

EVALUATION ON DEGREE OF BIODEGRADATION OF AZO DYE BY EXTRA-CELLULAR AND INTRACELLULAR ENZYMES FROM HALOTOLERANT BACTERIAL STRAIN.

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Article Received on
14 Jan 2016,

Revised on 04 Feb 2016,
Accepted on 24 Feb 2016

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ABSTRACT

Numerous biotechnological approaches have been suggested to overcome the problem of physiochemical treatment methods using microorganisms for the treatment of textile dyes and industry effluent as microorganisms play crucial roles in the mineralization of xenobiotic compounds. In this present study halotolerant bacterial strains were isolated and the identification process of halotolerant strain *Bacillus flexus* was selected on the basis on adaptation of identified strains over different NaCl concentrations and the time required for the formation of well-developed colonies under extreme saline conditions. Enzymatic degradation shows higher potential in degradation of azo dyes. Acid Blue 113 biodegradation by *B. flexus* at 25 ppm, 50 ppm and 100 ppm shows higher degree of degradation by Extra-cellular enzyme at all interval times (0th, 16th, 24th, 40th, 48th, 64th

and 72nd hours) than Intra-cellular enzymes. Intra-cellular enzymes degradation activity was greater in the time intervals of 0th, 16th, 24th and 40th hrs. From the time interval of 48th, 64th and 72nd hours Extra-cellular enzyme predominately degraded Acid Blue 113.

KEYWORDS: *Bacillus flexus*, halotolerant bacteria, azo dye, Acid Blue 113, and biodegradation.

INTRODUCTION

Studies carried out, especially throughout the last few decade, have amplified our present knowledge about different features of moderately halophilic bacteria, such as their

physiology, ecology, taxonomy or phylogenetic relationships with other microorganisms and to be slighter extent, their genetics. Besides, there are numerous fields in which their industrial applications are more promising, and as in the case of other extremophilic microorganisms, they have an important biotechnological potential as a source of compatible solutes, enzymes, and other compounds of industrial interest. Enzymatic analysis showed two fold and four fold increase in the activity of azoreductase and laccase enzymes, respectively, indicating involvement of both reductive and oxidative enzymes in biodegradation of Red HE7B (Thakur *et al.*, 2014).

Numerous biotechnological approaches have been suggested to overcome the problem of physiochemical treatment methods using microorganisms for the treatment of textile dyes and industry effluent as microorganisms play crucial roles in the mineralization of xenobiotic compounds (Kurade *et al.*, 2012).

Much of the experimental work involving the anaerobic decolorization of dyes (predominantly azo dyes) was conducted using mono cultures. Species of *Bacillus*, *Pseudomonas*, *Aeromonas*, *Proteus*, *Micrococcus* and purple non-sulphur photosynthetic bacteria were found to be effective in the anaerobic degradation of a number of dyes (Chang *et al.*, 2001; Saratale *et al.*, 2009). The anaerobic reduction of azo dyes utilized in the textile industry by a strain of *Bacillus cereus* isolated from soil. However, the permeation of the dyes through biological membrane into the microbial cells was cited as the principal rate-limiting factor for decolorization.

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.

Serial dilution

A serial dilution was made up to 10^{-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.

Spread plate technique

100 μ L 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 sametime period.

Strain selection

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 everyday. Further, the colonies are quadrant-streaked on fresh agar plate for strain purification and incubated for 24 hr.

Identification of the strain

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 and a Gram-negative were 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.

Pre-culture

Pre-culture was done in order to perform growth experiments. 5ml of nutrient broth was taken in 5 test tubes and autoclaved at 121°C and 15 psi pressure. The loopful of growth was removed from bacterial strain preserved agar slants and introduced into the test tubes containing nutrient broth. The tubes was labeled and incubated at 37°C for 24 h in a mechanical shaker for the oxygen supply required for the growth. The growth studies were carried out with this culture.

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

Ultrasonication (used 20% amplitude), a mechanical method used for cell disruption due to the influence of liquid shear created by high frequency ultra sound (ie., 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 degradation

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.

Biodegradation 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,

C_i initial concentration of the dye (ppm)

C_f 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

Dilutions of 10^{-4} , 10^{-5} , 10^{-6} and 10^{-7} were taken due to their uniform 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 process of halotolerant strain *Bacillus flexus* (Gram-positive, rod-shaped) 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 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.

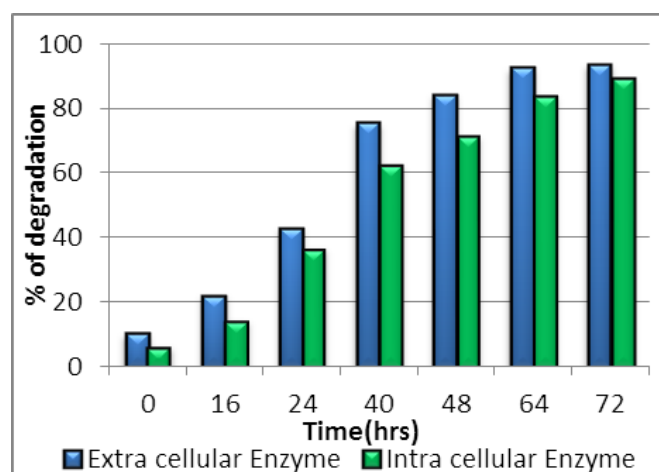


Fig 1: Comparison of Acid Blue 113 biodegradation between Extra-cellular and Intra-cellular enzymes of *B. flexus* at 25 ppm.

