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INVESTIGATION OF ENZYMATIC DEGRADATION OF ACID BLUE 113 BY HALOTOLERANT STRAIN *PSEUDOMONAS AERUGINOSA*

V. Pushpa Rani*[1], K. Shanmuga Priya[2], A. Anitha Nancy[3] and G. Meena Kumari[4]

[1-5]P.G. & Research Department of Advanced Zoology & Biotechnology, Loyola College, Chennai-600 034, Tamil Nadu, India.

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***Correspondence for Author**
V. Pushpa Rani
P.G. & Research Department of Advanced Zoology & Biotechnology, Loyola College, Chennai-600 034, Tamil Nadu, India.

ABSTRACT

The azo dyes are some of the most commonly used in the leather and textile industries as they are quite versatile in nature. Microbial degradation of azo dyes is mediated by enzymes. In this present study an attempt primed for the evaluation on degree of biodegradation of Acid Blue 113 azo dye by extra-cellular and intracellular enzymes from halotolerant bacterial strain. Preceding studies on the enzymatic biodegradation of azo dyes werenot distinguished extra-cellular and intra-cellular enzymes. The results found in this study shows that Extra-cellular enzymes exhibits higher degree of degradation than Intra-cellular enzymes at different interval of time (0th, 16th, 24th, 40th, 48th, 64th and 72nd hours) with varies dye concentration (25ppm, 50ppm, 75ppm &100ppm).

KEY WORDS: Biodegradation, halotolerant bacteria, Acid Blue 113, Extra-cellular enzyme, Intra-cellular enzyme and *Pseudomonas aeruginosa*.

INTRODUCTION

The azo dyes are some of the most commonly used in the leather and textile industries as they are quite versatile in nature (**Vijayaraghavan *et al.*, 2006**). Azo dyes are water-soluble synthetic organic compounds. Generally, azo dyes contain one, two or three azo linkages, linking phenyl, naphthyl rings that are usually substituted with some functional groups including triazine amine, chloro, hydroxyl, methyl, nitro and sulphonate (**Bell *et al.*, 2000**). There are more than 3000 azo dyes; about 80% of azo dyes are used in the dyeing process of textile industries. It had been estimated that approximately 10% of the dyes used in dyeing

process donot bind to the fiber and are released into the environment (**Asad *et al.*, 2007**). They possess toxicity like lethal effect, genotoxicity, mutagenicity, and carcinogenicity to plants and animals (**Puvaneswari *et al.*, 2006**). Roughly half of all known dyes are azo dyes, making them the largest group of synthetic colourants. They are xenobiotic compounds and resist biodegradation in conventional aerobic treatment plants. The recalcitrance of azo dyes results in contamination of ground water. In the dyeing processes, dyes are neither completely utilized nor recovered in the downstream processes and as a result the discharge of dyes in the effluent stream usually contains more than 10-15%.

Discharge of effluents without adequate removal of these dyes will remain in theenvironment and cause serious issues. Most of these dyes are toxic and potentially carcinogenic in nature and their removal from the industrial effluents is a major problem and causes serious environment threat (**Stolz 2001**).

Current state of the art employs several physical and chemical methods to treat the dye effluents but the major problems in these processes are that they are time consuming, costly and more importantly it generates secondary pollutants (**Forgacs *et al.*, 2004**) . Hence, there is an immediate need to remove the residual dye from aqueous effluent in a more eco-friendly manner.

Microbial degradation of azo dyes is mediated by enzymes. The predominant enzymes are azoreductase, laccases, lignin peroxidase, manganese peroxidase, and hydroxylases. Laccase and azoreductase have been shown to degenerate azo dyes (**Rodriguez *et al.*, 1999; Reyes *et al.*,1999**).Enzymatic processes are very promising for the decolorization of synthetic azo dyes.To understand the decolorization and degradation mechanism of azo compounds under aerobicconditions (redox-active), exhibit relatively wide substrate specificities (**Duran and Esposito 2000; Mester and Tien 2000)**. Wide variety of microorganisms excretedifferent active enzymes like laccases, phenolic oxidases, peroxidases and variety of azo-dye reductases enzymes.

In this present study an attempt primed for the evaluation on degree of biodegradation of Acid Blue 113 azo dye by extra-cellular and intracellular enzymes from halotolerant bacterial strain. Preceding studies on the enzymatic biodegradation of azo dyes werenot distinguished extra-cellular and intra-cellular enzymes. The previous study by **Vijaykumar *et al.*, (2006)** in

the enzymatic degradation of Acid Blue 113 carried out by Laccase enzyme source from *Cladosporioum cladosporioides*.

METHODOLOGY

Sample Collection

Soil sample from salt lake around the coastal area in Mahabalipuram (60kms from Chennai) was collected in airtight polythene bags under aseptic condition and used for bacterial screening within 2 hrs.

