Immobilization of microbes to various supports and its application in BOD sensor

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Introduction

Advancement of biotechnology and the developments in its various branches have made immobilization an important technique in the last few decades. Broadly immobilization can be defined as the confinement or localization of viable microbial cells to a certain defined region of space in such a way as to exhibit hydrodynamic characteristics which differ from those of the surrounding environment (Webb and Dervakos 1996) or retention of a biologically active catalyst (enzyme/enzymes or cell/cells) on a solid support material which should be mechanically strong and having good diffusional characteristics. Moreover impart a good interaction between the catalyst and the support material. Immobilization excels over conventional techniques because the immobilized catalyst can be used time and again for longer duration reducing the cost and time involved to perform a particular task.

Interest in immobilization and its application in various fields have sharply increased in last few years owing to the development of a wide array of support materials and different methods for immobilization. There are several methods available for immobilization e.g. adsorption, covalent binding, entrapment, cross linking and encapsulation (D'Souza 1999). During adsorption, the catalyst binds reversibly to the surface of the support material by the involvement of different forces like electrostatic, ionic and van der Waal's forces. Covalent binding takes place with aid of formation of covalent bond between catalyst and the solid support (eg: immobilization on inorganic supports, Moreno 1994). Likewise, entrapment is one in which the catalyst is in the freeform in the solution but there is only controlled movement (eg: use of copper alginate gel, Palmieri 1994) whereas cross linking involves joining of the cells/enzymes to each other to form a large structure (eg: enzyme & protein immobilization, Walt 1994). Encapsulation is achieved by enveloping the catalyst within various form of a semipermeable membrane (eg: encapsulation on silica gel, Livage 2001).

Immobilization has widespread application in various fields. One of them is in the field of environmental biotechnology where it plays an important role in detecting pollutant level by using biosensor. Biosensor is an analytical device which is used for detection of an analyte in a sample. The sensor has mainly three components a biocatalyst, a transducer and a detector. Biocatalyst is nothing but the immobilized microorganism whose metabolic activity is exploited to detect the pollution load in the mixture. The biocatalyst can be immobilized onto different support materials like charged membranes etc.

Therefore, for the successful development of a biosensor, in addition to analyzing various parameters like the enzyme/cell characteristics and behavior, we should be extra cautious while selecting the solid support material for immobilization because it has a lot of impact on the catalytic activity of the cell/enzyme after immobilization. To provide good reproducibility and sensing characteristics to the biosensor, the immobilized microbial membrane must have a long shelf life, high viability and stability and should allow the diffusion of substrates in and out of the membrane.

In the present study, different support materials were tested with respect to their stability, viability and leaching characteristics. The functional response of the membranes was then analyzed with GGA in order to select the best supporting material for the development of BOD biosensor.

Material and methods

Three bacteria namely *Aeromonas hydrophilla, Enterobacter cloacca, ,Yersinia enterocolitica,* were used for immobilization. Two different support materials were tested nitrocellulose filters & nylon membrane.

The inoculum was prepared by inoculating one loopful of all the three individual bacterial isolates in 50 ml of sterilized nutrient broth. The inoculated broths were incubated in an orbital shaker at 37 ^{o}C for 16-24 hrs so as to obtain actively growing mother cultures. The above cultures were inoculated separately in 100ml of sterilized nutrient broth and incubated at 37 ^{o}C for 16-24 hrs. Finally, these bacterial cultures were mixed in equal proportions on the basis of their optical density values at 625 nm. This microbial mixture was centrifuged at 6500 rpm for 20 min at 4 ^{o}C. The cell pellet was washed twice with 50 mM phosphate buffer and suspended in small volume of the same buffer. The resultant cell slurry was immobilized on different support materials.

Immobilization in nitrocellulose and in nylon membrane: For immobilization appropriate aliquots of the cell suspension were filtered under vacuum on the two different commercially available membranes. The immobilized microbial membranes were allowed to dry overnight at room temperature.

