



A Review on Membrane based Sensor used for production of enzyme.

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Abstract – The study of indigenously prepared membrane for bacterial affinity and for the detection of bacterial contamination in water sample had been carried out. The proposed work was initiated with the selection of specific polymeric membrane with enhanced affinity towards selected gram positive and gram negative bacteria. And the development of polymeric membrane based sensors for the detection of bacterial contamination in any water sample was carried out. Eventually, indigenous bacterial isolates retained on the above membrane will be tested for the ability to produce industrially important enzyme. Enzymes play a pivotal role in detergent, pharmaceutical, leather, food, metal recovery, and waste treatment and agriculture industries. The research activity in this area would contribute towards developing advanced bioprocess technology to reduce the toxicity of the pollutants and also to obtain novel useful substances through enzyme catalysis.

Keywords: membrane preparation, membrane characterization, Bacterial affinity membrane, Enzymes, Protease producing Enzymes.

I. INTRODUCTION

The ability of specific microorganism to produce specialized enzyme and protein has been exploited for many purpose in industries. Industrial important microorganisms are used to produce many things including food, cosmetics, and pharmaceuticals and constructing materials. Microorganisms abundantly present in water and soil. Membrane are defined a discrete, a thin interface that helps in the separation of chemical species or ions in a selective manner that passes through it. Membrane are used for various purposes but it is famous for being used in water purification like R.O Technology.

There is an inherent characteristics of bacteria to attach RO membranes. Because there are no transmembrane pressure differentials; the bacteria get attach to the membranes. Attachment between bacteria & membrane occurs due to accumulation of trace organic nutrients at the solid-liquid interface that is enough for the bacteria to survive.

II. LITERATURE REVIEW

A. Preparation of membrane

Sweta Binod Kumara (2016), suggested that Polysulfone (PSF) having different concentrations (15, 18, 20 % W/W) in dimethyl formamide were prepared through slow dissolution in heating for 4 h. Subsequently, the viscous polymeric solutions were casted

on a polyester support fixed to glass support and all the membranes were immediately immersed in non-solvent medium (water) as mentioned elsewhere [1].

B. Preparation of Enzyme

Suganthi (2013) Proteolytic production of the bacterial strains was screened on agar plates supplemented with 5% NaCl and 1% casein (MNA). The plates were incubated overnight at 37 °C. The protease producing strains were selected based on the zone of clearance [2].

Gaurav Pant (2014) The culture was harvested after 2 days' growth at room temperature. The crude enzyme preparation was subjected to ammonium sulphate precipitation, and the harvested culture was filtered through Whatman no. 1 filter paper of pore size 125 mm and centrifuged (REMI C-24BL) at 5000 rpm for 30 min at 4 °C. Ammonium sulphate was added slowly to the cell-free culture filtrate at 75% saturation to precipitate the protein, with continuous shaking and a magnetic stirrer [3].

Navneet Batra (2014) Production of the protease from the selected strains of bacteria was carried out in a medium containing peptone (1.5%), malt extract (1%) and NaCl (0.5%) at temperature of 40°C and 150 rpm. The pH of the medium was adjusted to 10 with 0.1 N NaOH. Samples were taken out at regular interval of 6 h and estimated for optical density, protein and protease activity [4].

Gitishree Das (2010) The culture media used for mass production of protease contains Dextrose 1%(w/v), peptone 0.5%, KH₂PO₄ 0.2%, MgSO₄ . 7H₂O 0.2%, Casein 1% and pH 8.0. It was maintained at 37°C for 48hrs. in a shaking incubator. After inoculation, fermentation was carried out at 37°C at 200 rpm for 48hrs. At the end of each fermentation period, the whole culture broth was centrifuged at 10,000 rpm for 15 minute, to remove the cellular debris and the clear supernatant was used as enzyme preparation (Olajuyigbe and Ajela, 2005). The purification of the enzyme was done by ammonium sulphate precipitation method [5].

Varjani Sunita J (2014) All experiments were carried out for 72 hours, every 24 hours sample was removed from flasks and enzyme assay was performed to check protease activity against respective blank sample. For each experiment blank/control flask was kept without inoculums [6].

