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A CASE STUTY

Decolourisation of textile effluent by *Pseudomonas aeruginosa* in low nutrient medium

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SUMMARY

Water soluble azo dyes are important class of synthetic organic compounds. The coloured pigments produced by these dyes are first sign of the contamination of waste water or any other water bodies. This paper describes the use of bacterial cell, as pure culture of *Pseudomonas aeruginosa* in its ability to remove colour from textile effluent aerobically. Degradation of reactive red, an azo dye, was used for the experiment at the concentration of 50 ppm and 100 ppm. The decolourisation efficiency achieved for these concentrations of dyes were 51.71 per cent and 65.42 per cent, respectively in five days of study. The effect of co-substrate glucose was also investigated, with modification in the medium by lowering nutrient content.

Key Words : Decolourisation, Primary degradation, *Pseudomonas aeruginosa*, Glucose, Reactive red

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World production of synthetic dyes is estimated at over 0.7 million tons annually and about 10,000 different dyes and pigments are produced worldwide (Mac Mullan *et al*., 2001). It is observed that about 10-90 per cent of the dyestuff used during process do not bind to the fabric and, therefore, lost in effluent (Reisech, 1996). There is about 2 per cent loss encountered when basic dyes are used and 50 per cent loss while using reactive dyes (Mac Mullan *et al*., 2001). Major pollutants in textile waste water are high suspended solids, COD, heat, acidity and other soluble substances (Dae-Hee *et al*.,1999) along with residual dye stuff *i.e.* colouring agent and heavy metals associated with dyeing and printing process (Correia *et al*.,1994).

Dyes are natural and xenobiotic compounds that makes the world more beautiful through coloured substances but the same pigment is the first contaminant that can easily be recognized in water bodies and for this reason waste water becomes serious environmental problem and of public health concern (Padmavathy *et al*., 2003; dos Santos *et al*., 2007). Besides the aesthetic problem, dyes obstruct light and reduce oxygen mass transfer. Discharge of highly coloured synthetic dyes effluent could damage the receiving water bodies (Nigam *et al*., 1996). These also have their impact on photosynthesis of aquatic plants and consequently affect the growth of primary producers and consumers. The increase in temperature in addition leads to depletion of dissolved oxygen concentration (Yeh and Thomas, 1995; Cunningham and Saigo, 2001).

The increased use of dyes and synthesis of new one is coupled with the higher resistant to environmental degradation and their recalcitrant nature leads to pollution problem. The industrial discharge is currently recognized as serious environmental issue (Stolz, 2001). The mill discharge lead to choronic and acute toxicity (Padmavathy *et al*., 2003). The azo dyes in the textile effluent can be toxic to microbial population and exhibit mutagenic effect on animals and produce carcinogenic compound degraded

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in the human digestive system (Pavan *et al*., 2008).

There are several treatment methodologies which have been evolved and are commercially used. The research till date have shown that azo dyes can be completely decolourised and some intermediates such as aromatic amines with side groups (-SO^3, -OH, -COOH, -Cl, -N) containing metabolites were quantitatively detected (O'Neill *et al.,* 2000).The combination of treatment methods involves physical or chemical method such as coagulation preparation, adsorption by charcoal. These methods are economically unfeasible and also transfer waste from one form to another form. The remaining undegraded products may be more toxic than the parent compound (Yeh and Thomas, 1995; Chen *et al.,* 1999).

