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Influence of various process parameters on the biosorptive foam separation performance of *o*-cresol onto *Bacillus cereus* and Cetyl Trimethyl Ammonium Bromide

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ABSTRACT

Response surface methodology was used to optimize the removal of o-cresol from aqueous solution by Bacillus cereus and Cetyl Trimethyl Ammonium Bromide based hybrid technique biosorptive foam separation. The biosorptive foam separation was carried out in two stages namely biosorption and foam separation. A minimum run resolution *V* central composite design with five variables (initial feed cresol concentration, pH of the feed, biosorbent dosage, time and agitation speed) for biosorption and three variables (liquid pool height, surfactant concentration and airflow rate) for biosorptive foam separation were applied to optimize the process. The optimized conditions for maximum removal of cresol for biosorption were, Initial feed cresol concentration 467 mg/l, pH of the feed 6.44, biosorbent dosage 1.11 g, time 5.25 days and agitation speed of 195 rpm for biosorption, and that for biosorptive foam separation were liquid pool height 28.38 cm, surfactant concentration 0.29% and airflow rate 1.8 lpm. The results showed a good fit with the proposed statistical model for removal of o-cresol ($R^2 = 0.9387$) for biosorption and ($R^2 = 0.9980$) for biosorptive foam separation.

1. Introduction

Phenolic compounds constitute a very large group of organic pollutants in the waste water. Auxiliary phenolic compound such as resorcinol, catechol, and cresols are considered to be major pollutants in waste water as they are used as raw materials in many chemical industries. The presence of such persistent chemical in the effluent causes increasing anxiety due to the evidence of adverse ecological and public health effects. These pollutants are recognized as carcinogens and are known to be toxic to the environment even at a very low concentration. Cresol can be found in several types of industrial waste water such as pharmaceutical, oil refineries, coal mines, gasoline, pesticides and chemical spills. Skin is a major route of occupational exposure resulting in the development of white patches and blistering. It may act as a promoter for stomach tumors. Lungs exposed to cresol show signs of emphysema, edema, bronchopneumonia and hemorrhage. It may cause adverse effects on the central nervous system, cardiovascular system and kidney. Liver showed signs of centrilobular and midzonal necrosis. Woman exposed to tri-cresol reported increased gynecological problems, menstrual disturbances, hormonal disturbance, perinatal mortality and increased abnormal development of new born. Exposure at higher levels may result in lowering consciousness and death. The worldwide production *o*-cresol is 37,000–38,000 tons/annum and over 45 million pounds of cresols are estimated to be released into the environment (EPA, 1983). USEPA (US Environmental protection agency) listed cresol as a priority pollutant and as a pollutant of group C (possible human carcinogens) [1]. The Indian standard specifications for drinking water have set the maximum allowable limit of 0.001 ppm for phenols. It is therefore necessary to reduce or eliminate cresol from wastewater to acceptable level before disposal to water bodies [2]. Various technologies for the removal of phenolic compounds available in the literature include physicochemical treatment processes such as adsorption, ion-exchange resin, activated carbon, aerobic and anaerobic biodegradation processes. Amongst these technologies, the adsorptive removal by using granular or powdered activated carbon has gained wide acceptance and popularity [3].

The search for new technologies for the removal of toxic metals and aromatic compounds from contaminated sites has focused on biosorption. Das et al. evaluated low temperature

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Abbreviations: ANOVA, analysis of variance; CCD, central composite design; CTAB, Cetyl Trimethyl Ammonium Bromide; RSM, response surface methodology; 4-AAP, 4-amino antipyrine; MTCC, Microbial Type Culture Collection and Gene Bank.

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carbonization process to diminish p-cresol from aqueous system. Giirses et al. studied adsorption of *o*-cresol onto lignite and a bituminous coal and found bituminous to be the best. Hadjara et al. demonstrated the use of hybrid (diatomite/carbon) adsorbent for the removal of p-cresol from aqueous solution, and proved the affinity and suitability of these composites. Zhu et al. suggested that physicochemical-activated coconut char is highly effective in the mitigation of p-cresol. Kennedy et al. investigated the adsorption behavior of m-cresol on a porous carbon prepared from rice husk (RHAC) [4–8]. Because of higher regeneration costs we have to think over alternate biosorbents. Algae, bacteria, fungi and yeasts have all proven to be potential biosorbent. Zhang et al. investigated the biosorption of eight aromatic compounds with different functional groups by *penicillium oxalicum* biomass. Surkatti et al. studied the biodegradation of simulated wastewater containing p-cresol using *pseudomonas putida* immobilized in PVA gel in batch as well as continuous reactors. Yan et al. showed that biodegradation of phenol and m-cresol using a pure culture of *Candida tropicalis* could degrade 2000 mg/l phenol and 280 mg/l m-cresol alone in 66 and 52 h. The uptake and accumulation of hazardous organics by microorganism offers an economical, practical and efficient alternative to existing methods [9–11].

