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Kinetics of Acid Black 24 Biodegradation

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An aerobic consortium CN-1 has been constructed using five different bacteria namely *Pseudomonas aeruginosa* CLRI BL22, *Moraxella osloensis, Citrobacter freundii* (SUB3 & SUB6) and *Pseudomonas aeruginosa* isolated from textile effluent. The enhanced degradation of Acid Black 24 by this constructed aerobic consortium CN-1 compared to the constituent strains, which is evident from the kinetic studies, may be attributed to the concerted activities of the bacterial strains. The consortium CN-1 degraded 83% in 48h of incubation whereas the individual bacteria required more than 120 h of incubation to achieve the same level of degradation. The degradation was found to follow first order with the K_1 value of 0.101332h^{-1} which is very high compared to the K_1 values of AB24 decolorization mediated by individual constituent microorganisms.

Key words: Biodegradation, Consortium CN-1, Azo dye, Kinetics.

Human race cannot imagine the world without colors. Starting from textile industries to pharmaceutical industries to food industries, dyes have become indispensible. Absence of dyes will make the fashion world non-existent, make it difficult for layman to select his dosages, make the food non-appealing etc. In spite of all these credits, these dyes when released into the environment (water bodies) create havoc making the scarcely available resource unusable for the human kind. Textile industries and dye manufacturing industries are the major contributors for this havoc.

The discharges of dye house wastewater into the environment is aesthetically displeasing, impede light penetration, damage the quality of the receiving streams and may be toxic to treatment processes, to food chain organisms and to aquatic life[1] . While numbers of physical and chemical treatment methods are available for the removal of dyes from the effluent, they have their own advantages and disadvantages, the most common being the cost involved, requirement of energy input. Further they mediate phase transfer of the pollutants rather than their removal from the environment [2]. Biodegradation has always been looked in as an alternative for the above mentioned processes since it is eco-friendly, cost effective and does not require energy input and results in innocuous end products[3].

In most of the cases the individual bacterial strain cannot degrade different groups of azo dyes as most of the bacteria are specific for particular group of dyes. Therefore, individual organisms cannot be depended for the treatment of textile effluent which contains different groups of dyes making the construction of consortium mandatory for the biological treatment process [4]. A bacterial consortium containing five different bacterial strains isolated from textile effluent has been constructed for the degradation of Acid Blue 113[5]. This consortium was also found to be capable of degrading Acid Black 24. In this study the time course of Acid Black 24 degradation by consortium as well as the constituent bacterial strains and the rates in which these reactions proceed were analyzed.

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MATERIALS AND METHODS

Chemicals

All the solvents used were of analytical grade, purchased from Merck Pvt. Ltd. The chemicals used for the preparation of reagent, solutions and microbiological growth media were purchased from SISCO Research and Hi-Media Laboratories Pvt. Ltd, Mumbai, India. Acid black 24 [CAS Registry Number 3071-73-6] was obtained from Saujanya dye chemicals Ahmedabad, Gujarat, India.

Microorganisms and culture conditions

The bacteria present in the effluent were isolated by serial dilution method and their dye degrading ability was checked by pour plate method on nutrient agar containing Acid Black 24 (AB 24). All the bacterial cultures were maintained on nutrient agar slants and were used for biodegradation studies after preculturing in nutrient broth for 12 h. The bacterial cells were cultivated in nutrient broth for 24 h and the cells after centrifugation were resuspended in 20% glycerol and stored at –20^{0}C as stock cultures. The purity of the glycerol stocks were checked on nutrient agar plates before sub culturing on nutrient agar slants for inoculum preparation.

The consortia was developed by aseptically transferring the 2% inoculum containing approximately 2 x 10^8 cells per ml of 12 h grown culture of each individual strains in 250 ml Erlenmeyer flasks containing 50 ml of medium and 50 mg l^{-1} dye solution at optimized conditions [6]. Individual strains were inoculated with 2% (v/v) aliquots of 12 h grown culture, respectively, to maintain the same cell count in the pure culture and in the consortium. The bacterial strains in the consortia are identified by the standard 16S rRNA analysis by isolating and sequencing the 16S rDNA sequence followed by comparison with the public databases (Genbank, EMBL and DDBJ) and BLASTN sequence match routines.

