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Response surface methodology for decolorization of azo dye Methyl Orange by bacterial consortium: Produced enzymes and metabolites characterization

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ABSTRACT

The use of chemometric methods such as response surface methodology (RSM) based on statistical design of experiments (DOEs) is becoming increasingly widespread in several sciences such as analytical chemistry, engineering and environmental chemistry. In the present study, the decolorization and the degradation efficiency of Methyl Orange (MO) was studied using a microbial consortium. The microbial growth of *Sphingomonas paucimobilis*, *Bacillus cereus* ATCC14579, *Bacillus cereus* ATCC11778 is well in the presence of MO (750 ppm) within 48 h at pH 7 and 30 °C. In fact, these microorganisms were able to decolorize and to degrade MO to 92%. The degradation pathway and the metabolic products formed during the degradation were also predicted using UV–vis, Fourier transform infrared (FTIR) spectroscopy and nuclear magnetic resonance (NMR) spectroscopy analysis. Under optimal conditions, the bacterial consortium was able to decolorize completely (>84%) the dye within 48 h. The color and COD removal were 84.83% and 92.22%, respectively. A significant increase in azoreductase, lignin peroxidase and laccase activities in the cells were obtained after complete decolorization. Phytotoxicity study using plants showed no toxicity of the produced products.

1. Introduction

The textile industry wastewater is rated as the most polluting among all industrial sectors in terms of both volume and composition of the effluents [1,2]. The color in these discharged wastewater is due to synthetic dyes left unused due to industrial inefficiencies. Presently over 10,000 different dyes and pigments are used in dyeing and printing industries all over the world. Many of them are believed to be toxic and carcinogenic [3].

Almost 7×10^5 tones of dyes are produced around the world every year, and most of them are azo dyes containing one or more azo groups (R1–N=N–R2), which are extensively used as industrial raw materials [4]. It is quite undesirable to discharge azo dyes with different color into the environment due to their higher pollution and toxic intermediates produced [5]. Compared with chemical and physical methods, the biological treatment has been the main focus for the degradation of these dyes, which can produce lower costs and fewer toxic resultants [6,7]. Many microorganisms, belonging to bacteria, fungi, even yeasts proved their ability to decolorize azo dyes by bioadsorption or degradation. Among these microorganisms, bacteria and fungi which played a key roles in the treatment of wastewater containing dyes, and it has been proved that can they decolorize dyes with different types of enzymes [8].

Recently, azoreductase activity was detected in many bacteria, such as *Sphingomonas xenophaga* BN6, *Pigmentiphaga kullae* K24 and *Caulobacter subvibrioides* C7-D [9–11]. There is a little application for practical treatment although many pure cultures are available to decolorize azo dyes, which can be supported by three reasons [12]. Firstly, fungi cannot use azo dyes as sole carbon and energy source, and their growth is time consuming. Secondly, low efficiency of bacteria degrading azo dyes is achieved under aerobic conditions, because oxygen is a more efficient electron acceptor compared with azo dye. Thirdly, single strains cannot adapt the complex and variable environment conditions. Therefore, mixed microbial populations are expected to perform better than single microorganisms [13]. However, there is a little information about using fungal–bacterial consortium to decolorize azo dyes.

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Table 1
Mixture design matrix with the experimental analysis.

Assay	*Sphingomonas paucimobilis*	*Bacillus cereus* ATCC14579	*Bacillus cereus* ATCC11778	Total	COD removal (%)	Decolorization (%)	Germination (%)
1	1.00	0.00	0.00	1.00	89.67	82.80	70
2	0.00	1.00	0.00	1.00	90.40	84.83	85
3	0.00	0.00	1.00	1.00	90.18	81.84	82
4	0.50	0.50	0.00	1.00	88.43	80.21	65
5	0.50	0.00	0.50	1.00	92.22	79.86	87
6	0.00	0.50	0.50	1.00	91.57	84.83	85
7	0.33	0.33	0.33	1.00	88.98	84.06	67
8	0.66	0.16	0.16	1.00	90.11	79.67	83
9	0.16	0.66	0.16	1.00	90.25	82.40	84
10	0.16	0.16	0.66	1.00	89.71	77.67	75

Statistical optimization method (a central composite design coupled with response surface methodology (RSM)) overcomes the limitations of classical methods and was successfully employed to obtain the optimum process conditions while the interactions between process variables were demonstrated [14].

The importance and theoretical concepts behind the optimization through experimental design as well as RSM in research and development efforts have been thoroughly discussed in a number of informative articles and the sequential steps of RSM are also highlighted in subsequent sections of this study. RSM is nowadays, a promising as well as a powerful tool for multivariate optimization through sequential experimentation. Several researchers have already been using various RSM approaches to explain optimization process [15].

