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Research article

Assessment on the decolourization of textile dye (Reactive Yellow) using *Pseudomonas* sp. immobilized on fly ash: Response surface methodology optimization and toxicity evaluation

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ABSTRACT

This study focuses on the investigation of removal of textile dye (Reactive Yellow) by a combined approach of sorption integrated with biodegradation using low cost adsorbent fly ash immobilized with *Pseudomonas* sp. To ensure immobilization of bacterial species on treated fly ash, fly ash with immobilized bacterial cells was characterized using Fourier transform infrared (FTIR) spectroscopy, scanning electron microscopy (SEM), and fluorescence microscopy. Comparative batch studies were carried out using *Pseudomonas* sp, fly ash and immobilized *Pseudomonas* sp on flyash and were observed that immobilized *Pseudomonas* sp on flyash acted as better decolourizing agent. The optimized pH, temperature, and immobilized adsorbent dosage for highest percentage of dye removal were observed to be pH 6, 303 K, 1.2 g/L in all the cases. At optimum condition, the highest percentage of dye removal was found to be 88.51%, 92.62% and 98.72% for sorption (flyash), biodegradation (*Pseudomonas* sp) and integral approach (*Pseudomonas* sp on flyash) respectively. Optimization of operating parameters of textile dye decolourization was done by response surface methodology (RSM) using Design Expert 7 software. Phytotoxicity evaluation with *Cicer arietinum* revealed that seeds exposed to untreated dye effluents showed considerably lower growth, inhibited biochemical, and enzyme parameters with compared to those exposed to treated textile effluents. Thus this immobilized inexpensive technique could be used for removal of synthetic dyes present in textile wastewater.

1. Introduction

Increasing environmental pollution coming from rapid industrialization is one of the extreme challenges facing today's world (Das et al., 2015a,b; Marković et al., 2015; Shah et al., 2013; Wang et al., 2018). Over next several decades worldwide water scarcity will affect the population of different countries and India has already been counted as one of these water deficit countries. Along with proper utilization of fresh water reuse and recycling of wastewater releasing from industries, different agricultural fields and domestic activities is also important to abate the deficiency of fresh water. Dye is one of the major water pollutants discharged from the industries which considerably damage ecosystem functioning, biodiversity. They are widely used in variety of industries such as textile, pharmaceutical, solvents, acrylic, printing, paper, cosmetics, food, pigment, and pulp. These industries are increasing in number with increase in human demands on these substances (Chowdhury et al., 2011). Dyes present in water even at concentration of 1 mg/L are purely unhygienic for human health (Vijayaraghavan and Yun, 2008). The toxicological aspects of dyes can cause acute (for the short span of time harmful effects of single or multiple exposures of substances on a living body) or chronic (harmful long-term effects of substances on a living creature that results either from repeated exposures) toxicity to living organism (Burgeron et al., 2015). Dyes may be mutagenic, genotoxic, teratogenic, and cause severe health issues to human bodies like disturbance in renal system, dysfunction in digestive tract system, damage in cerebral system, hepatic system, skin and central nervous system (Srinivasan et al., 2014; Satapathy and Das, 2013). It minimizes food intake capability, inhibits growth and fertility rates in many mammalian cells. Dyes affects photosynthesis by inhibiting the entry of sunlight and their accumulation through aquatic food chain system decreases concentration of dissolved oxygen providing anoxic conditions. This results in severe disturbances on aquatic eco-systems (Vijayaraghavan and Yun, 2008). The dyes diminish aesthetic properties of water by providing significant colour

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changes. The presence of dyes in aquatic system could alter the chemical oxygen demand (COD), biochemical oxygen demand (BOD), dissolved and suspended solids. However, due to the adverse effects of dyes in water nowadays the industries realising them are challenged by the demands to fulfil the requirements of increasingly stringent legislation and controls monitored by governments and regulatory bodies (Satapathy et al., 2015).

Different types of physical methods (flocculation, coagulation, sedimentation, ultra filtration) and many chemical methods- electrolysis, ion-exchange neutralization, etc have been used to remove dye present in solution but complete dye decolourization has not been achieved due to complex molecular structure coupled with the by-product associated toxicological aspects (Chowdhury et al., 2011). There are many other drawbacks such as high reagent and energy production of toxic products, more capital and operating cost and time consuming in those conventional methods (Martins et al., 1999). Biological treatment have often been utilized as non-hazardous ability, high capacity for pollutant intake, regeneration capability, and economically viable option utilizing culture of different microbial strains (Wang and Chen, 2009). Pure culture and consortium of bacterial species can uptake dye particles, metals, and other toxic elements as their sole nutritional carbon source or nitrogen source (Fernando et al., 2013; Pathak and Dikshit, 2013). Several bacterial trophic groups have been reported for their capacity to decolorize dyes such as *Staphylococcus hominus, Vibrio* sp., *Proteus vulgaris, Dietzia* sp., *Pseudomonas aeruginosa*, *Bacillus subtilis*, *Stenotrophomonas* sp., *Halomonas* sp. etc. and many other species have been reported to have developed their enzyme systems for degrading the dye compounds (Ghosh and Saha, 2012). *Pseudomonas* sp. has been reported to degrade the varieties of dyes such as Crystal Violet, Methyl Violet, Methylene Blue, Safranin, CI Direct Blue 6 etc. (Banerjee et al., 2017; Sarnaik and Kanekar, 1999).