Decolorization studies

The dye degradation studies were carried out in 250 ml conical flasks containing 50 ml mineral salt media containing 50 mg l^{-1} of AB 24. The media was inoculated with 2% inoculum of the bacterial consortium and incubated in orbitary shaker at 150 rpm at 30^{0}C. Degradation of the AB 24 was followed spectrophotometrically by reading the n-butanol extract of the culture medium at 622 nm [6]. Intracellular protein content which is used as an index of bacterial growth was estimated using the Lowry method (1957).

RESULTS AND DISCUSSION

The isolates for the consortium developed were selected based on three factors, their ability to degrade the dyes efficiently (>90%), rapidity and their ability to degrade a wide variety of dyes. Further many researchers have mentioned that a higher degree of biodegradation and mineralization can be expected when co-metabolic activities within a microbial community complement each other[7,8].

Five different bacterial cultures have been isolated from effluent samples collected from Suntex processing mills (Gummidipoondi), Professional fabrics (Tirupur) and Kafer Textile mills (Tirupur). These bacteria were identified as *Citrobacter freundii* (two strains SUB 3 and SUB 6), *Moraxella osloensis* and *Pseuodomonas aeruginosa* based on 16s rRNA sequencing and were used for the development of consortium CN-1along with *Pseuodomonas aeruginosa* CLRI BL22 obtained from CLRI. The construction of consortium CN-1 was explained in detail elsewhere[5].

Table 1. Kinetics of AB 24 biodegradation by Consortium CN-1

Organism	Decolorization (%)	K_1 (h^{-1})	R^2
Pseudomonas aeruginosa CLRI BL22	72	0.071390	0.809
Moraxella osloensis	54	0.062181	0.858
Citrobacter freundii SUB 3	57	0.04606	0.873
Citrobacter freundii SUB 6	63	0.036848	0.772
Pseudomonas aeruginosa	74	0.039151	0.971
Consortium CN-1	83	0.101332	0.954

Pseudomonas aeruginosa, belonging to the family of Pseudomonadaceae, is a Gram-negative, aerobic, coccobacillus bacterium with unipolar motility and is an opportunistic human pathogen. *Pseudomonas aeruginosa* is an excellent candidate for xenobiotics biodegradation

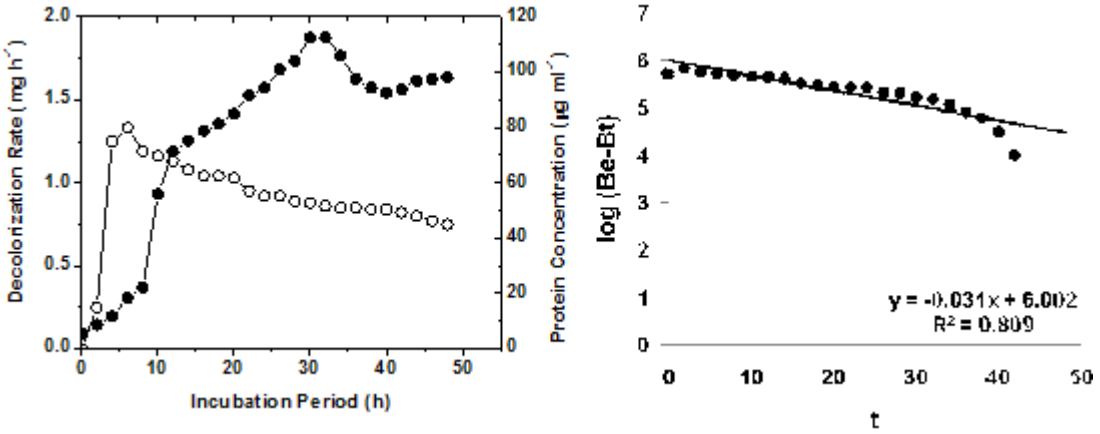

Fig. 1. a) Time course of AB 24 (50 mg l^{-1}) degradation by *Pseudomonas aeruginosa* CLRI BL22 (b) Kinetic study of AB 24 (50 mg l^{-1}) degradation by *Pseudomonas aeruginosa* CLRI BL22

