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# USE OF ENZYME TECHNOLOGY FOR THE DEGRADATION OF HARMFUL XENOBIOTICS (AROMATIC DYE)

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**Abstract:**

*The basic objective of the present investigation is to study the enzymatic pre-treatment as alternative physicochemical methods, for enhancing the biodegradability of aromatic dye compounds and was found to be more effective (Alexander et al., 1997). Horseradish peroxidase was extracted and studies for azo degradation at batch scale. The batch studies performed to study the process parameters during azo dye degradation revealed, effect of repeated application of alginate immobilized enzyme on dye degradation as 52%removal of dye in first batch which was reduced to 22.2%in the fifth batch .Where as acrylamide Gel immobilized HRP degraded 80%in first batch which was reduced to 15%in the fifth batch .Based upon the results the studies were further conformed to optimize the immobilized enzyme procedure. There was an increase in percent degradation of dye with increase in temperature as 4ºc showed 47.7% degradation & 24ºshowed 72.2% increased degradation , similarly at acidic pH of 2 approximately 77.7% degradation was observed & at basic pH 8.9 it was 50.5%.Two types of immobilizing materials, alginate & acrylamide gel used for immobilizing the enzyme. Immobilized beads showed effective degradation rates compared with free enzyme. Repeated application of beads showed consistently reduced activity. By comparing gel immobilized matrix is effective in degradation than at alginate.*

**Key words:** *Alginate, Acrylamide Gel, immobilization, horseradish peroxidase.*

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## INTRODUCTION:

The industrial use of enzyme dates back to early 1900's (Radzicka & Wolfenden 1995, Kraut 1988). Enzymes, protenaceous materials are defined as temperature sensitive organic catalysts produced by living cells and capable of acting outside and inside cell (Knowles 1991, Neet 2018). The catalytic action of enzymes is extremely efficient, selective and to a greater extent is the consequence of its protein structure (Wand 2001, Zavodszky et al., 1998, Zaccai 2000), as compared to chemical catalyst as enzymes demonstrate higher reaction rate, milder reaction conditions and greater stereospecificity (Benkovic and Hammes 20003, Agrawal et al., 2002; 2004; 2005). Xenobiotic compounds normally encountered in waste water adversely affect the ongoing biological process by inhibitory growth of microbial population and the performance. Xenobiotic compounds are not potential enough to conventional biological wastewater treatment. Possible reasons for their non-biodegradability are lack of requisite enzymes in the conventional biological treatment plant.

A bioreactor is an important part of biochemical processes, as it is efficient to convert raw constituents into economically viable finished products (Butter 2005, Eva and Ralf 2008, Zaushitsyna *et al.*, 2013). Its structure is designed to maintain biological environmental conditions required for cells or other biomolecules. Bioreactors cre classified as batch, fed batch or continuous based on modus operand. The bioreactor used in this study is Packed Bed bioreactor **(Figure 1)** which has a solid immobilized enzyme packed column. A nutrient broth is suspended as flowing liquid over the immobilized bed of enzyme.

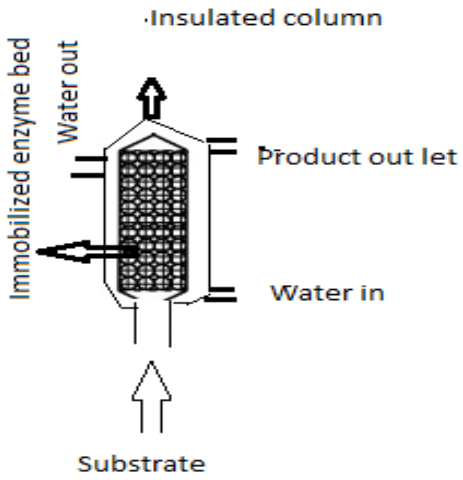


Figure :1. Bed Bioreator for Dye degradation

The economical product so obtained is in liquid form collected at the other end of the reactor. The movement of the fluid can be up or down, but due to gravity downward streaming is preferred. An insulating column with water circulation helps to maintain the temperature. Though not sufficient to maintain the pH it needs to be checked at frequent intervals.

