Journal of Water Process Engineering 16 (2017) 199–205

Contents lists available at ScienceDirect

Journal of Water Process Engineering

journal homepage: www.elsevier.com/locate/jwpe

Concocted bacterial consortium for the detoxification and mineralization of azoic-cum-sulfonic textile mill effluent

Madhava Anil Kumar[a], Ganesan Vigneshwaran[a], Muzhumathi Ezhil Priya[d], Muthulingam Seenuvasan[a], Vaidyanathan Vinoth Kumar[b], Dhanasekaran Anuradha[c], Subramanian Sivanesan[d,*]

[a] Department of Biotechnology, Madha Engineering College, Chennai, India
[b] School of Bioengineering, Department of Biotechnology, SRM University, Chennai, India
[c] Centre for Biotechnology, Alagappa College of Technology, Anna University, Chennai, India
[d] Department of Applied Science and Technology, Alagappa College of Technology, Anna University, Chennai, India

ARTICLE INFO

Article history:
Received 22 December 2016
Received in revised form 21 January 2017
Accepted 24 January 2017
Available online 2 February 2017

Keywords:
Achromobacter xylosoxidans
Klebsiella pneumoniae
Bacillus mannanilyticus
Concoction
Consortium
Mineralization
Textile mill effluent

ABSTRACT

A bacterial consortium, comprising of three isolates; *Achromobacter xylosoxidans* strain APZ, *Klebsiella pneumoniae* strain AHM and *Bacillus mannanilyticus* strain AVS was concocted using the mixture design (MD) matrix. The concocted consortium was employed to degrade and mineralize a textile mill effluent (TME) having sulfone and azo groups. The average mineralization rate (AMR_{TME}) of the liberated aromatic amines were more rapid than that of the individual strains, among them *K. pneumoniae* strain AHM was predominant. The analytical techniques confirmed that TME decolorization was due to biodegradation by the extracellular oxidoreductases, induced by the bacterial consortium. The azoic groups of the TME and the sulfones were not found in the treated solution which was analyzed by the mass spectral analyses. The phyto-toxicological parameters revealed the reduced toxic nature of the biotransformed products.

1. Introduction

Textile colorants and their intermediates are xenobiotic and recalcitrant compounds and their existence in natural ecosystem may create abnormalities and aesthetic problems [1]. Bioremediation is widely preferred than the conventional physico-chemical treatments like filtration, adsorption, coagulation/flocculation, electrolysis, ozonation, and photolysis [2–4]. Researches reveal that bacterial strains mediated dye degradation is faster and rapid than fungal treatment [2]. During biodegradation of azo dyes, there is a release of colorless aromatic amines which are toxic and mutagenic to biological systems [5]. A bacterial strain find difficulties in oxidizing the aromatic amines, thus a system comprising two or more bacterial strains would help in ameliorating the mineralization of the aromatic amines [6]. The individual bacterial strains in the consortium would attack the molecules at different positions, thus making the intermediates to get decomposed by the other strains [7,8].

MD matrix under design of experiments (DOE) is used to optimize the ingredients in any formulations and validates the relationship between the formulation and their relative performance in lesser experiments [9]. Formulation of a bacterial consortium using procured strains with the aid of MD matrix has been reported to decolorize the textile dyes [10,11].

In the present study, the ability of a chemometrically concocted bacterial consortium in mineralizing a TME comprising of sulfone and azo groups were to be investigated. The optimal concoction of the ingredients in the bacterial consortium was selected on the basis of experiments possessing higher AMR_{TME} values, using the MD matrix. The study also aims to elucidate the ability of the consortium in decolorizing TME by understanding role of oxidoreductases. The products of TME were to be characterized and consequently, phytotoxicity assays were to be performed, to assess the toxicity of the biotransformed products.

* Corresponding author.
E-mail addresses: siva@annauniv.edu, sivanesh1963@gmail.com (S. Sivanesan).

http://dx.doi.org/10.1016/j.jwpe.2017.01.008
2214-7144/

2. Material and methods

2.1. Chemicals and bacterial strains

ABTS (2,2′–azino–bis(3–ethyl–benzothiazoline–6 sulfonic acid)), catechol, methyl red and *n*-propyl alcohol were obtained from Sigma-Aldrich (Bangalore, India). TME (λ_{max} = 519 nm and pH 8.4) from textile industry outfall site was collected, neutralized to pH 7.0 ± 0.2 and stored in the dark at room temperature to control the growth of the non-indigenous microorganisms. All chemicals were of analytical grade with highest purity. The three isolated bacterial strains; *A. xylosoxidans* (KJ010764), *K. pneumoniae* (KJ558434) and *B. mannanilyticus* (KT895346) shown in Supplementary Materials Fig. S1 were used to formulate the bacterial consortium.