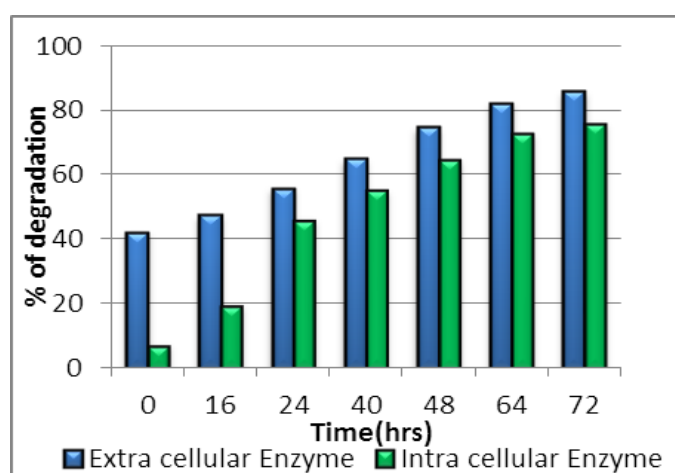


Fig 2: Comparison of Acid Blue 113 biodegradation between Extra-cellular and Intra-cellular enzymes of *B. flexus* at 50 ppm.

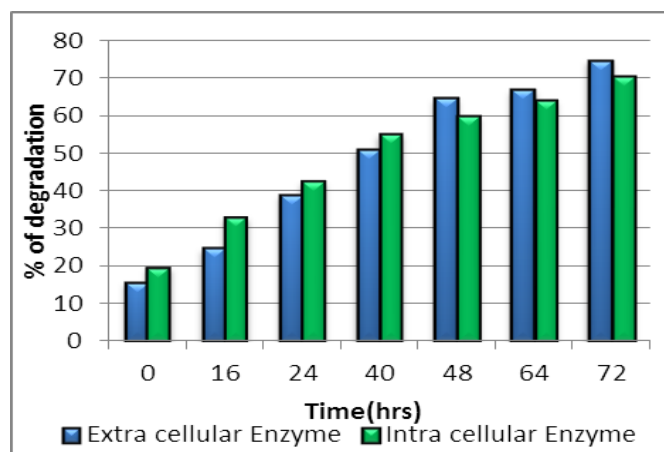


Fig 3: Comparison of Acid Blue 113 biodegradation between Extra-cellular and Intra-cellular enzymes of *B. flexus* at 75 ppm.

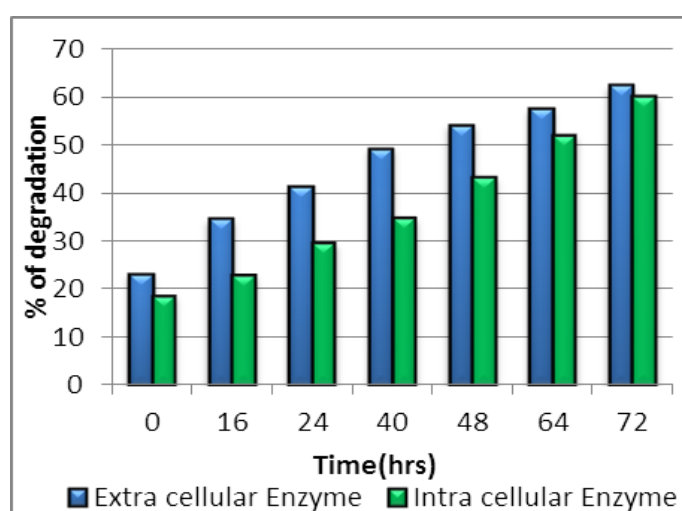


Fig 4: Comparison of Acid Blue 113 biodegradation between Extra-cellular and Intra-cellular enzymes of *B. flexus* at 50 ppm.

DISCUSSION

Acid Blue 113 biodegradation by *B. flexus* at 25 ppm (**Fig: 1**) shows higher degree of degradation by Extra-cellular enzyme at all interval times (0th, 16th, 24th, 40th, 48th, 64th and 72nd hours) than Intra-cellular enzymes.

Enzymatic degradation at 50 ppm (**Fig: 2**) exhibits the similar result as 25 ppm. The Extra-cellular enzyme at all interval times (0th, 16th, 24th, 40th, 48th, 64th and 72nd hours) than Intra-cellular enzymes.

At 75 ppm (**Fig: 3**) degree of degradation differ between Extra-cellular and Intra-cellular enzymes of *B. flexus*. Intra-cellular enzymes degradation activity was greater in the time

intervals of 0th, 16th, 24th and 40th hrs. From the time interval of 48th, 64th and 72nd hours Extra-cellular enzyme predominately degraded Acid Blue 113.

At 100 ppm Extra-cellular enzyme reached the maximum degradation percentage at all time intervals (0th, 16th, 24th, 40th, 48th, 64th and 72nd hours) than Intra-cellular enzymes.

CONCULSION

In the present study, Extra-cellular enzyme reached the maximum degradation percentage at most of the dye concentration. Numerous biotechnological approaches have been suggested to overcome the problem of physiochemical treatment methods using microorganisms for the treatment of textile dyes and industry effluent as microorganisms play crucial roles in the mineralization of xenobiotic compounds. Enzymatic degradation shows higher potential in degradation of azo dyes.

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