Isolation and Optimization of Bacterial Strains

A serial dilution was made up to $^{10\text{-}10}$ from sample. As per the serial dilution procedure 0.85% saline water prepared to isolate halotolerant bacteria. Dilutions of 10^{-4} 10^{-5}, 10^{-6} and 10^{-7}were taken due to their uniform distribution of colonies. 100 µL of each dilution was spread on to pre-sterilized nutrient agar Petri plates which containing different NaCl concentrations (0.5%, 5%, 10%, 15% and 20%) for minimum saline condition for the optimal growth as well in order to isolate all halotolerant, moderately halophilic and halophilic bacteria present in the sample. The petri plates were incubated at 37°C for 24 hrs. A control plate was also prepared and incubated for the same time period.

Halotolerant Bacteria Screening

To screen the halotolerant bacteria, the colonies in the 10% and 15% saline agar plates were streaked over 0.5% and 5% agar plates and incubated at 37°C for 24 hrs. Colonies developed even in lower NaCl concentrations, which provide evidence that those strains are acclimatized in different NaCl concentrations, which is constructive for biological treatment of dye effluent, as the effluent salinity concentration varies every day. Further, the colonies are quadrant-streaked on fresh agar plate for strain purification and incubated for 24 hr.

Identification of Halotolerant Bacteria

The isolated strain was studied for its morphological characteristics by Gram staining technique. From Gram staining procedure and morphological identity, 10 halotolerant/halophilic bacterial strains were identified. Out of 10 strains a Gram-positive was selected. Biochemical tests were performed for the observation of strains. The isolated and purified strains were preserved in agar slants in aseptic conditions at 5°C in a refrigerator.

Extraction of Extra-Cellular Enzyme

After the bacterial growth it was centrifuged at 14,000 RPM for 15 min at 4°C in order to decant the supernatant which has been used as extra-cellular enzymes.

Extraction of Intra-Cellular Enzyme

Ultra-sonication (used 20% amplitude), a mechanical method used for cell disruption due to the influence of liquid shear created by high frequency ultra sound(i.e., above 16 KHz). The harvested cell pellets were re-suspended in phosphate buffer (pH 7). The disruption period was upto 60 min with pause of 10 seconds in an ice bath.

Dye Stock

100 ppm stock solution: 10mg Acid Blue 113 was added to 100ml of Mineral Salt Medium (MS Medium). Using this stock solution, various concentrations viz., 1 ppm, 2 ppm, 3 ppm, 5 ppm, 10 ppm, 15 ppm, 25 ppm, 50 ppm, 75 ppm and 100 ppm of dyesolutions were prepared.

Degradation of Acid Blue 113

Four different dye concentrations (25 ppm, 50 ppm, 75 ppm and 100 ppm) were prepared in 50 ml of MS Medium which contains of glucose, the main substrate. A volume of 2 ml inoculum was added and incubated at 37°C in a rotary shaker and kept as control. The samples were drawn at regular intervals (0th, 16th, 24th, 40th, 48th, 64th and 72nd hours) and the absorbance of the supernatant was measured after centrifugation.

The percentage of dye degradation was then calculated as follows:

$$C_f = \frac{\text{Absorbance}}{\text{Slope of calibration plot}}$$

$$\% \text{ Degradation} = \frac{(C_i - C_f) \times 100}{C_i}$$

Where,

Ci initial concentration of the dye(ppm)

Cf final concentration of the dye(ppm)

Simultaneously, the amount of biomass generated each time was also noted by measuring the dry weights of the pellets formed after centrifugation.

RESULT

Serial dilutions of 10^{-4} 10^{-5}, 10^{-6} and 10^{-7}were taken due to their even distribution of colonies. After 24 hr incubation, 0.5% saline agar plate develop cluster of colonies. In the duration of 2 to 3 days 5%, 10% and 15% saline agar plates were found to develop individual yellow, light orange and orange colonies. After the identification halotolerant strain *Pseudomonas aeruginosa* (Gram-negative, cocci) was selected on the basis of halotolerance limits (adaptation of identified strains over different NaCl concentrations) and the time required for the formation of well-developed colonies under extreme saline conditions. The concentration of inoculum necessary for any parameter study was obtained from this after 24 h. The appearance of turbidity in the test tube shows the growth of the bacteria. The absorbances of the standard dye concentrations were measured and a standard graph was plotted. The slope of the calibration line was determined.