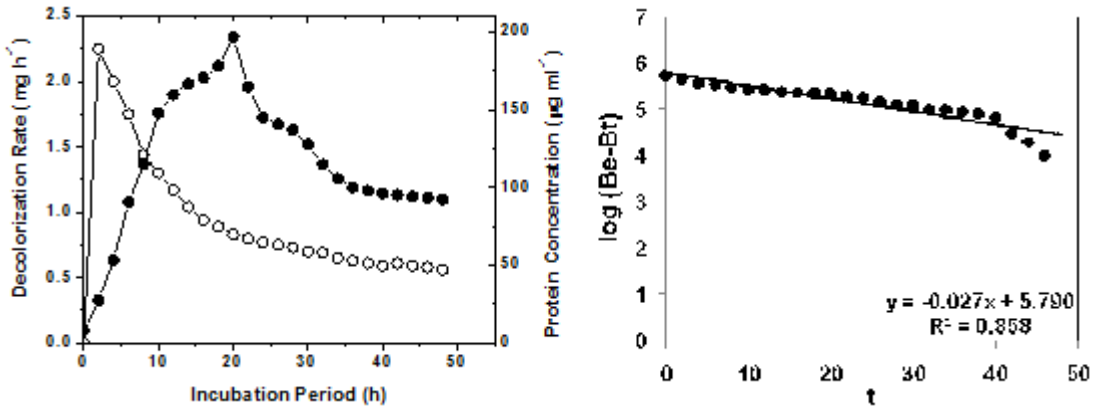

Fig. 2. a) Time course of AB 24 (50 mg l^{-1}) degradation by *Moraxella osloensis* (b) Kinetic study of AB 24 (50 mg l^{-1}) degradation by *Moraxella osloensis*

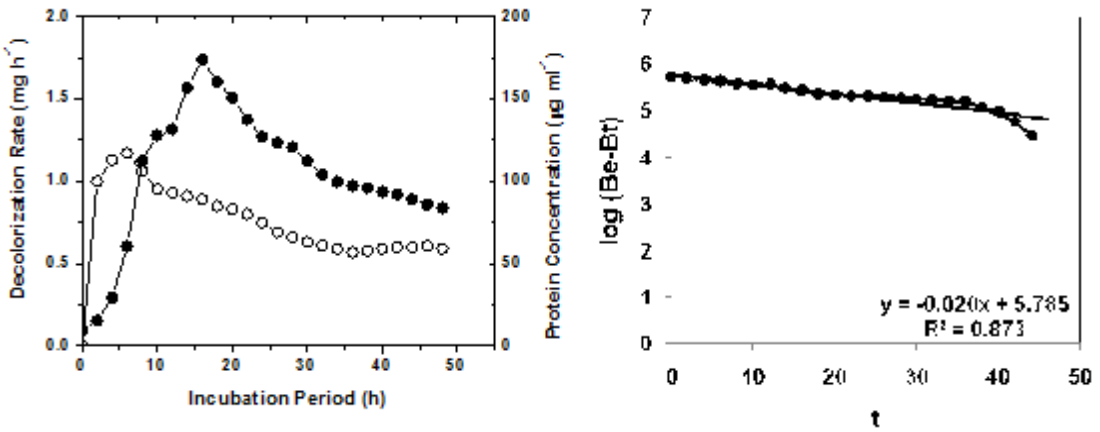

Fig. 3. a) Time course of AB 24 (50 mg l^{-1}) degradation by *Citrobacter freundii* SUB 3 (b) Kinetic study of AB 24 (50 mg l^{-1}) degradation by *Citrobacter freundii* SUB 3

studies and is also extensively studied for their ability to degrade a wide variety of azo dyes[9-11] including the pathways[12]. *Pseudomonas aeruginosa* CLRI BL22 has already been proven for their degradation potential. Valli Nachiyar and Suseela Rajakumar[6] have reported the