The immobilized microbial membrane was stored at different temperatures i.e., 4 ^{o}C - 37 ^{o}C and different pH in order to study the stability and viability of the membrane. The membrane was also studied for leaching studies which is an important parameter for long shelf life of the biosensor.

The immobilized membranes were checked for their response in terms of change in current using GGA as a reference standard in BOD analysis. The response was measured by coupling the immobilized microbial membrane to cathode of the oxygen probe. For this, the immobilized membrane attached with electrode was dipped in stirred phosphate buffer solution, 50 mM (pH 6.8). Aliquots of stock GGA samples were added after a stable current was attained for 30 min (initial steady state current). The current change (decrease) was observed after addition of the samples until a steady state was reached. Consumption of oxygen by the immobilized microbial membrane caused decrease in dissolved oxygen in system. The response was calculated on the basis of current difference between the initial steady state current and final steady state current

Results and Discussion

It is well known that the cells are negatively charged (Bickerstaff, 1997) and this property has been exploited to immobilize whole cells on positively charged supports such as nylon membranes/nitrocellulose membrane.

Microbial consortia were immobilized on different supports- nylon and nitrocellulose. The immobilization in nitrocellulose membranes involves the entrapment of microbes, whereas, cell immobilization on nylon membranes involve dual modes of immobilization i.e. entrapment as well as adsorption.

The immobilized microbial membranes were checked for their viability and stability prior to their use as the biocomponent for the construction of the BOD biosensor. The results for stability study of immobilized microbial membranes stored at different temperatures under constant pH (6.8) show that the microbes immobilized on nylon membranes exhibit more stability as compared to those

immobilized on the nitrocellulose membrane. The stability studies done at four different temperatures i.e, 4 ^{o}C – 37 ^{o}C showed that better result was obtained when the membranes were stored at 4 ^{o}C in comparison to the membrane stored at higher temperatures. The stability of all the prepared membranes at different pH but at constant temperature was also assessed simultaneously. The results showed that the phosphate buffer (pH 6.8) was best suited for the storage of microbial membrane.

The results of viability studies showed that the nylon membrane stored in phosphate buffer pH 6.8 at 4 ^{o}C remained viable for extended periods of time (up to 400 days) in comparison to the membranes stored at other temperature and pH. Nylon membrane gave more promising results than nitrocellulose membrane. In case of leaching studies the nylon membrane shows better results as compared to nitrocellulose membrane as negligible leaching was observed as evident by the optical density measurements of the storage buffer.

The immobilized membranes were checked for their response in terms of change in current using GGA as a reference standard in BOD analysis.

Table1. Comparison of nylon nitrocellulose membrane.

S.No.	Parameters	Membranes	Time in Days							
			15	60	120	180	240	300	360	400
1	Stability at 4^{o}C and pH 6.8	N1*	+++	+++	++	+++	++	++	++	++
		N2*	+++	+	-	-	-	-	-	-
2	Viability at 4^{o}C and pH 6.8	N1*	+++	+++	+++	++	++	++	++	+
		N2*	++	++	+	+	-	-	-	-
3	Leaching§	N1*	0.00	0.02	0.05	0.09	0.16	0.18	0.24	0.28
		N2*	0.10	0.22	0.45	0.59	0.95	1.25	1.35	1.42
4	Response of membrane (ΔI) at 60mg/lGGA	N1*	360	372	370	375	365	362	360	372
		N2*	300	225	185	12	-	-	-	-

*N1 – Nylon Membrane
*N2 – Nitrocellulose membrane
§Optical density of storage buffer at 625nm
+ 70-50% (stability)
++ 90-70% (stability)
+++ 100-90% (stability)
+ Poor Growth
++ Good Growth
+++ Excellent Growth

Conclusions

The results of present study show that the nylon membrane performs better as compared to the nitrocellulose membrane with respect to the stability, viability, leaching and current response and is therefore better suited for the development of membrane based BOD biosensor.

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