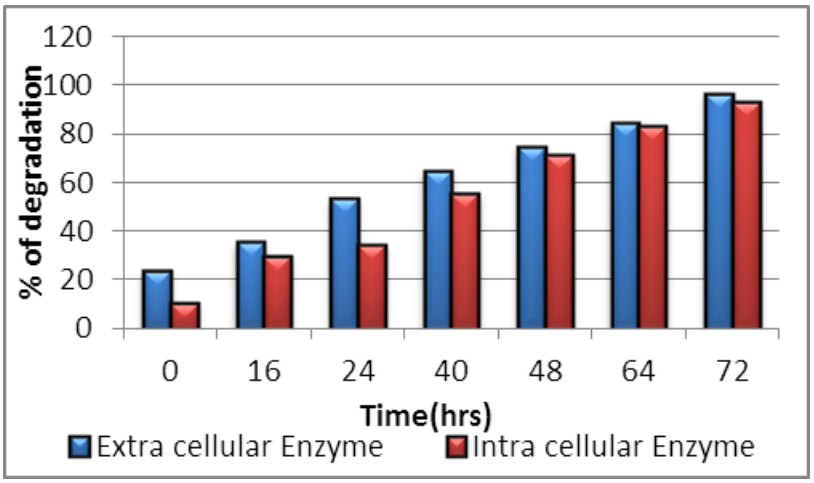

Fig 1: Comparison of Acid Blue 113 biodegradation between Extra-cellular and Intra-cellular enzymes of *P. aeruginosa*at 25 ppm

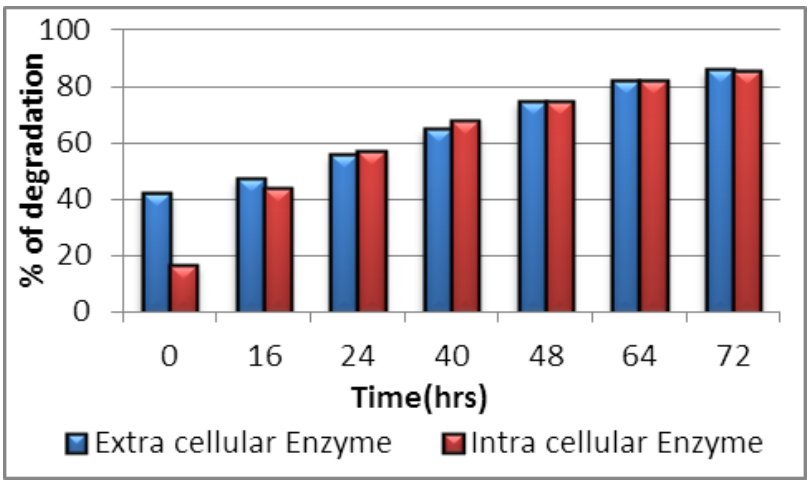

Fig 2: Comparison of Acid Blue 113 biodegradation between Extra-cellular and Intra-cellular enzymes of *P. aeruginosa*at 50 ppm.

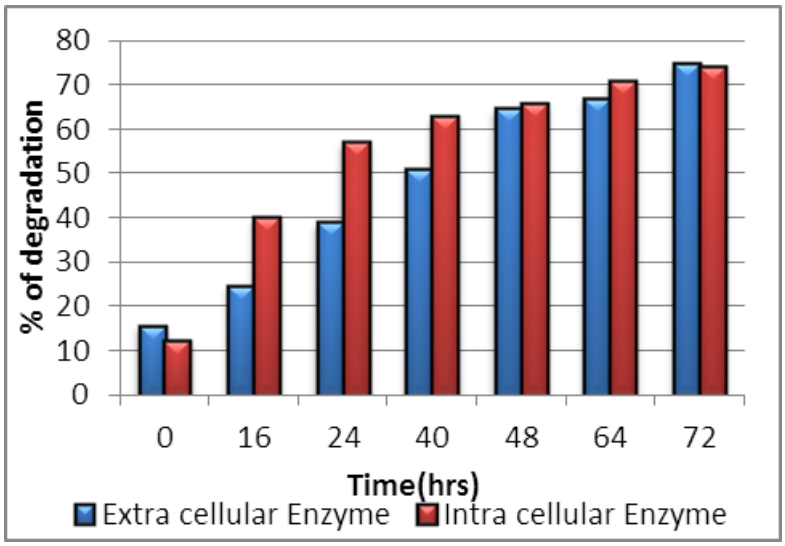

Fig 3: Comparison of Acid Blue 113 biodegradation between Extra-cellular and Intra-cellular enzymes of *P. aeruginosa* at 75 ppm