Enzyme immobilization refers to the entrapment of enzyme with an inert, insoluble material (Wu Hong *et al.*, 2013, Gray *et al.*, 2013 Engelmark 2014, Zucca and Sanjust 2014).Immobilized enzyme has been a very important part of pharmaceutical industry (Schmidt 2004, Peinado *et al.*, 2006, Aragao *et al.*, 2014). Degradation of dye using enzyme has been an effective method (Venkaramohan 2002, James and Monika 2002). Acritical analysis of protein dynamics and structure is an advantage in this experiment (Schramm and Shi 2001, Heller 2005, Eisenmesser *et al.*, 2002).It is very useful for continuous process and serves best for bioreactor operation. It can be used for single or batch studies.

The present work encompasses investigation of enzymatic pretreatment as a potential alternative to physicochemical method of treatment for enhancing biodegradability of aromatic dye bearing waste water.

Also, optimizing the immobilization technique for peroxidase enzyme and subsequent studies for dye degradation and optimizing process parameters.

## MATERIALS AND METHODS:

**Dye preparation:**

Dye Acid Black 10 BX, was purchased from sigma. The dye effluent was prepared prior to experiment by mix stock of dye at 18 mg/l ml distilled water.

2.2. Horseradish peroxidase enzyme extraction:

Horseradish peroxidase enzyme was extracted by using the protocol of Bhunia et al. (2001). In brief the procedure followed was as follows- The Horseradish roots were crushed in grinder after removing all the dust and impurities. The extract was centrifuged at 10,000 rpm for 6 min at 4 °C. The supernatant was collected and dialyzed by using 12 KD membranes against 0.1 M acetate buffer (pH 4.5) buffer then the purified enzyme was stored at 0 to -4°C for further use.

**Enzyme activity measurement:**

The HRP enzyme activity was measured by using Nicell and Wright, (1997) method. In brief the phenol and $H_2O_2$ were used as substrate and 4-aminoantipyrine was used as chromogen dye. The enzyme activity assay was performed in 0.1 mol/l phosphate buffer (pH 7) containing 1.0 x $10^{-2}$ mol/l phenol, 2.4 x $10^{-3}$ mol/l 4-aminopyretene and 2 x $10^{-4}$ mol/l $H_2O_2$. The rate of $H_2O_2$ consumption was calculated by the color formation at 510 nm.

**Immobilization of Horse reddish peroxidase (HRP) by using alginate method:**

$CaCl_2$ and alginate were prepared and alginate was mixed with enzyme and leads were formed in stirred $CaCl_2$. Beads were weighed and stored in phosphate buffer. Dye degradation by immobilized beads batch studies were performed, 4.5 ml of dye was taken in a vial 0.1 ml of $H_2O_2$ and 2gms of immobilized beads were added subjected to agitation on shaking for a period of 45 minutes. Residual dye concentrations were measured at 617 nm OD. Repeated usage of beads was studied for 5 times by some procedure.

**Immobilization of HRP by using acrylamide gel:**

Phosphate buffer (pH 7): acrylamide and bis-acrylamide were dissolved in this buffer and finally ammonium persulfate was added. Polymerization was brought about after adding TEMED within 20 to 30 min. Gel was washed with water. Gel was broken by as pirating with Pasteur pipette centrifuge at 100 to 1200 ppm weigh dry.

Effect of pH on dye degradation was estimated by taking concentration of dye at varied pH: 2, 3, 4, 6 and 7. OD was observed at 617 nm.

Effect of repeated application of immobilized enzyme was observed at least 5 times.

Effect of temperature on dye degradation with immobilized HRP was observed at temperature 4°C, 8°C, 12°C, 16°C, 20°C and at room temperature at 617 nm OD.

