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Immobilization technique of natural dyes, as a novel method to preserve industrially important *E. coli* and *Bacillus* species

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ABSTRACT

Immobilization is a method used for the preservation of enzymes as this method provides enhanced resistance to the changes in certain environmental conditions like pH/temperature. The advantage of immobilization (IMB) of live bacterial cells is especially high due to their role in environmental monitoring due to their low cost, easy handling and high sensitivity to the environment. Through our project we tried to show that this method of IMB is effective in the preservation of microorganisms. We used natural dyes during the course of our project which were extracted from grinding of fruits and vegetables such as pomegranate, beetroot and carrot. We used Sodium Alginate beads so that there were a good number of beads formed that helped for the proper entrapment of microorganisms *E. coli* and bacillus for future use. Natural dyes were chosen over synthetic dyes due to their environmental friendliness, cost effectiveness and less complexity. To confirm if the method had been effective, we prepared a growth curve to check the growth of the microorganism and found out that this method could be used to store the microbes for a month's duration.

INTRODUCTION

Immobilization is a very practical and frequently used technique for the preservation of enzymes. Enzymes immobilized by this method could be preserved and utilized even after long time durations. Immobilization of the entire cell (Kawaguti *et al.*, 2006) has been shown to be a better alternative as compared to immobilization of isolated enzymes because it helps in avoiding the lengthy and expensive procedures of enzyme purification, preservation and sustenance by simply preserving the enzyme in its natural environment and hence protecting it from inactivation (Sanjay *et al.*, 2012). Immobilization of living microorganisms has been described by several investigators as being helpful in the production of specialty chemicals for industrial use (Hyde *et al.*, 1991). Through our project, we aim at incorporating this technique for preservation of microorganisms so that they can be used in the future without any change in their physiological or chemical behaviour. Using this technique a microorganism could be attached to an inert, insoluble material because of the reaction between sodium alginate and calcium chloride. After the reaction completes, an inert layer of calcium alginate is formed which is what we call as a Bead (Yang *et al.*, 2007). In the past few years many different methods of immobilization have been used such as Affinity-tag binding (Sardar *et al.,* 2000) technique in which cells may be immobilized to the surface, like a porous material, using non-covalent or covalent protein tags and has been used for protein purification purposes. Adsorption (Spahn and Minteer, 2008) on glass, alginate beads or matrix in which the cell is bound to the outside of an inert material. Entrapment method using which the cell is trapped inside insoluble beads or microspheres, such as calcium alginate beads. Cross-linkage method is the technique used in which the enzyme molecules covalently bind to each other to and create a matrix that consists of almost only enzyme.

We have used the entrapment method to trap the microorganisms into the bead. Matrices for entrapment include calcium alginate, carrageenan, agar, cellulose, polyacrylate, and polyamide (Hyde *et al.*, 1991). Immobilization has found its applications in various fields these days ranging from the production of a chiral molecule for pharmaceutical and research purposes to the use of immobilized glucose isomerase in food industry for production of high fructose syrup.

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MATERIALS AND METHODS

Extraction of Natural Dye

Here, we used beetroot, carrot and pomegranate inorder to extract the dye. The three different samples were taken, washed and cut into fine pieces and placed separately on top of the filter paper. We then placed them inside an electronic grinder in order to derive a paste out of it. Then the semi-solid extract was collectedand poured inside different conical flasks that had been labelled accordingly. We covered the heads of the conical flasks with aluminium foil and kept it in the refrigerator for their storage (Fig 1and Fig 2).

Fig. 1, Fig. 2: Extraction of Dye.

Microbes Used

The immobilization of two bacterial strains was performed. The bacterial strains used were *E.Coli* and Bacillus species (*Bacillus cereus*: NCBI Genbank under the accession number KC571175 as *Bacillus cereus* Isolate GS2).

BEAD FORMATION AND IMMOBILIZATION TECHNIQUE

Sodium alginate, calcium chloride and sodium chloride (NaCl) were used for this technique. 4% $CaCl_2$ by weight was prepared by mixing 4 gm. of $CaCl_2$ into 100ml water and stirred well. After this 0.1N NaCl sodium alginate solution was prepared by mixing 0.585gm of NaCl into 100ml water.

Fig 3, Fig. 4: Bead formation.

Fig. 5: Immobilization technique.

