# Escherichia Coli –Polymer Bead for Setting out Bioremediation Process

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**Abstract--Microbial cell immobilization in a polymeric bead is a technique in which microorganisms are trapped within the complex matrix of water- insoluble gel. The microbial cells are entrapped into the matrix. They are not directly attached to the support surface, rather simply encapsulated inside the polymer matrix. Polymer beads are completely biodegradable and have no environmental pollution effect. Moreover it does not change any genetics characters of species, increases stability, protection against contamination. Our experiment, immobilization of Gram (-) bacterium *E. coli* was carried out by using sodium alginate gel beads entrapment technique where the *E .coli* cells are entrapped within a solution of sodium alginate and PVA (Poly vinyl alcohol) mixer. *E. coli* beads are produced with in calcium chloride solution. It looks like a circular, white, soft gel. *E. coli* cell are present in viable form for a long time period in that polymer bead. It will be used as a stock culture in laboratory, On treatment it significantly reduced the microbial contamination from effluent and increased dissolved oxygen concentration, therefore may be applicable in waste water treatment and detergent industry to remove dirty.**

***Key words: Cell immobilization, PVA-Sodium alginate bead, Escherichia coli, BOD level, Dissolved oxygen.***

## I. INTRODUCTION

Microbial bead production or cell immobilization process is a technique in which microorganisms are trapped within the complex matrix of water-insoluble gel polymerized pores by polymerization and precipitation .The network lattice of the gel polymer prevents cells from leaking, while allowing substrates to infiltrate and the products to escape[1]. Microbial bead production is done for long time preservation of microbial culture so that microbial cells are not able to divide but remain viable for so many weeks. Mainly bacterial species have ability to remain alive after the completion of growth (e.g., after 24 hr at 37°C ). but maintaining optimum and suitable environment may vary species to species. This problem may overcome by production of microbial bead [2]. Microbial beads give a protection against contamination and retain their original characters. Immobilization technique is necessary to eliminate genetic instability. Most laboratories maintain a large collection of pure cultures by making bead formation which is frequently referred as stock culture collection [1].

The beads are biodegradable and produce no environmental pollution. The released bacteria that is available for root colonization immediately at their germination. Microbial beads are stored at ambient temperature over a long period without loss of bacterial content. Polyvinyl Alcohol (PVA) and sodium alginates both are non-toxic; therefore they are used to entrapment of E coli bacteria. This *E coli* bacterial beads is used for elimination of microbial contaminant from effluents [1,2].

## II. MATERIALS AND METHODS

### *Escherichia coli* culture and Growth curve plot

Small freshly saturated cultures of *E. coli* BL21(DE3) strain remove the cap from a sterile 16- or 18-mm culture tube. 5ml of aliquot was transferred into another tube containing nutrient broth. Grow the culture at 37°C with vigorous agitation. Under these conditions, the cells will reproduce rapidly and the microbial growth curve was constructed by plotting the increased cell numbers versus time of incubation. In every predetermined time the microbial culture was taken for spectrophotometric analysis at 600nm [4].

### Bead Formation

PVA and sodium alginate were mixed in a equal ratio and poured in a boiling water for proper mixing. It was autoclaved at 121°C for 15 minutes for proper sterilization. Inoculated with *E. coli* cell were taken to at its mid logarithmic growth phase. The particular time was obtained by measuring the O.D value of *E. coli* culture. It was then mixed thoroughly with PVA- Sodium alginate solution. The mixture was taken in a sterile 5mL syringe and extruded drop by drop into a cold sterile 5% $CaCl_2$ solutionn. The Beads are filtered by using filter paper and finally washed with distilled water. It was air dried and stored in a transparent glass Jar for future use [1].

### BOD & Dissolved Oxygen measurement

The RO water and fresh pond water were taken as control and standard respectively. The sample effluent was collected from nearest industrial area. The entire water sample was collected in a 300mL BOD bottle. The dissolved oxygen amount was measured by The Winkler Method, briefly Winkler's A ($MgSO_4$ Solution) and Winkler's B (Alkaline KI solution) were added to the all labeled bottle (Control, Standard, Effluent Sample) preciously for avoiding possible air contact. It was mixed by inverting the bottle 3-4 times and keeps it in dark. A precipitate was appeared and allowed the precipitate to settle down. Then concentrated $H_2SO_4$ was added and closed the bottle followed by shaking till the precipitate dissolved. The color change was observed. It was

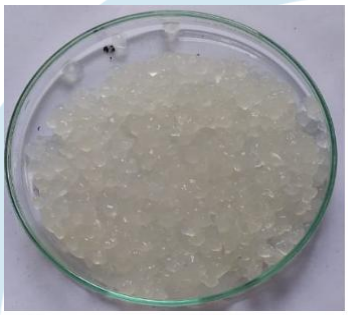
titrate with 0.005 (N) $Na_2S_2O_3$ solution using starch as indicator. The colored solution turned into colourless at the end point. Repeat the titration to get 2 or 3 concurrent readings [4].