The biological or microbial treatment of these effluents of dye stuff is more significant (Mac Mullan *et. al*., 2001; Stolz, 2001). It is environmental friendly and cost effective alternative of chemical treatment and more over without leaving any residue (Verma and Madamwar, 2003)**.** Biological decolourisation involves anaerobic or aerobic treatment process (O' Neil *et al*., 2000; Stolz, 2001). The reductive cleavage under anaerobic condition yields aromatic amines which are mutagenic and toxic to humans (Chung and Stevens, 1993). Aerobic degradation of dyes could be achieved by oxidation of substituent located on the aromatic ring or on the side chain (Ekici *et al*., 2001; Rajaguru *et al.,* 2000). Several continuous aerobic and anaerobic processes are also being carrying out these days (O' Neil *et al*., 2000; Stolz, 2001). Anaerobic reduction can be followed by aerobic oxidation for complete mineralisaton (Rajaguru *et al.,* 2000). It is thus, important to search out the possible aerobic degraders for decolourisation. The use of whole bacterial cell (Pearce *et al*., 2003) and bacterial consortium is being worked out for the purpose (Carliell *et al*., 1995; Moosvi *et al.,* 2005).

Pseudomonas aeruginosa has been observed as novel bacterial strain capable for decolorizing textile dyes by several workers (Bhatt *et al*., 2005; Leena and Raj, 2008; Sheth and Deve, 2009). Other sp. including *P.oleovorans* and *P.putida* has also been successfully traced (Silveira *et al*., 2009) and *P.luleola* (Hu, 1998 and 2001).

In the present work *Pseudomonas aeruginosa* has been used for the decolourisation of dye, reactive red, an azo dye at different time intervals.

MATERIAL AND METHODS

Micro-organism:

Pseudomonas aeruginosa was collected from Dept. of Microbiology, IMS, BHU, Varanasi. Stock culture was maintained on nutrient agar plate at 4^0C in a refrigerator. The colony is of light green colour, on the culture plate.

Dye:

Reactive red dye was used as a model dye for fundamental experiments. The concentrations of dye used, were 50 ppm and 100 ppm.

Medium:

Nutrient broth medium which is generally used for growing bacteria was modified for present experiment. The aim was to checkout decolourisation with lower nutrients content in optimized condition.

Peptone	-	8 g/li.
NaCl	-	20 g/li.
Yeast extract	-	8 g/li.
Glucose	-	8 g/li.
pH	-	7 ±0.2.

The addition of glucose as co-substrate was preferred to increase the decolourisation rate. This was earlier reported (Yeh and Thomas, 1995; Coughlin *et al.*,1997; Russ *et al.*, 2000; Hu, 2001; Padmavathy *et al*., 2003).

Stock medium was sterilized by autoclaving at 121º C for 15 min at 15 p.s.i.

On cooling, medium was inoculated by culture of *Pseudomonas aeruginosa* with the help of sterilized inoculation needle and incubated at 37^0 C for 48 hrs.

After incubation the turbidity was evaluated for the growth of micro-organisms.

Addition of dye:

After incubation the culture was treated with different concentration of dye (*i.e.* 50 ppm and 100 ppm).

Screening of decolourisation of dye:

The dye concentrations used for treatment were 50 ppm and 100 ppm. The absorbance was observed at λ_{max} of dye *i.e.* 550nm (green filter).

Initially at t_0 and after 24 (t_{24}), 72 (t_{72}) and lastly 120 (t_{120}) hours.

The percentage of color removal was estimated with the formula based on the reduction of absorbance:

% decolourisation = initial OD – final OD …(1)

where,

Initial OD = at 0 time and final OD = at each sampling time.

The uninoculated dye solution was used as control for measurement of actual decolourisation of dye. Decolourisation per cent of different concentration by bacterium was monitored for 5 days (120 hrs.).

Tubes were observed and evaluated at sampling time *i.e.* 0 day, on 3rd day (72 hrs) and 5th day (120 hrs) for turbidity (evidence of growth) in medium and decolourisation (primary degradation) of sampling tubes.

Determination of optical density (OD) of supernatant:

Samples were withdrawn from experimental tubes and centrifuged at 6000 rpm for 10 min. The OD of resultant

supernatant was determined spectrophotometrically, at λ max of the dye *i.e.* 550 nm (at green filter).