Qasim Khalil Beg (2013) After optimizing the values of various physical and nutritional factors by a 'one at a time method', five most important factors, namely, casamino acids (A), glucose (B), inoculum age (C), incubation time (D) and agitation (E) were observed, which mainly controlled protease production by *B. mojavensis* under batch fermentation. Taking the above factors in consideration, a response surface methodology using a two-step experimental design was adopted for improving total protease production [7].

Hema. T. A (2012) Protease activity was assayed by the modified method of Keay et al., 1970; 1ml of the diluted enzyme was mixed thoroughly with 1ml of 2% casein solution. The mixture was incubated at 37°C for 10 min. Then the reaction was terminated by the addition of 2ml of 0.4 M trichloro acetic acid and the mixture was again incubated for 20 min at 37°C. The incubated solution was filtered through Whatmann no:1 filter paper. Then 1 ml from the filtrate with 5ml of 0.4M sodium carbonate and 1ml of 0.5 N folin phenol reagents was

added and mixed thoroughly. Again the mixture was incubated at 37°C for 20 min and the final solution was measured at 660nm. One unit of protease activity was defined as the amount of enzyme required to liberate 1 μ of tyrosine in 20 min at 37°C) [8].

Nihan Sevinc (2008) Two culture media used for protease production and compared. One of them contained (% w/v) : glucose- 0.1, peptone-1, yeast extract-0.02, MgSO₄-0.01, CaCl₂-0.01, K₂HPO₄-0.05 (pH 7.0) (Qadar et al. 2009). Other composed of (% w/v) : glucose-1, peptone-0.5, yeast extract-0.3, MgCl₂-0.02, CaCl₂-0.04 (pH 7.0) (Sangeetha et al. 2008) [9].

Baby Joseph (2011) The production of proteases was carried out by solid state fermentation (SSF) method. Ground nut shell medium(sieved ground nut shell – 100gms, Sodium chloride – 0.5gms, Casein – 0.5 gms, Distilled water -250ml, wheat bran medium(sieved Wheat bran – 100 gms; sodium chloride, casein – 0.5gms; Distilled water – 250ml) and chemical medium (Glucose – 100mg; peptone – 0.5g; KH₂PO₄ - 0.5gms; MgSO₄.7H₂O – 0.2gms; FeSO₄.7H₂O – 0.01gms; Casein digest – 1.5gms; Lactose – 2.0 gms; Distilled water – 100gms) were used in this study. The contents of the flask were inoculated with 1mL of inoculums (1x10⁸ cells/mL) after autoclaving. The contents were mixed thoroughly by gently beating the flasks on the palm of the hand and incubated in different incubation temperatures (12, 24, 36, 48 and 72 hrs) [10].

Sathiya G (2013) Dyes synthetic medium was used for the proteolytic enzyme production studies. A quantity of 250 ml broth was dispensed in 500 ml of Erlenmeyer flask and inoculated with the *Bacillus subtilis* culture. It was then incubated at 37°C for 48 hrs. Then the medium was centrifuged at 15000 rpm for 10 minutes at 40°C and the supernatant was taken for the experiment [11].

F Soundra Josephine (2011) Production of protease from *Bacillus*, Sp was carried out in a medium containing the following: glucose, 0.5% (wt/vol); peptone, 0.75% (wt/vol); and salt solution, 5% (vol/vol) (MgSO₄ .7H₂O, 0.5% [wt/vol]; KH₂ PO₄ , 0.5% [wt/vol]; and FeSO₄ .7H₂O, 0.01% [wt/vol]), pH-7.0 and maintained at 37 °C for 24 hours in a shaker incubator (200 rpm) [12].

Chinnasamy Muthulakshmi (2012) Precultures at 10% of the working volume were prepared by 5544 Afr. J. Microbiol. Res. inoculating 200 ml of modified M162 medium with a loopful of the pure isolate from an agar plate in 1000 ml Erlenmeyer flasks. The flasks were incubated for 24 h at 45°C, 200 rpm in a New Brunswick Scientific Innova 4000 incubator shaker. The precultures were microscopically checked for contamination using a Zeiss Olympus Phase Contrast Microscope [13].

III. CONCLUSION

The bacterial species in water attach on the polymeric membrane surface based on several physicochemical properties of bacteria, membrane and environment. The aim of this study was to check bacterial adhesion on different polymeric membranes with its modified hydrophobicity and use it to concentrate bacteria in water sample. In future, membrane with selective carbon source or mixture of carbon sources for the detection of targeted bacteria or group of bacteria can be prepared.

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