To this solution 3.5gm of Sodium Alginate was mixed. For mixing the sodium alginate properly we used a magnetic stirrer at 100 rpm. The Sodium Alginate was separated into three different beakers in three equal portions. Then CaCl2 solution was poured in three petri-plates. Each of the three dyes was added to the sodium alginate beakers. Two strains of bacteria were inoculated into this mixture (Fig 3, Fig 4 and Fig 5).

Growth Kinetics of the Bacteria

To check the viability of micro-organisms, a growth curve was prepared by using growth kinetics. For this, 1ml of Citrate buffer was added to the Nutrient broth. The beads were then dissolved in this mixture and kept for overnight incubation. The growth of bacteria was observed by using colorimeter and the growth kinetics was measured at a wavelength of 610 nm. The OD values were measured and confirmed for the culture the next day. The OD value was checked at a regular interval of 30 minutes and a standardized growth curve of the organism was plotted with time on the X-axis and Absorbance values on the Y-axis.

RESULTS AND DISCUSSION

The immobilisation technique was performed on *E. coli* and Bacillus using dyes extracted from pomegranate, carrot and beetroot. A whatman filter paper-No 1 was used during the extraction of dyes instead of newspaper etc. to prevent any synthetic dyes from the newspaper to add to the natural dye which we were extracting and thus preventing contamination from occurring. The immobilized bacillus cereus (Fig 6 and Fig 7) was then tested by comparing a normal growth curve (Graph 1) to the growth curve observed for the immobilized bacteria (Graph 2). The growth curve values were noted down (Table 1) and a standard graph was plotted for the same.

Table 1: Growth curve values for bacillus cereus strain.

Time(hours)	Colorimeter reading for bacillus cereus strain (nm)
0	0.23
½	0.30
1	0.50
1 ½	0.60
2	0.67
2 ½	0.70
3	0.76
3 ½	0.83
4	0.89
4 ½	0.94
5	0.90
6	1.00
7	1.05
8	1.12
9	1.23
10	1.28
11	1.31
12	1.36
13	1.41
14	1.38
15	1.28

The purpose of doing this was to check if this method could be used as good preservation technique for bacteria with natural dyes instead of synthetic dyes. It was then observed that the growth curve for the immobilised bacillus species seemed to be

more or less similar to a normal growth curve for any other bacteria. Hence it was concluded that this method could be used for preservation of bacterial strains by natural dyes.

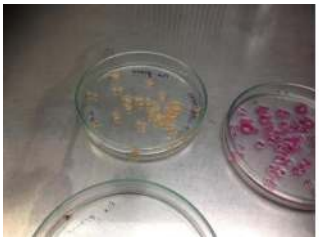
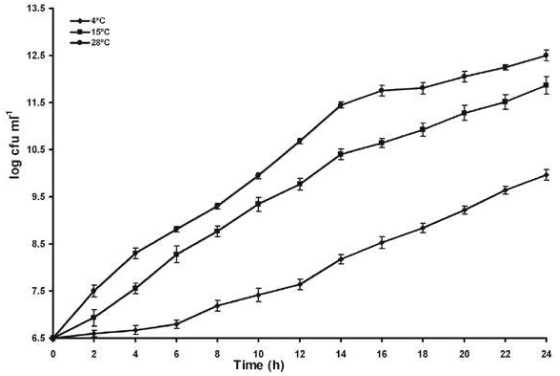
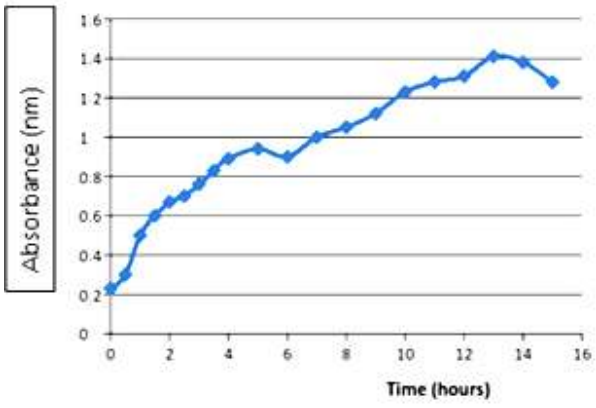



Fig 6, Fig. 7: Immobilized bacillus cereus strains on plates.