RESULTS AND DISCUSSION

The Table 1 depicts the results obtained when *Pseudomonas aeruginosa* was cultured in presence of different concentration of dyes. The highest percentage of decolourisation was obtained with 100 ppm dye concentration which was 65.42 per cent on 5th day (120 hrs) and the culture with 50 ppm dye concentration showed lower decolourisation *i.e.* 51.71 per cent only.

After 24 hrs. the decolourisation percentage was 9.34 per cent for 50 ppm and 17.96 per cent for 100 ppm dye concentration, respectively.

It was found in the present study that the concentrations showed significant decolourisation on 1st day which continue on various time periods.

Effect of dye concentration on the decolourisation efficiency was also seen which is necessary to investigate the optimal degrading capacity of the bacteria. Per cent decolourisation increases with an increase in dyes amount (Bhatt *et al*., 2005; Sheth and Deve, 2009).

The technologies investigated in waste cleaning bioremediation has emerged the most desirable approach for cleaning up environmental pollutants (Glazer, 1997). A number of micro-organisms studied so far for the bioremediation with the use of bacteria is being most studied, as the bacteria are easier to culture and grow quickly than fungus (Glazer, 1997).

Uses of azo dyes are wide because of their ease of synthesis, versatility and cost- effectiveness (Ekici *et al*., 2001). These dyes represent a major group of dyes causing environmental concern because of their colour, biorecalcitrance, potential toxicity and carcinogenicity to animals and humans.

The aim of the work was to study the efficiency and response of the cells of *Pseudomonas aeruginosa*, to decolourise reactive azo dye in lab condition.

The result (Table 1) showed that the dye is being decolorized or primarily degraded up to certain extent.

The control showed no decolourisation which confirms that decolourisation was as a result of metabolic activity of introduced bacterial cells and not due to abiotic factor as reported earlier (Nachiyar and Rajkumar, 2003; Sheth and Deve, 2009). Lowering in the absorbance of the observed decrease in visible and UV absorbance peaks signify degradation of dye and their subsequent aromatic compounds (Mac Mullan *et al*., 2001; Togo *et al.*, 2008).

As the result showed (Table 1 and Fig. 1) that decolourisation could be achieve by the bacterial cells *i.e. Pseudomonas aeruginosa* with about 65.42 per cent decolourisation, which was significantly considerable.

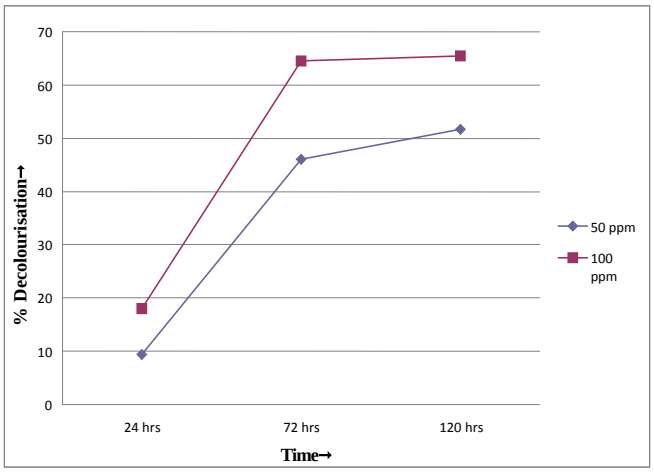

Fig. 1 : Percentage decolourisation of different concentration of dye by *Pseudomonas aeruginosa* at different period of time

The effective degradation by the use of *Pseudomonas* has been approved in different studies and research, with different decolourisation rate (Hu, 1998 and 2001; Nachiyar and Rajkumar, 2003; Oranusi and Ogugbue, 2003 and 2005; Bhatt *et al*., 2005; Silveira *et al*., 2009; Leena and Raj, 2008; Sheth and Deve, 2009).