After measuring the dissolved oxygen (DO) amount in the water, waters were treated with 0.5gm of Microbial beads.Then all of them were kept in BOD incubator for 5 days for further measurement of DO. After 5 days, water color was observed and titration experiment performed same as before.

**Data Management and Statistical Analysis**

All experiment was carried out thrice and data were electronically captured into Microsoft excel sheet. One way Anova were performed for analysis of data. Group analysis was also performed. Data were represented as Mean ± SD value followed by significance were written alphabetically upon it. Same letter represented non-significant and different letter assure significance.

## III. RESULT AND DISCUSSION



E-coli immmobilized in polymeric beads

Dried Bead after autoclave

A fine white coloured round shaped, approximately 2 nm in size ,soft gel like structure of microbial bead were produced (Fig. 1). it was autoclaved and the bead colour was changed into light yellow colour. Further it was air dried and kept in a glass jar for future use.

**Figure 1: *E coli* Bead for production**

*E. coli* growth curve suggested that cell doubling occurs between 20-40 min. This time is required for hatching out the cells for developing beads because it releases enzyme pectin-lyase and others enzyme. Enzymes secrete into extracellular growth medium and are covalently bonded to each other and outside of the inert materials to create a matrix.

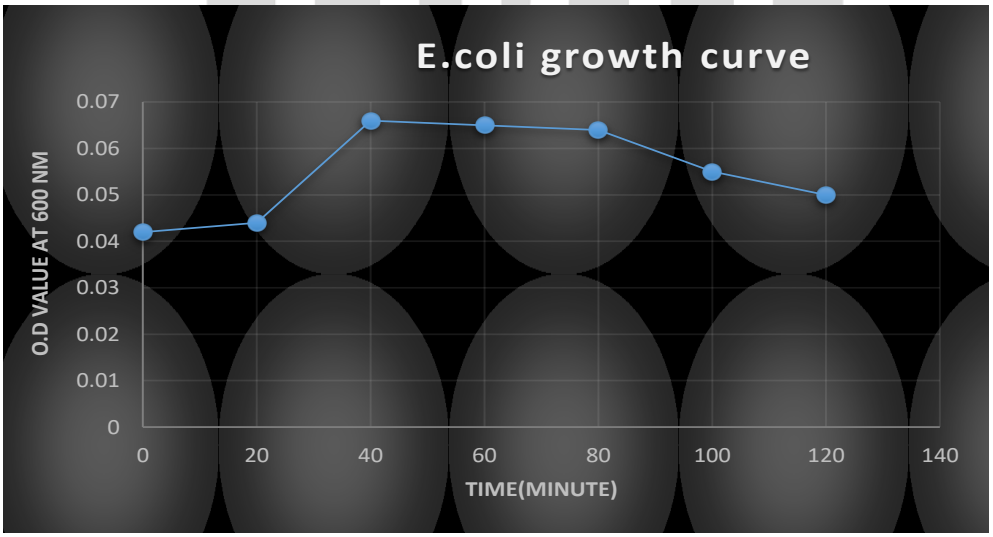



**Figure 2: *E. coli* growth curve**

For *in-situ* bioremediation, we found that 5 days treatment with Microbial bead reduced BOD level of effluent significantly compared to standard (Pond water) and control RO water. It was significantly increase DO level from day 0 to day 5 both in standard and effluent water (Figure 3). pH value significantly reduced in effluent water after treatment (figure 4).

