

To Study the Effect of pH on Lipase

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ABSTRACT

Bacterial lipases are commercially more important mainly because of the ease of their cultivation and optimization to obtain higher yield. The industrial demand of new sources of lipases with different catalytic characteristics stimulated the isolation and selection of *Bacillus subtilis*. This study was designed to investigate physicochemical parameters for optimized lipase production. Microbial lipases catalyze both the hydrolysis and synthesis of long-chain acylglycerols. They are currently given much attention with the rapid development of enzyme technology. The characterization of these enzymes of paramount importance to establish the process conditions for subsequent application. To determine the optimum pH, we used the sodium citrate buffer pH 3, 3.6 and 5.6 and sodium phosphate buffer at pH 7.3 and 8. Change in pH also alters an enzyme's shape. Different enzymes work best at different pH values. The enzyme can continue locking with a substrate and continue its catalytic activity. If the pH is too high or too low it can change the shape of the enzyme. The most favourable pH value the point where the enzyme is most active is known as the optimum pH. Extremely high or low pH values generally result in complete loss of activity for most enzymes pH is also a factor in the stability of enzymes. As with activity for each enzyme, there is also a region of pH optimal stability.

Keywords: Lipase, enzymes, *Bacillus subtilis*, optimal, pH.

INTRODUCTION

Lipase (triacylglycerol acylhydrolases, EC 3.1.1.3) is an important group of enzymes mainly due to a large number of industrial applications. From the industrial point of view, lipase enzymes are considered very important, due to their greater production potential on a large scale. The isolation from lipases by *Bacillus subtilis* from different soil samples namely Ruchi Soya Oil Mill, Bajrang Soya Oil Mill, Ghatabillo, Madhya Pradesh and Dairy farms and characterization of bacteria, determine maximum lipase producer by qualitative and quantitative means were investigated. [1] Lipases are widely found in animals, plants and microorganisms especially those originated from bacteria, are more stable than others. Many microorganisms are known as good producers of extracellular lipases. [2] Studies

on the production of extracellular lipases with *Bacillus* have shown variations among different strains. [3] Lipases occur widely in bacteria, yeast and fungi. [4] Bacterial lipases are commercially more important mainly because of the ease of their cultivation and optimization to obtain higher yield. Microbial lipases are high in demand due to their specificity of the reaction, stereo-specificity, and less energy consumption than conventional methods. [5] High enzyme activity lipase can replace the traditional catalyst in processing biodiesel as this enzyme replaces chemicals in a process which is otherwise highly energy intensive. [6] The applications of lipase also affected by temperature and pH stability. The enzyme is used as a catalyst for production of different products used in cosmetic industry, [7] in pulp and paper industry, [8] in the synthesis of biodiesel, [9] degreasing of

leather and in pharmaceutical industry. [10] They are also employed in organic chemical processing, biosurfactant synthesis, nutrition and biomedical sciences. [11] Lipases enzyme also accelerates cheese ripening and the lipolysis of butter, fat, and cream. [12] They are mostly used in the detergent, food, pharmaceutical industries. The objective of this study was the production of lipase and characterization of the enzyme with regards to its stability in relation to pH and the optimization of pH conditions for obtaining higher lipase activity. In the present study, pH was varied using different substrates concentration. [13] In connection with their biochemical properties, both lipolytic enzymes display different substrate specificities. [14]

MATERIALS AND METHODS

Collection of Sample:

The soil sample was collected from the oil mill namely Ruchi Soya Oil Mill, Bajrang Soya Mill, Ghatabillod, Madhya Pradesh and Dairy firms. All the samples were transported in sterile plastic bags to the laboratory.

Effect of pH on enzyme activity:

The pH activity profile of a lipase enzyme was studied over a wide range from 5.0 – 8.0. 0.01M citrate buffer was employed in pH range 5.0-5.5 whereas 0.01M phosphate buffer was employed for pH range 6.0-8.0.

5ml of enzyme preparation was incubated with 10ml of PVA emulsified substrate and 5ml of the buffer (phosphate/citrate) of the desired pH at 37 degree Celsius and 100 rpm for 2 hr. The reaction was stopped at the end of the incubation period by adding 30ml 1:1 alcohol: acetone solution to the above reaction mixture and then titrated with 0.02N NaOH in the presence of phenolphthalein as indicator. Blank values were obtained by titrating under identical conditions separately.

Blank preparation: 5ml of PVA emulsified substrate and 1ml of enzyme extract. Controls were determined in the same way with enzyme preparation pre- incubated

with 30ml of 1:1 alcohol: acetone solution at room temperature for 1 hr.

RESULT AND DISCUSSION

Effect of pH on enzyme activity (isolates):

Table No. 1

Sample	pH	Blank	Test
Dairy	6.0	1ml	24ml
Dairy	7.0	1ml	25ml
Dairy	8.0	1ml	30ml(max)

Table No.2

Sample	pH	Blank	Test
Bajrang Soya	6.0	1ml	22ml
Bajrang Soya	7.0	1ml	24ml
Bajrang Soya	8.0	1ml	29ml(max)

Table No.3

Sample	pH	Blank	Test
Ruchi Soya	6.0	1ml	29ml
Ruchi Soya	7.0	1ml	30ml
Ruchi Soya	8.0	1ml	34ml(max)

The pH activity profile of lipase enzymes has been illustrated in the above table in the range of pH 6.0 to 8.0. It has been observed that the enzyme exhibit maximum activity at pH 8.0. The graph of the above data was drawn and annexed with this report for at a glance review.



Fig: 1 Effect of pH on enzyme

(Test Tubes from left to right)

1 to 3: Bajrang Soya mill

4 to 6: Dairy Firm

7 to 9: Ruchi Soya mill

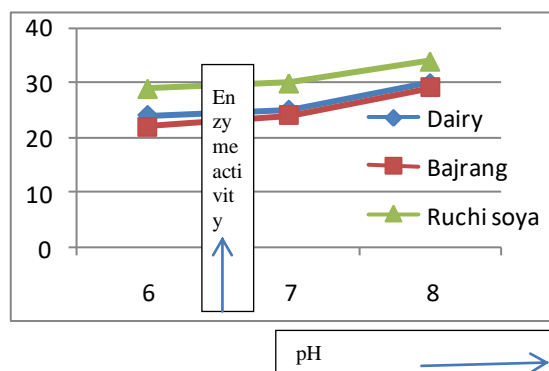


Fig 2: Effect of pH on enzyme activity

CONCLUSION

Thus from the above study, it's concluded that pH is one of the important physical conditions that affect both bacterial growth rates as well as the enzyme production. Each enzyme also has an optimum pH at which it works best. However, the optimum is not the same for each enzyme. The optimum pH of lipase is 8.

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