The present work aims to study the ability of *Sphingomonas paucimobilis*, *Bacillus cereus* ATCC14579 and *Bacillus cereus* ATCC11778 to decolorize MO. However, the selection of optimal conditions for the growth and the different proportions of the three microorganisms using the response surface methodology can ameliorate the decolorization performances of the cells for MO. The produced metabolites during the degradation were also predicted using UV–vis FTIR spectroscopy and nuclear magnetic resonance (NMR) spectroscopy analysis. These metabolites were studied for their toxicity using the plants phytotoxicity.

2. Material and methods

2.1. Dye and chemicals

The commercially used textile azo dye Methyl Orange ($C_{14}H_{14}N_3NaO_3S$, E.C. No. 2089253; λ_{max} = 466 nm) was purchased from the Sigma–Aldrich (Chemical Company, MO, USA) and used for the study without any further purification.

Reduced nicotinamide adenine dinucleotide (NADH), 2,2′-azinobis (3-ethylbenzthiazoline)-6-sulfonate (ABTS) and methyl sulfoxide-*d*6, 99.9 at% D (contains 0.03% v/v TMS) (DMSO *d*6) were purchased from Sigma–Aldrich Chemicals, USA. Sodium phosphate buffer (PBS), tartaric acid, acetate buffer and n-propanol were purchased from Biorad, USA. All chemicals used were of the highest purity available and of analytical grade.

2.2. Microorganisms and culture media

Three microorganisms, *S. paucimobilis*, *Bacillus cereus* ATCC14579, *Bacillus cereus* ATCC11778 were used in this study. *S. paucimobilis* was isolated in previous works of Ayed et al. [16,17] with the ability of degrading azo and triphenylmethane dyes (Congo Red, Methyl Red, Malachite Green and Crystal Violet). *Bacillus cereus* ATCC14579 and *Bacillus cereus* ATCC11778 are a reference strains. The used medium was composed in 1000 ml of distilled water: glucose (1250 mg/L), yeast extract (3000 mg/L), $MgSO_4$ (100 mg/L); $(NH_4)_2SO_4$ (600 mg/L); NaCl (500 mg/L); K_2HPO_4 (1360 mg/L); $CaCl_2$ (20 mg/L); $MnSO_4$ (1.1 mg/L); $ZnSO_4$ (0.2 mg/L); $CuSO_4$ (0.2 mg/L); $FeSO_4$ (0.14 mg/L) and it was maintained at a constant pH of 7 by the addition phosphate buffer [16,17].

2.3. Experimental design and methods

The D-optional method in the experimental design, provided by the software Minitab (Ver. 14.0, U.S. Federal Government Commonwealth of Pennsylvania, USA), was used to optimize the formulation of the microbial consortium. Generally, the mixture design was used to study the relationships between the proportion of different variables and responses. Ever since Scheffe devised a single-lattice and single-core design in 1958, the mixture design has developed a variety of methods [18,19].

Response surface methodology (RSM) is usually applied following a screening study to explore the region of interest of the factors identified by the preceding study [15]. The mixture design is widely used in the formulation of food experiment, chemicals, fertilizer, pesticides, and other products. It can estimate the relationship between formulation and performance through regression analysis in fewer experiment times [20].

In this study, *S. paucimobilis*, *Bacillus cereus* ATCC14579 and *Bacillus cereus* ATCC11778 were used as mixture starters, with different proportions ranging from 0 to 100%, as shown in Table 1. Decolorization experiments were taken according to the ratio given by the experimental design, and 10% of mixed culture were inoculated into the Mineral Salt Medium (MSM) (3.0 g/L yeast extract and 1.25 g/L glucose and 750 ppm MO) at 37 °C for 10 h in shaking conditions (150 rpm) [21,22].

2.4. Statistical analysis

The statistical analyses were performed by the use of multiple regressions and ANOVA with the softwares Minitab v 14.0 and Essential Regression v 2.2. The significance of each variable was determined by applying Student's *t*-test [23,24].

The *P*-value is the probability that the magnitude of a contrast coefficient is due to random process variability. A low *P*-value indicated a "real" or significant effect.

2.5. Color and COD removal

Chemical oxygen demand (COD) was determined spectrometrically by 5B-1 Quick COD analyzer (LianHua Environmental Instrument Institute, Langzhou, PR China). The color of the influent and the effluent was monitored spectrophotometrically (Hach DR 2000). Color and COD removal were determined using the following equations [17].

$$\text{Color removal}\,(\%) = \frac{A_i - A_t}{A_i} \times 100 \quad (1)$$