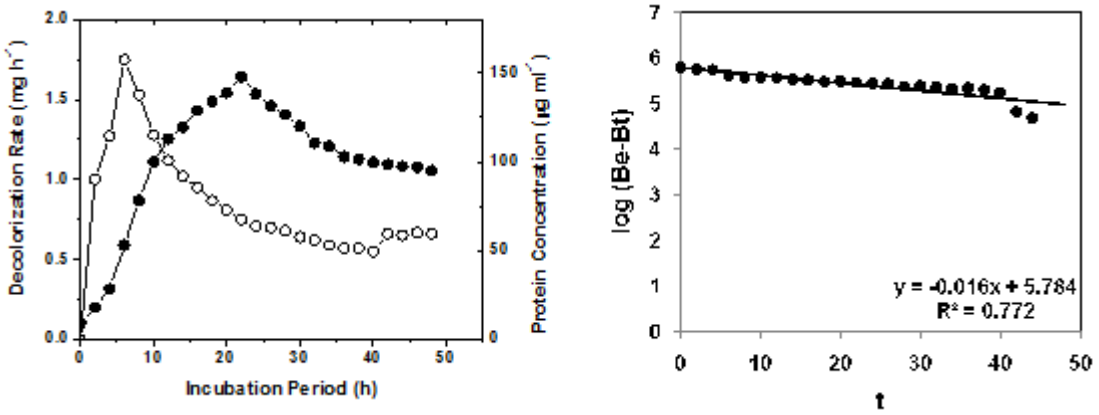

Fig. 4. a) Time course of AB 24 (50 mg l^{-1}) degradation by *Citrobacter freundii* SUB 6 (b) Kinetic study of AB 24 (50 mg l^{-1}) degradation by *Citrobacter freundii* SUB 6

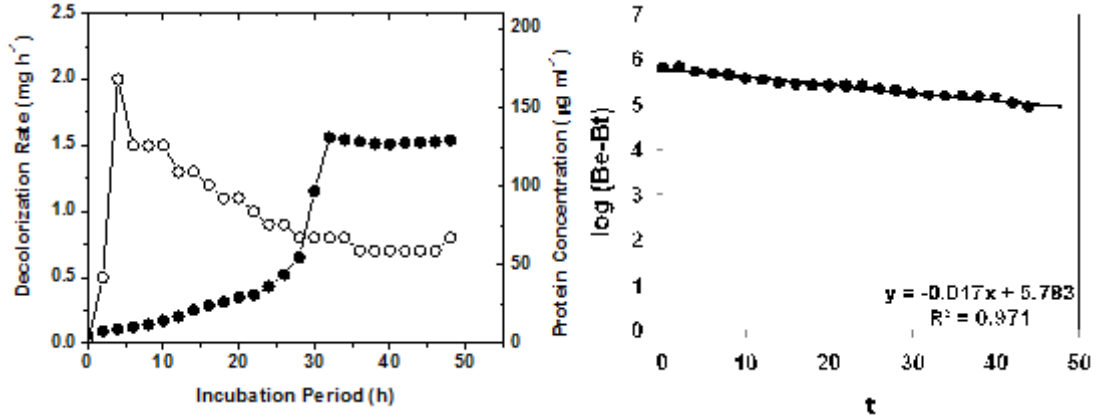

Fig. 5. a) Time course of AB 24 (50 mg l^{-1}) degradation by *Pseudomonas aeruginosa* (b) Kinetic study of AB 24 (50 mg l^{-1}) degradation by *Pseudomonas aeruginosa*

