4	113.99
11.0	2.0	0.80	0.99	0.82	2.61	47.8	83.26
11.0	2.5	0.68	0.81	0.79	2.28	54.4	88.32
H_3BO_3 %	$CaCl_2$ %						
3.5	1.5	0.86	1.16	0.92	2.94	41.2	80.23
3.5	2.0	0.79	1.13	0.85	2.77	44.6	78.31
3.5	2.5	1.05	1.14	0.93	3.12	37.6	69.52
4.0	1.5	0.65	0.89	0.80	2.34	53.2	85.10
4.0	2.0	0.52	0.88	0.84	2.24	55.2	87.28
4.0	2.5	0.75	0.97	1.10	2.82	43.6	71.33
4.5	1.5	1.08	0.99	0.89	2.96	40.8	70.65
4.5	2.0	0.86	0.80	0.85	2.51	49.8	78.52
4.5	2.5	0.79	1.00	0.82	2.61	47.8	68.44

Conclusion

The results have shown formation of porous biosupport materials by blending polyvinyl alcohol with AlgNa, for lipase immobilization. Preparation of mechanically stable polyvinyl alcohol/AlgNa biosupport materials demonstrated the significance of their use in bioreactors. Immobilized lipase hydrolyses p-NPP is faster than the free lipase. In industrial processes, if we use immobilized lipase along with polyvinyl alcohol/AlgNa biosupport materials, the capital and operational costs can be reduced. The highest immobilized lipase activity and loading efficiency were 87.28 (U/g) and 55.2% shown by 10% (w/v) polyvinyl alcohol and 2% (w/v) AlgNa biosupport material blended with 4% (w/v) H_3BO_3 and 2% (w/v) $CaCl_2$ solution for 30 min i.e. optimum condition for the experiment. At elevated temperature, the polyvinyl alcohol/AlgNa biosupport catalysts showed an extravagant mechanical stability and activity of lipase in the p-NPP substrate. This study focused on lipase immobilization due to its potential application in industry lipase from *Pseudomonas cepacia* was successfully immobilized by hydrophobically binding with polyvinyl alcohol/AlgNa biosupport materials. The optimum calculated temperature free and immobilized lipase activity was 40 and 50 °C, respectively. Free and immobilized lipase activity was observed at optimal pH 8.0. After six cycles of operation, the immobilized biosupport catalyst could retain 73.12% of its initial activity.

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Conflict of interest

The authors declare no conflict of interests.

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