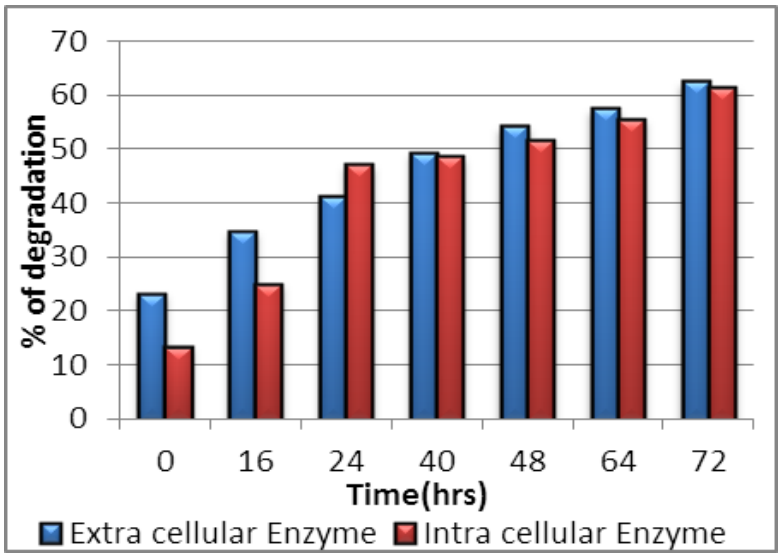

Fig 4: Comparison of Acid Blue 113 biodegradation between Extra-cellular and Intra-cellular enzymes of *P. aeruginosa*at 100 ppm

DISCUSION

In the attempt to enzymatic degradation of Acid Blue 113 azo dye with the help of ***P. aeruginosa*** a halotolerant bacterial strains. As the enzymatic degradation has potential effect over biodegradtion of textile dyes. To check the ability and compare the degree of degradation between Extra-cellular and Intra-cellular enzymes of *P. aeruginosa*at different time interval (0, 16, 24 40, 48, 64 & 72 hours) and different concentration of dye (25ppm, 50ppm, 75ppm &100ppm) utilized for the study under optimized condition.

Biodegradation range at 25ppm gradually shows increase in degradation with time by Extra-cellular and Intra-cellular enzymes. But solely extra-cellular enzymes exhibit the higher degree of degradation as compared to Intra-cellular enzymes in all time intervals (0, 16, 24 40, 48, 64 & 72 hours) in 25ppm concentration of dye.

The percentage of biodegradation at 50ppm shows a different trend in degradation with time by Extra-cellular and Intra-cellular enzymes. At the initial 0 hours Extra-cellular enzymes demonstrate higher level of degradation than Intra-cellular enzymes. In the initial time intervals (0 & 16 hours) Intra-cellular enzymes shows lesser activity than Extra-cellular enzymes but at the time interval of 24 & 40 hours percentage increased with time. At 48 &64 hours Extra-cellular and Intra-cellular enzymes exhibits the similar degree of degradation. In 72 hours Extra-cellular enzyme shows slight elevation than Intra-cellular enzymes.

Biodegradation between Extra-cellular and Intra-cellular enzymes of *P. aeruginosa* at 75 ppm shows the increase trend of Intra-cellular enzymes in degree of degradation as the time interval increase from 16, 24, 40 & 48 hours. In 0 hour and 72 hours Extra-cellular enzymes presented the higher trend.

The degree of biodegradation at 100ppm Extra-cellular and Intra-cellular enzymes exhibits different range of degradation at time intervals. Extra-cellular enzymes shows increase trend at time intervals of 0, 16, 40, 48, 64 & 72 hours. At 24 hours Intra-cellular enzymes shows the sudden elevation.

CONCULSION

The results found in this study shows that Extra-cellular enzymes exhibits higher degree of degradation than Intra-cellular enzymes at different interval of time with varies dye concentration. Mostly microbial degradation of azo dyes is mediated by enzymes. The predominant enzymes are azoreductase, laccases, lignin peroxidase, manganese peroxidase, and hydroxylases. This current study is the effort to identify the degradation strength of Extra-cellular and Intra-cellular enzymes.

REFERENCE

1. Asad S, Amoozegar MA, Pourbabaee AA, Sarbolouki MN, Dastgheib SM Decolorization of textile dyes by newly isolated halophilic and halotolerant bacteria. *Bioresources Technology* 2007; 98: 2082-2088.

2. Bell, J., Plumb, J J., Buckley, C A., Stuckey, D C. Treatment and decolorization of dyes in an anaerobic baffled reactors. *J.Environ Eng Div* ABCE 2000; 126: 1026- 32.
3. Forgacs, E., Cserhati, T. and Oros, G. Removal of synthetic dyes from wastewaters: a review, Environ. Int., 2004; 30 953-971.
4. Puvaneswari, N., Muthukrishnan, J., and Gunasekaran, P. Toxicity assessment and microbial degradation of azo dyes.*Indian J Exp Biol.* 2006; 44: 618 626.
5. Stolz, A. Basic and applied aspects in the microbial degradation of azo dyes, Appl. Microbiol.Biot., 2001; 56: 201.
6. Vijayraghavan, R., Vedaraman, N., Surianarayanan, M. and MacFarlane, D. R. Extraction and recovery of azo dyes into an ionic liquid, Talanta, 2006; 69: 1059-062.