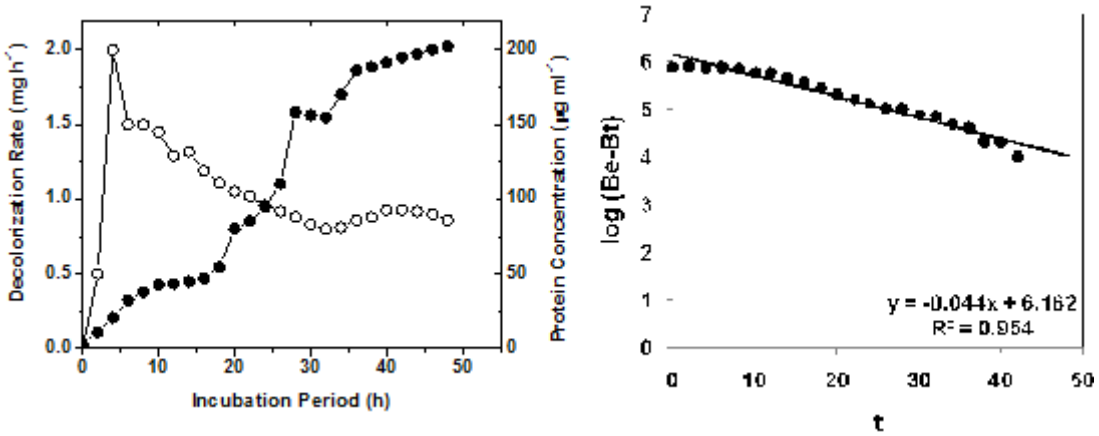

Fig. 6. a) Time course of AB 24 (50 mg l^{-1}) degradation by Consortium CN-1 (b) Kinetic study of AB 24 (50 mg l^{-1}) degradation by Consortium CN-1

biodegradation of Acid Blue 113, and its degradation products metanilic acid[13] 8-anilino 1-naphthalene sulphonic acid[14].

Moraxella osloensis is a species of Gram-negative, oxidase positive, aerobic bacteria within the family Moraxellaceae in the gamma subdivision of the purple bacteria. *Moraxella* sps. have been reported to be a potential biodegrading organism degrading variety of anthropogenic compounds like nitrophenols [15], substituted anilines[16] and polythene [17]. But only few reports are available on the degradation of aniline [18] and naphthalene 2, 6 and naphthalene 1, 6 disulfonic acid[19], the intermediate compounds in dye manufacture/ degradation by *Moraxella* sps. Nachiyar *et al.* [5] have reported the decolourization of acid blue 113 by a microbial consortium comprising *Moraxella osloensis* as one of the constituent.

Citrobacter freundii is a facultative anaerobic Gram-negative bacillus of the Enterobacteriaceae family. The bacteria are long rod-shaped with a typical length of 1-5 ìm. Most *C. freundii* cells are surrounded by several flagella used for locomotion, but a few are non-motile. Wahab et al.[20] have investigated on the enzymatic system involved in the azo dye biodegradation of *Citrobacter freundii* strain A1 using three model dyes namely Acid Red 27, Direct Blue 15 and Reactive Black 5. The potential of this strain to operate as a bacterial consortium was explored and the syntropic interaction between strain A1, *Enterococcus casseliflavus* C1, and *Enterobacter cloacae* L17 was reported to enhance the biodegradation of azo dye Amaranth[21].

The degradation of AB24 at 50 mg l^{-1} by individual as well as the consortium CN-1 was given in Table 1. It is clear from the table that the consortium was capable of bringing about 83% degradation of the dye after 48h of incubation whereas the degradation was found to be in the range of 54-72% for individual organisms.

The biodegradation rates of dye by consortium and individual organisms were calculated by testing the kinetic data with first-order model[22].

$$\frac{dB}{dt} = -k_1(B_s - B)$$

Integrating the above Equation between the limits 0 to t from B=0 to B=Bt, the above kinetic expressions becomes as

$$\log(B_s - B_t) = \log B_s - \left[\frac{k_1}{2.303}\right]t$$

where, Bt is the biodegradation of dye (mg) at time t and k_1 (h^{-1}) is the first-order rate constant.

Of all the constituent microorganisms *Pseudomonas aeruginosa* CLRI BL22 (Fig. 1) followed by *Moraxella osloensis* (Fig. 2) displayed high decolorizing ability showing 63% & 54% decolorization respectively by 48 h of incubation reaching a maximum of 83% decolorization by 120 h. While *Pseudomonas aeruginosa* CLRI BL 22 started to degrade the AB 24 in its late lag phase, *Moraxella osloensis* which exhibited a very short lag phase started to degrade the dye immediately. The decolorization rate was found to be slow and steady in case of *Pseudomonas aeruginosa* CLRI BL 22 reaching a maximum 1.3 mg l^{-1} by 4h after which it decreased gradually. But in case of *Moraxella osloensis* decolorization rate was so rapid that it reached a maximum of 2.3 mg l^{-1} by 2h which came down rapidly to half of that by 12h. The K_1 values were calculated to be 0.071393 h^{-1} and 0.062181 h^{-1} for *Pseudomonas aeruginosa* and *Moraxella osloensis* respectively with the R^2 values of 0.809 and 0.858 indicating that the reactions were slow and less consistent compared to that of consortia.