## RESULTS & DISCUSSION:

**3.1 Effect of repeated application of alginate immobilized enzyme on dye degradation: R**epeated usability of alginate immobilized enzyme was tested in experimental conditions (18 mg/l) dye 0.1 ml $H_2O_2$ and 2 gms of enzymes beads, with first stage shows almost 55.5% of removal, after repeated applications there was gradual decrease in activity. **(Table 1 and figure 2)**

**Table –1 : Repeated Application of Alginate beads (Dye concentration :18 mg/l; Enzyme Conc. 2 gms; pH 2; Temp. room temp.; Time 45 min; $H_2O_2$ dos 0.1 µg/l)**

| No. of applications | Amount of dye degraded mg/l | Amount of dye left mg/l | % of dye degradation |
|---|---|---|---|
| 1 | 10 | 8.0 | 55.5 |
| 2 | 6.5 | 11.5 | 36.1 |
| 3 | 5.6 | 12.4 | 31.1 |
| 4. | 5.1 | 12.9 | 28.3 |
| 5 | 4.0 | 14.0 | 22.2 |

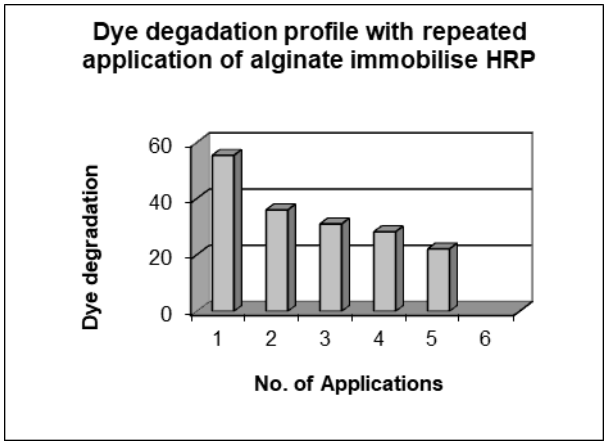


**Figure: 2 Repeated Application of Alginate beads (Dye concentration :18 mg/l; Enzyme Conc. 2 gms; pH 2; Temp. room temp.; Time 45 min; $H_2O_2$ dos 0.1 µg/l)**

**Effect of repeated application of acrylamide gel immobilized HRP:**

With every repeated applications of acrylamide gel immobilized enzyme there was a constant reduction in dye degradation efficiency (app. 12%). Study was performed for 5 applications and dye degradation from 58.8% reduced to 15% **(Table 2: Fig.3)**

**Table –2 : Repeated application of Gel beads (Dye concentration 18 mg/l. Enzyme – 2 gms of beads, $H_2O_2$ 0.1 µg/l; Temp. room temp.; Time 45 min.)**

| No. of applications | Amount of dye left mg/l | Amount of dye degraded mg/l | % of dye degradation |
|---|---|---|---|
| 1 | 3.6 | 14.4 | 58.8 |
| 2 | 7.4 | 10.6 | 48.3 |
| 3 | 9.3 | 8.7 | 48.3 |
| 4. | 13.2 | 4.8 | 26.6 |
| 5 | 15.3 | 2.7 | 15.0 |

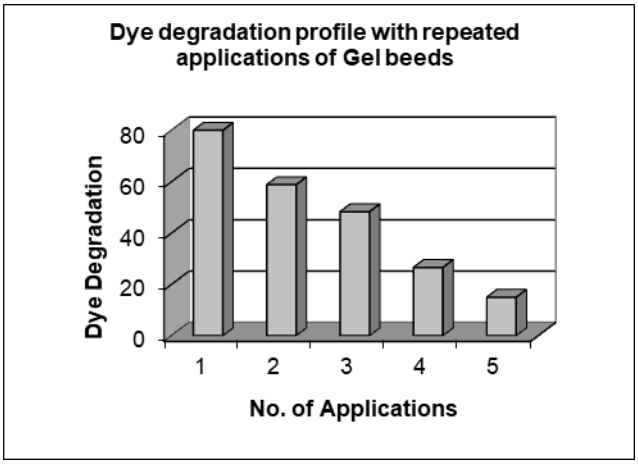


**Figure:3 : Repeated application of Gel beads (Dye concentration 18 mg/l. Enzyme – 2 gms of beads, $H_2O_2$ 0.1 µg/l; Temp. room temp.; Time 45 min.)**