Due to the increased awareness on therapeutic properties of natural dyes in public, the worldwide demand of natural dyes has become of great interest. Natural dyes are derived from sources which occur naturally in nature such as plants, insects, animals and minerals. Various synthetic colorants have been banned because they have been found to cause allergy-like symptoms or are carcinogenic. Thus natural dyes are a good resort to use in place of synthetic dyes as they serve the same purpose with potentially no harmful effects to the people working on the method of immobilization. Also the use of natural dyes in place of synthetic dyes has brought down the cost of chemicals etc. which were earlier required to make the synthetic dyes or to buy the readymade synthetic dyes for immobilizing bacterial cells or their respective enzymes.



Graph. 1: Normal growth curve.



Graph. 2: Growth curve obtained from immobilized bacillus strain.

CONCLUSION

Thus the use of immobilized cells is of more interest to the scientists due to its better exploitation under less maintenance as compared to isolated proteins or other nucleic acids which are isolated from the bacterial cells for studies. Not only for research purposes, but also for various applications in different industries, the immobilized cells are finding much use in.

One of the major fields where immobilization method is being much used is for Atomic Force Microscopy (AFM) which has the unique characteristic of imaging cells, biomolecules in a liquid environment without the need to treat the sample chemically. Such imaging techniques use immobilisation of the sample to the mounting surface. There is wide use of bacteria which has been immobilized to treat industrial wastewater containing a chlorinated pyridinol (Meyer et al, 2010). 3, 5, 6-trichloro-2-pyridinol (TCP) was removed from industrial wastewater by using Pseudomonas sp. strain M285 immobilized on diatomaceous earth beads. Silica gel and polyurethane immobilised bacteria play a very important role in healing cracks in concrete the moment they appear.$CaCO_3$can be precipitated by the bacteria after being immobilized. Higher activity of bacteria has been observed in silica gel than in polyurethane. $CaCO_3$can be precipitated by the bacteria in a mimic self-healing process. When cracked specimens were treated by polyurethane immobilized bacteria, more self-healing efficiency was observed. The beads which are formed through this method can be stored for about 15 to 20 days in which the microorganism would still remain in an active state. With further advancement in technology and new age developments in engineering science, this method will find potential use in almost every work field looking for higher efficiency, safety and economically friendly studies.

REFERENCES

AniIdris , Wahidin Suzana. Effect of sodium alginate concentration, bead diameter, initial pH and temperature on lactic acid production from pineapple waste using immobilized *Lactobacillus delbrueckii*, Process Biochemistry, 2006, 41(5): 1117–1123

F. W. Hyde, G. R. Hunt, And L. A. Errede. Immobilization of Bacteria and Saccharomyces cerevisiae in Poly (Tetrafluoroethylene) Membranes. Appl Environ Microbiol. 199; 57(1): 219-22.

Kawaguti HY, Manrich E, Sato HH. Production of isomaltulose using Erwinia sp. D12 cells: culture medium optimization and cell immobilization in alginate. Biochem Eng J. 2006; 29:270–277.

Louise Meyer, Rikke; Zhou, Xingfei; Tang, Lone; Arpanaei, Ayyoob; Kingshott, Peter; Besenbacher Flemming. Immobilisation of living bacteria for AFM imaging under physiological conditions.Ultramicroscopy, Ultramicroscopy. 2010; 110(11):1349-57.

M. Kierstan, C. Bucke. 1977. The immobilization of microbial cells, subcellular organelles, and enzymes in calcium alginate gels, John Wiley & Sons, Inc. pp. 387–397

Qingxiang Yang, Chunmao Li, Huijun Li, Yuhui Li, and Ning Yu. Degradation of synthetic reactive azo dyes and treatment of textile wastewater by a fungi consortium reactor. Biochemical Engineering Journal, 2009; 43(3): 225–230

Sardar M, Roy I, Gupta MN. Simultaneous purification and immobilization of Aspergillusniger xylanase on the reversibly soluble polymer Eudragit(TM) L-100. Enzyme Microb.Tech. 2000;27:672–679.

Singh, D, Mondal, K.K., and Gopalakrishnan, J. 2008. A Practical Manual on Detection of Bacterial Plant Pathogens: Symptomatology to Advanced Techniques. Division of Plant Pathology, IARI, New Delhi. 80pp.

Singh Sanjay, Amod Kumar, V Suneetha, Bishwambhar Mishra, Gopinath R, Sharad Yadav and Bhaskar Mitra.Synthesis and activation of Immobilized beads by natural dye extracts. Int. J. Drug Dev. & Res., 2012; 4(1): 115-128

Spahn C, Minteer SD. Enzyme immobilization in biotechnology. Recent Pat Eng. 2008;2:195–200.

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