The other three organisms of the consortium namely *Citrobacter freundii* (both SUB3 and SUB6) (Fig. 3 & Fig. 4) and *Pseuodomonas aeruginosa* SUB10 (Fig. 5) brought about 57, 63 and 72% decolorization respectively after 48h of incubation and they required 120h of incubation for bring about 83% (*Citrobacter freundii* SUB3 and SUB6) and 81% (*Pseudomoas aeruginosa*). Even though the decolorization percentages were similar to *Pseudomoas aeruginosa* CLRI BL22 and *Moraxella osloensis* the K_1 values were found to be 0.04606 h^{-1}, 0.036848 h^{-1} and 0.039151 h^{-1} with the R^2 values of 0.873, 0.772 and 0.971 indicating that the rates in which they carried out the reaction were slow.

The growth of microbial consortium CN-1 was found to be slow and steady which was accelerated after 18 h of incubation (Fig. 6). This delayed acceleration may be attributed to the different growth profiles of the constituent microorganisms. Further, they might have taken time to get adapted to the presence of each other.

This consortium CN-1 was found to be highly efficient in removing 83% of AB 24 after 48 h of incubation with the maximum rate of 2 mg l^{-1} by 4h which decreased gradually. The decolorization by this consortium CN-1 followed first order kinetics with the K_1 value of 0.101332h^{-1} which is very high compared to the K_1 values of AB24 decolorization mediated by individual constituent microorganisms. The high R^2 value (0.954) indicates the consistency of the system. The higher decolorization efficiency of consortium may be attributed to the concerted activities of the constituent strains where the cometabolic activities complement each other. Number of reports is available on the degradation of dyes using microbial consortium which displayed enhanced degradation compared to the individual microorganisms [21,23,24].

CONCLUSION

Biodegradation is always looked upon as an alternative to physical and chemical methods as this is cost effective, environmentally friendly and produce less sludge. Biodegradation using bacterial consortium is more advantageous compared to individual strains as this can handle pollutants belonging to different classes. The bacterial consortium CN-1 containing five different organisms was found to be capable of degrading AB24 efficiently compared to the individual organisms which is clear from the K_1 and R^2 values.