**Comparative merging of two immobilized HRP activity on dye degradation by two different immobilized methods:**

The matrixes used for immobilization were alginate and acrylamide in the experiment and it was observed that immobilized beads with acrylamide gel showed comparatively high enzymatic degradations rate of azo dye constantly. The study was performed for 5 applications but after 4th application capacity degradation of acrylamide gel decreased but in case of alginate beads in second application only the rate of dye degradation fall and remained constant thereafter.**(Table 3)**

**Table –3 : Comparison between application of Gel beads formed by Alginate and Acrylamide (Dye concentration 18 mg/l. Enzyme – 2 gm of beads, $H_2O_2$ 0.1 µg/l; Temp. room temp.; Time 45 min.)**

| No. of applications | Amount of dye left mg/l | Amount of dye degraded mg/l | % of dye degradation |
|---|---|---|---|
| ALGINATE | | | |
| 1 | 3.6 | 14.4 | 58.8 |
| 2 | 7.4 | 10.6 | 48.3 |
| 3 | 9.3 | 8.7 | 48.3 |
| 4. | 13.2 | 4.8 | 26.6 |
| 5 | 15.3 | 2.7 | 15.0 |
| ACRYLAMIDE | 4.7 | 13.8 | 57.6 |
| 1. | 7.2 | 11.5 | 47.8 |
| 2. | 8.1 | 9 | 47.5 |
| 3. | 13 | 5 | 22.3 |
| 4. | 15.9 | 3.2 | 14.2 |
| 5. | 19.3 | 1.6 | 10.0 |

**Effect of temperature on dye degradation with acrylamide gel immobilized HRP**

There was no effect of temperature increase on acrylamide gel immobilized HRP in the range of 4°C to 20 °C. At 20 °C degradation rate showed an increasing trend from 47.7% to72.4%of dye degradation **(Table 4).**

**Table –4 : Effect of temperature on immobilized enzyme activity.**

| Temp. | Dye remained | Dye degraded | % of degradation |
|---|---|---|---|
| 4 | 9.4 | 8.6 | 47.7 |
| 8 | 9.2 | 8.8 | 48.8 |
| 12 | 8.6 | 9.4 | 52.2 |
| 16 | 8.4 | 9.6 | 53.3 |
| 20 | 7.9 | 10.1 | 56.1 |
| 24 | 5.0 | 13.0 | 72.2 |

**Effect of pH on dye degradation with acrylamide gel immobilized HRP**

The increase in the pH from 2 to 7 was analyzed. The enzymatic activity was reduced from 77.7% to 50.5% as the pH was increased. **(Table 5)**

**Table –5: Effect of pH on immobilized enzyme activity (Dye conc. 18 mg/l; Enzyme 2 grams of 4.5 ml of dye; $H_2O_2$ 0.1 mg/l)**

| PH | Amount of Dye remaining (mg/l) | Amount of Dye degraded (mg/l) | % of dye degradation |
|---|---|---|---|
| 2 | 4.0 | 14.0 | 77.7 |
| 3 | 5.2 | 12.8 | 71.1 |
| 4 | 6.0 | 12.0 | 66.6 |
| 16 | 7.2 | 10.8 | 60.0 |
| 20 | 8.4 | 9.6 | 53.3 |
| 24 | 8.9 | 9.1 | 50.5 |

## CONCLUSION:

The enzymatic pretreatment is a promising alternative to physico chemical treatment in enhancing biodegradability of xenobiotic compounds. Peroxidase enzyme of HRP is effect on azo dye and enzymatic degradation is dependent on pH and temperature. The observations are in conformity with Bhunia A *et.al*.(2000) who worked on Horseradish peroxides catalyzed degradation of industrially important dyes.

It was also observed that batch immobilization degradation is effective till 5 times repetition and Acrylamide gel immobilized system is more effective in degradation as compared to alginate immobilization system.

## CONFLICT OF INTEREST:

The authors showed no conflict of interest.

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