Peptone was used as organic nitrogen source supplemented with yeast extract for better result. This result for peptone addition is supported by other researchers (Padmavathy *et al*., 2003; Ola *et al.*, 2010). In this paper the degradation has been achieved by optimisation of media, without micro nutrient other than NaCl in it, which make decolourisation economically more adoptable by dyeing industries.

The preferred pH for medium is 7.0 (Bhatt *et al*., 2005; Sheth and Deve, 2009; Ola *et al.*, 2010).

The use of cosubstrate in association with bacterial cells in culture medium, enhanced the decolourisation rate (Padmavathy *et al*., 2003; Oranusi and Ogugbue, 2003 and 2005; Moosvi *et al.*,2005; Ola *et al.*, 2010). The metabolism which causes the decolourisation is the generation of redox equivalents that results in reductive cleaves of reactive dye by their catalytic activity (Russ *et al.*, 2000; Oranusi and

Table 1: Percentage decolourisation of reactive red dye by *Pseudomonas aeruginosa*

Sr. No.	Dye concentration	% Decolourisation of reactive red on days		
		1st day (24 h)	3nd day (72 h)	5rd day (120 h)
1.	50 ppm	9.34	46.00	51.71
2.	100 ppm	17.96	64.53	65.42

Data ± 0.03- 0.085 OD.

Ogugbue, 2005).

In present work, the co-substrate which was used for the purpose was glucose. Glucose is monosaccharide and is preferred metabolisable substrate with higher redox equivalents and hence, leads to higher decolourisation efficiency by accelerating decolourisation reaction (Carliell *et al*., 1995; Donlon *et al.*, 1997; Oranusi and Ogugbue, 2003; Boonyakamol *et al.*, 2009). Use of glucose indicates dependence of *Pseudomonas aeruginosa* on carbohydrate metabolism (Kulla, 1981; Nachiyar and Rajkumar, 2003). This conversion of co-substrate in redox equivalent is seen earlier both in aerobic (Rajaguru *et al.*, 2000; Padmavathy *et al*., 2003; Nachiyar and Rajkumar, 2003) and in anaerobic condition (Boonyakamol *et al.*, 2009). The redox mediators catalyses transfer of redox equivalents like NADH, NADPH or FADH to choromophoric group of the dyes (Russ *et al.*, 2000). On the other hand, a high concentration of redox mediator (0.1 mM) had an inhibitory effect on decolourisation especially under thermophilic condition (Boonyakamol *et al.*,2009).

Decolourisation by microbes requires an unspecific enzymatic capacity, found in wide variety of micro-organisms (Chung and Stevens, 1993). The decolourisation of synthetic dyes were achieved by reductive cleavage of the characteristic choromophoric group by the enzyme azo reductases, laccases and peroxidases (Stolz, 2001; Bhatt *et al*., 2005; Chen *et al.*,1999; Oranusi and Ogugbue, 2003 and 2005). *Pseudomona*s produces the enzyme azoreductases (Zimmermann *et al.*,1982; Hu,1998 and 2001; Sheth and Deve, 2009; Nachiyar and Rajkumar, 2003). In the presence of oxygen an oxygen insensitive azoreductases was seems to be involved (Kulla, 1981; Banat *et al.*,1996) in reduction of azo bonds (Hu,1998 and 2001; Nachiyar and Rajkumar, 2003; Nakanishi *et al.*, 2001). This reductive cleavage of azo bonds results in formation of colourless amines (Stolz, 2001) because aerobic bacteria may easily remove the aromatic amines in aerobic condition (Knackmuss, 1996). Thus, making this bacterium favourable for the development of azo dye bioremediation systems. Some researchers had observed the decolourisation of azo- dyes follows the first- order kinetic model (Hu, 2001; Carliell *et al*., 1995) where as some others were found that it follow zero-order kinetics (Dubin and Wright, 1975).

Thus, the present study shows that the azo dyes can be removed significantly with the help microbial cell aerobically and for this *Pseudomonas aeruginosa* can be a useful tool.

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