Sorption is also one of the major and worldwide methods for wastewater treatment. Fly ash, red mud, CuO nanoparticles, chitosan, etc. are efficient and cost effective adsorbents for proper wastewater treatment (Liu et al., 2015; McDonald et al., 2014; Saha et al., 2012; Singh et al., 2013a,b). However, economically viable efficient adsorbents are required. Fly ash is a major by-products coming from crushed coal combustion in thermal power plants. Fly ash particles vary between less than 1 μm–150 μm in diameter and are basically composed of silicon, aluminium, magnesium, and iron (Shaobin et al., 2004). In India, per year almost 200 million tons of coal produces ash areas that occupy 65,000 acres of land (Mohanty and Patra, 2015). Rapid increment of power requirement for industrial purposes of this country provides increased amount of fly ash particles. Therefore fly ash management constitutes a serious environmental hazard for India and requires remarkable research and development. Thus, fly ash utilized as an adsorbent is of environmentally as well as cost effective approach for a sustainable use of an industrially hazardous waste (Wang and Chen, 2009).

Immobilization of bacterial cells can be done by natural phenomenon or artificial process. Among the several types of artificial processes, physical adsorption, flocculation, chemical cross-linking, lattice entrapment, membrane entrapment, ionic binding, covalent binding, micro-encapsulation are the main and most reliable processes. This combined approach increases the dye degradation considerably by contacting dye molecules for better bacterial availability (Chen et al., 2003).

Pseudomonas sp. has been reported in previous studies to degrade the varieties of dyes (Banerjee et al., 2017; Sarnaik and Kanekar, 1999). Fly ash used as an adsorbent is of environmentally as well as inexpensive approach for a sustainable use of an industrial waste (Wang and Chen, 2009). *Pseudomonas putida* embedded on sodium alginate, *Pseudomonas aeruginosa* immobilized on chitosan-Fe_2O_3 Composite, *Bacillus licheniformis* attached on polyacrylamide gel beads, were reported in earlier studies to decolourize reactive red, cibacron brilliant red, and reactive yellow 17 dye respectively (Suganya and Revathi, 2016; Fetyan et al., 2017; Suganya and Revathi, 2016).Thus, for the first time the present study investigates on the removal of reactive yellow textile dye by an integrated approach which employs sorption coupled with biodegradation using bacterial immobilization process by immobilizing *Pseudomonas* sp. on fly ash. Different parameters such as effect of pH, temperature, and inoculum dosage were studied to evaluate the effect of these parameters on dye decolourizing capacity. The batch studies of different parameters were optimized using response surface methodology (RSM) and optimization conditions were validated with experimental responses. The chick pea seeds are major cultivation in Bengal and essential controlling factor in agricultural economy of India and have used in toxicity studies (Banerjee et al., 2017; Bhattacharya et al., 2012). Therefore, the comparative in vitro phytotoxicity assay induced by both treated and untreated samples on chick pea (*Cicer arietinum*) seeds was carried out.

2. Experimental

2.1. Dye and chemicals

Reactive yellow, a textile dye (RY; λ_{max} = 425 nm), was commercially purchased. Nutrient broth, Nutrient agar, the compositions of minimal media –Sodiumdihydrogen phosphate (NaH_2PO_4), Disodium hydrogen phosphate (Na_2HPO_4), Magnesium sulphateheptahydrate ($MgSO_4{\cdot}7H_2O$), Ferrous sulphateheptahydrate ($FeSO_4{\cdot}7H_2O$), Calcium chloride ($CaCl_2$)were purchased from Merck, India. Calcium hydroxide ($Ca(OH)_2$), Sodium borate ($Na_2B_4O_7{\cdot}H_2O$), TritonX-100, Ethyl acetate, Ethylenediaminetetraacetic acid (EDTA), Formaldehyde (HCHO)were obtained from Sigma Aldrich, India. Ethidium bromide was purchased from Invitrogen, India and Acridine orange, Potassium bromide (KBr) from Himedia, India. The bacterial strain (*Pseudomonas mendocina*) has been procured from Microbial Type Culture Collection, Chandigarh, India.

2.2. Collection and treatment of adsorbent (fly ash)

Fly ash was obtained from Thermal Power Station situated in Kolkata, West Bengal, India. After washing with distilled water the raw fly ash was treated with 10% calcium hydroxide so that the surface area and porosity of the adsorbent would further be increased (Chowdhury et al., 2011). Then the precipitated fly ash was separated from the suspension by filtration technique and dried at 70 °C in hot air oven for overnight. The dried fly ash was then crushed and finally used for the adsorption study.

2.3. Immobilization process

At first vial of the procured bacterial strain was broken and the strain was incubated on a nutrient agar plate for overnight growth. Then nutrient broth was prepared freshly and overnight bacterial culture was inoculated aseptically in it for 24 h. After that, 5 ml overnight culture was added to 95 ml of sterilized water and incubated again for 24 h. Afterwards, 5 g pre-treated sterilized fly ash was added to this bacterial culture and kept it in an incubator (REMI CIS-24 PLUS, India) for 24 h for 37 °C and agitation speed of 120 rpm. The culture was centrifuged (REMI PR-24, India) at 5000 rpm for 10 min and preserved at 4 °C for further studies.

2.4. Characterization of fly ash

The characterization of adsorbents (fly ash and fly ash with immobilized bacterial cells) was done by the method of Fourier transform infrared (FTIR) spectroscopy (PerkinElmer Spectrum Version 10.4.4, Waltham, United States), X-ray diffraction (Rigaku Ultima III, USA), energy-dispersive X-ray spectroscopy (Inca, ZEISS EVO-MA 10, Germany), scanning electron microscopy (ZEISS EVO-MA 10,