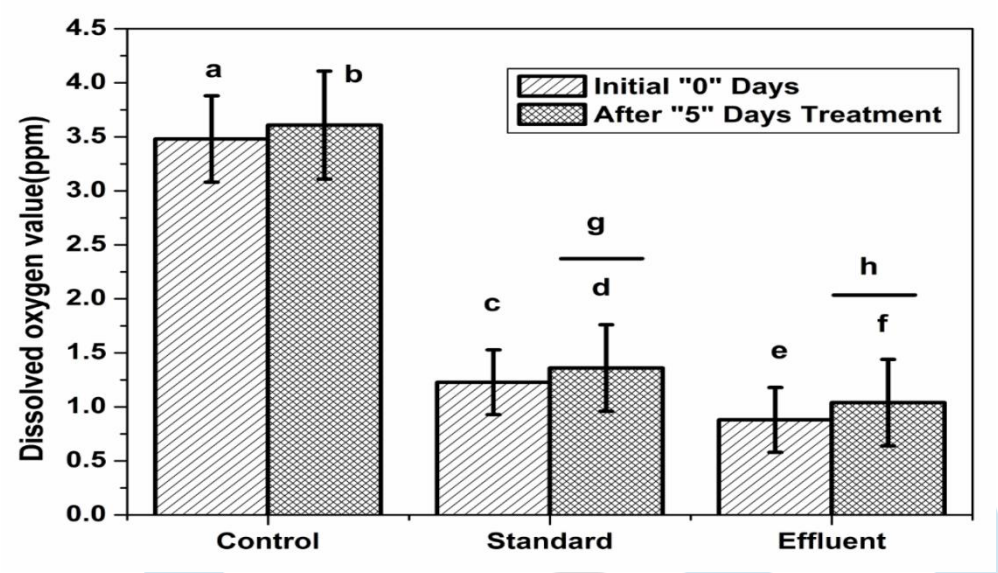



**Figure 3 Dissolved Oxygen value, Data Represneted as Meanss ± SD,**

Slightly alkaline water promote microbial growth as it contain minerals, whereas, in neutral or slightly acidic environment microbes released form bead and degraded the hydrocarbon and the bacterial, fungal biomass of the effluent. Therefore dissolved oxygen level was increased and water quality improved [5].

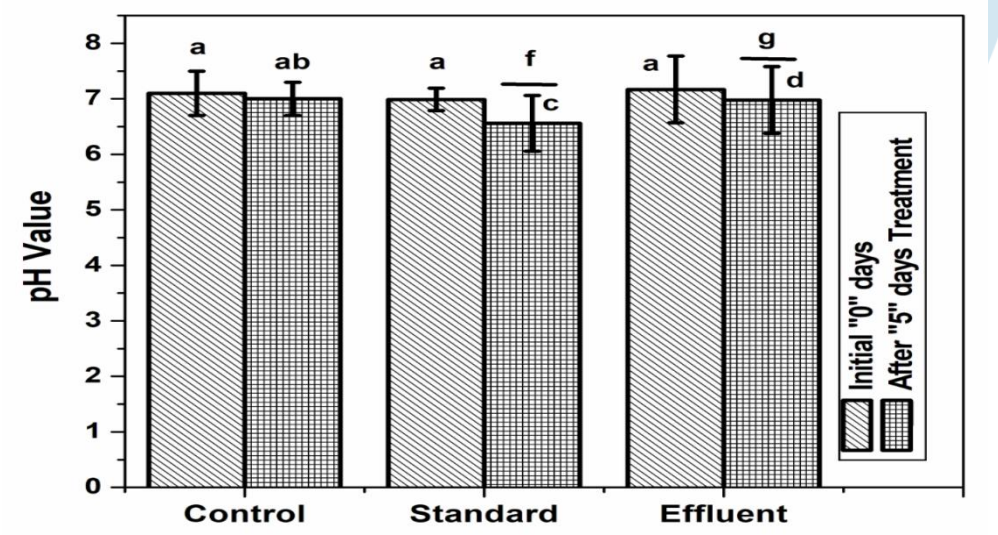



**Figure 4 pH value, Data Represneted as Meanss ± SD,**

## IV. CONCLUSION

Microbial bead production or enzyme immobilization is one of the most promising approaches for exploiting enzyme based processes in biotransformation, diagnostics, pharmaceutical and food industries. Several hundreds of enzymes have been immobilized in a variety of forms including penicillin G Acylase, lipases, proteases, invertase, etc. Research should be focused to overcome the current limitation related to immobilization techniques, so as to expand the horizon from all round application. Large beads have a potential use in industry for production of microbial compound, in wastewater treatment (removal of contaminants), in agriculture for production of bacterial inoculants.

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