REFERENCES

1. Natarajan, R. and Manivasagan, R. Kinetic Modeling of Dye Effluent Biodegradation by *Pseudomonas Stutzeri*. *ETASR - Engineering, Technology & Applied Science Research*. 2013; **3**:387-390.
2. Lucas, M.S., Dias, A.A., Sampaio, A., Amaral, C. and Peres, J.A. Degradation of a textile reactive azo dye by a combined chemical-biological process: Fenton's reagent-yeast. *Water research.* 2007; **41**:1103-1109.
3. Olaniran, A.O., Balgobind, A. and Pillay, B. Bioavailability of Heavy Metals in Soil: Impact on Microbial Biodegradation of Organic Compounds and Possible Improvement Strategies. *International Journal of Molecular Scicences*. 2013;**14**:10197-10228.
4. Tony, B.D., Goyal, D., Khanna, S. Decolorization of textile azo dyes by aerobic bacterial consortium. *International Biodeterioration and Biodegradation*. 2009; **63**:462-469.
5. Valli Nachiyar, C., Swetha, S., Narendra Kumar, G., Karunya, A., Ananth, P.B., Prakash, K. and Anuradha, S. Biodegradation of acid blue 113 containing textile effluent by constructed aerobic bacterial consortia: optimization and mechanism. *Journal of Bioremediation and Biodegradation.* 2012; **3**:162
6. Valli Nachiyar, C. and Suseela Rajkumar, G. Degradation of a tannery and textile dye, Navitan Fast Blue S5R by *Pseudomonas aeruginosa*. *World Journal of Microbiology and Biotechnology*. 2003;**19**:609-614.
7. Khadijah, O., Lee ,K.K., Mohd Faiz, F. and Abdullah. Isolation, screening and development of local bacterial consortia with azo dyes decolourising capability. *Malaysian Journal of Microbiology*. 2009; **5**:25-32.
8. Nigam, P., Mc Mullan, G., Banant, I. and Marchant, R. Decolourisation of effluent from the textile industry by a microbial consortium. *Biotechnology Letters*. 1996;**18**:117-120.
9. Bhatt, N., Patel, K.C., Keharia, H. and Madamwar, D. Decolorization of diazo-dye Reactive Blue 172 by *Pseudomonas aeruginosa* NBAR12. *Journal of Basic Microbiology.* 2005; **45**: 407-418.
10. Selvakumar, K.V., Basha, C.A., Prabhu, H.J., Kalaichelvi, P. and Sudha N. The potential of free cells of *Pseudomonas aeruginosa* on textile dye degradation. *Bioresource Technology*. 2010; **101**: 2678-2684
11. Jadhav, J.P., Phugare, S.S., Dhanve, R.S. and Jadhav, S.B. Rapid biodegradation and decolorization of Direct Orange 39 (Orange TGLL) by an isolated bacterium *Pseudomonas aeruginosa* strain BCH. *Biodegradation*. 2010; **21**:453-463.
12. Valli Nachiyar, C. and Suseela Rajakumar G. Mechanism of Navitan fast Blue S5R degradation by *Pseudomonas aeruginosa*. *Chemosphere*. 2004; **57**:165-169.
13. Valli Nachiyar, C., Vijayalakshmi, K., Muralidharan, D. and Suseela Rajkumar, G. Mineralization of metanilic acid by *Pseudomonas aeruginosa* CLRI BL22. *World Journal of Microbiology and Biotechnology*. 2007; **23**:1733-1738.
14. Valli Nachiyar. C. and Suseela Rajkumar, G. Biodegradation of ANSA by *Pseudomonas aeruginosa*. *Industrial Journal of Microbiology and Biotechnology*. 2006; **33**:845-849.
15. Jain, R.K., Dreisbach, J.H. and Spain, J.C.

Biodegradation of p-nitrophenol via 1,2,4-benzenetriol by an *Arthrobacter sp*. *Applied and Environmental Microbiology*. 1994; **60**:3030–3032.

16. Zeyer, J., Wasserfallen, A. and Timmis, K.N. Microbial mineralization of ring-substituted anilines through an ortho-cleavage pathway. *Applied and Environmental Microbiology* 1985; **50:**47-53.
17. Kathiresan, K. Polythene and plastic-degrading microbes in an Indian mangrove soil. *Revista de Biologia Tropical* 2003; **51:**629-633.
18. Zeyer, J. and Kearney, P.C. Microbial Metabolism of Propanyl-and 3,4-Dichloroaniline. Pesticide. *Biochemistry and Physiology* 1982; **17**:224-231.
19. Wittich, R.M., Rast, H.G. and Knackmuss, H.J. Degradation of naphthalene-2,6- and naphthalene-1,6-disulfonic acid by a *Moraxella sp*. *Applied and Environmental Microbiology* 1988; **54**:1842–1847.
20. Abdul-Wahab, M.F., Chan, G.F., Yusoff, A.R.M. and Rashid, N.A.A. Reduction of azo dyes by flavin reductase from Citrobacter freundii A1. *Journal of Xenobiotics*. 2013; **3**:e2.
21. Chan, G.F., Gan, H.M. and Rashid, N.A.A. Genome Sequence of *Citrobacter* sp. Strain A1, a Dye-Degrading Bacterium. *Journal of Bacteriology*. 2012; **194**:5485-5486.
22. Lagergren, S. About the theory of so-called adsorption of soluble substances. Kungliga Svenska Vetenskapsakademiens, *Handlingar*. 1898; 24:1–39.
23. Khehra, M.S., Saini, H.S., Sharma, D.K., Chadha, B.S. and Chimni, S.S. Comparative studies on potential of consortium and constituent pure bacterial isolates to decolorize azo dyes. *Water Research*. 2005; **39**:5135-5141.
24. Bhatt, N., Sapna, T. and Kshama, B. Biodegradation of reactive red M8B by bacterial consortium SpNb1. *Indian Journal of Science and Technology*. 2012; **5**:3047-3053.