2.2. MD modelling for the concoction of bacterial consortium for TME decolorization

MD matrix provided by Minitab software (Ver. 14.0, U.S. Federal Government Commonwealth of Pennsylvania, USA), was used in concocting the consortium and to understand the interrelationship between the ingredients [9]. TME decolorization were performed in Bushnell-Haas (BH) broth (pH 6.6 at 37° C) under static condition by inoculating 100 mL TME (without dilution) with fully grown cultures of the individual strains in varying proportions as shown in Table 1. The experimental responses were fitted to a regression model.

$$Y = \beta_1 A + \beta_2 B + \beta_3 C + \beta_{12} AB + \beta_{23} BC + \beta_{13} AC \quad (1)$$

where, Y is the observed response; A, B and C are the ingredients of the consortium; β_1, β_2, β_3, β_{12}, β_{13} and β_{23} are the linear and interaction coefficients respectively.

The adequacy of the regression model and the significance of the variables were evaluated by determining the regression correlation coefficients, analysis of variance (ANOVA), probability (P-value), Fisher variance ratio (F-value), Student's *t*-test, mixture and normal probability plots [12].

2.3. Analyses of TME decolorization and mineralization

An aliquot of the decolorized medium was withdrawn and centrifuged at 10,000 rpm for 15 min to separate the biomass. The decolorization was quantitatively analyzed on Shimadzu UV–1800 spectrophotometer (Tokyo, Japan) at 519 nm. The changes in absorbance of the decolorized medium were compared with the abiotic (without microorganisms) controls. TME mineralization was quantified by measuring percentage mineralization (%M) of the liberated aromatic amines [13]. The concentration of aromatic amines was determined spectrophotometrically at 560 nm by adding 5.0 mg of the lyophilized decolorized solution in 5 mL of 0.4% chloranil in dimethyl formamide and heated to 100 °C for 5 min. The concentration of the aromatic amines (mM/L) was estimated using aniline-2-sulfonic acid as a standard and subsequently AMR_{TME} was calculated;

$$AMR_{TME}\left(\mu M/min\right) = \frac{C \times \%M \times 1000}{100 \times t} \quad (2)$$

$$\%M = \frac{Aromatic\ amines_{(0h)} - Aromatic\quad amines_{(t)}}{Aromatic\ amines_{(0h)}} \times 100 \quad (3)$$

2.4. Assaying activity of oxidoreductases

The concocted bacterial consortium was grown in BH broth containing TME or without it (control) and the decolorized solutions was filtered to separate biomass followed by centrifugation at 10,000 rpm for 30 min at 4 °C. The supernatant was used for assaying the activities of laccase, lignin peroxidase (LiP), azoreductase and tyrosinase spectrophotometrically (at 420, 300, 430 and 410 nm) using ABTS, *n*-propyl alcohol, methyl red and catechol respectively as substrates [14–17]. One unit of enzyme activity is defined as the change in absorbance units/min/mg protein. The reference blanks contained all the components except the assayed enzyme and enzyme assays were carried out at room temperature in triplicate.

Table 1
MD matrix for the concoction of the bacterial consortium.

Run	*A. xylosoxidans* strain APZ	*K. pneumoniae* strain AHM	*B. mannanilyticus* strain AVS	Aromatic amines (mM/L) (at 480 min)	%M	AMR_{TME} (μM/min)
1	1.0	0	0	0.37	24.49	0.25
2	0	1.0	0	0.33	32.65	0.33
3	0	0	1.0	0.46	6.12	0.06
4	0.50	0.50	0	0.14	71.43	0.73
5	0.50	0	0.50	0.27	44.90	0.46
6	0	0.50	0.50	0.14	71.43	0.73
7	0.33	0.33	0.33	0.11	77.55	0.79
8	0.66	0.16	0.16	0.14	71.43	0.73
9	0.16	0.66	0.16	0.09	81.63	0.83
10	0.16	0.16	0.66	0.15	69.39	0.71

Table 2
ANOVA for AMR_{TME} (μM/min) for the regression model.

Source	Degrees of freedom	Sum of square	Sum of adjusted squares	Adjusted average squares	F-ratio	P-value
Regression	5	0.618	0.618	0.123	19.28	0.007
Linear	2	0.072	0.037	0.018	2.91	0.166
Quadratic	3	0.545	0.546	0.182	28.36	0.004
Residual error	4	0.025	0.025	0.006		
Total	9	0.644				
R^2 = 96.02%		$R^2_{adjusted}$ = 91.03%		$R^2_{predicted}$ = 72.19%		