Flotation, originated in minerals processing has nowadays found extensive use in wastewater technology due to its advantages. Gaudin was the first one to speak about the flotation of microorganisms due to their size and shape followed by Grieves who worked on the flotation of particulate, ferric oxide, bacteria, active carbon and clays. Recently, Guo et al. has attempted to foam fractionate phenol using CTAB and recovered 93.43%. Biosorptive flotation technique implemented by Zoubolis and Matis for toxic metal removal was applied for cresol separation ad worked well [12,13]. Here, sorptive phenolic removal has been achieved in the first stage ad efficient solid–liquid separation along with unadsorbed cresol in the second stage. One of the best possible techniques for direct discharge of waste water or recycling certainly constitutes sorptive flotation.

RSM has a major advantage over the one factor at a time approach, as it allows the evaluation of the effects of multiple variables and their interactions on the output variables with reduced number of trials. Response surface methodology is an effective statistical technique based on the fit of polynomial equation to the experimental. The major objective is to simultaneously optimize the levels of these variables to attain the best system performance. Central Composite Design (CCD) was used for generation of response surface analysis model. The statistical implication of the second-order model equation was determined by performing Fisher's statistical test for analysis of variance (ANOVA). In particular, a good model must be precise and significant based on *F*-value and *p*-value in contrast to the lack of fit. Moreover, the proportion of variance exhibited by correlation co-efficient (R^2) should be close to 1 as this would demonstrate better correlation between the experimental and predicted values [14].

In the present work hyper-phenol tolerant strain *Bacillus cereus* was tried to mitigate cresol and the interactions among the following eight operating variables namely initial feed cresol concentration, pH of the feed, biosorbent dosage, time, and agitation speed for biosorption and liquid pool height, surfactant concentration and airflow rate for biosorptive foam separation was studied. Statistically optimized conditions for maximum removal of *o*-cresol from aqueous solution was found. The optimized parameters of biosorption were initialized in foam separation and parameters such as liquid pool height, surfactant concentration and airflow rate were optimized. Residual cresol concentration in the aqueous solution was determined by the 4-amino anti pyrine method. The major advantages of biosorptive foam separation of cresol by *Bacillus cereus* and CTAB is that the cresol concentration in treated solution was reduced to a greater extent.

2. Materials and methods

2.1. Reagents and analysis

o-cresol, sodium hydroxide flakes (98%), Cetyl Trimethyl Ammonium Bromide (CTAB), ethanol, and Hydrochloric acid (HCl) were bought from SDFCL (India). Luria-Bertani broth (LB broth), and Nutrient agar were purchased from Hi-media (India). Reagents used in 4-amino antipyrine (4-AAP) procedure were of analytical grade and supplied by Merck (Germany).

Microbial culture, *Bacillus cereus* (MTCC 8312) was purchased from Microbial Type Culture Collection and Gene Bank and maintained in a standard nutrient agar medium. Cells were grown in 250 ml Erlenmeyer flasks containing 100 ml nutrient broth in an incubation shaker (Scigenics Biotech, India). All flasks and media were sterilized and heat inactivated in an autoclave at 121 °C, 15 psi for 20 min. Biomass was collected by centrifugation and the pellet was washed with 70% ethanol and air dried. Dried biomass was used as an adsorbent.

Sartorius basic meter PB-11 (Germany) was used for measuring pH and UV–vis spectrophotometer Shimadzu-UV-2450 (Japan) used for measuring the absorbance values of the phenolic solution.

2.2. Biosorption experimentations

Batch biosorption experiments were carried out in a series of Erlenmeyer flasks, where 100 ml of aqueous *o*-cresol (concentration 262–738 mg/l), pH (4.62–9.38) to be treated was placed in contact with a known mass of biosorbent (0.524–1.476 g in 100 ml). Flasks were cotton plugged and agitated in an incubation shaker for a time period of (2.62–7.38 days) at an agitation speed of (140.5–259.5) rpm and a temperature of 30 °C according to experimental design. The samples were filtered through 0.45 μm cellulose acetate membrane filter and analyzed for residual *o*-cresol concentration. The interactive effect of various operating parameters such as initial feed concentration, pH, biosorbent dose, equilibrium time, and agitation speed were studied.

2.3. Biosorptive foam separation experimentations

Biosorptive foam separation experiments were carried out in a cylindrical glass tube of inner diameter 2 cm with a top hemispherical bend to collect the foam. The Schematic representation of the entire process was shown in (Fig. 1). A known volume of *o*-cresol loaded biomass (21.6–38.4 cm) solution from stage I was taken into the foam column. Surfactant Cetyl Trimethyl Ammonium Bromide (0.132–0.468 w/v %) was added to the bulk solution. The air was then admitted into the feed solution at a controlled flow rate (1.16–2.84 lpm). After a time interval of 20 min, the residual pool liquid in the foam column was centrifuged and the supernatant was analyzed to determine the *o*-cresol concentration according to standard methods [15]. In the second stage, the interactive effects of liquid pool height, surfactant concentration, and air flow rate on percentage removal were studied in detail. Percentage removal was determined by Eq. (1)

$$\text{Removal of o} - \text{cresol (\%)} = \frac{C_o - C_f}{C_o} \times 100 \quad (1)$$

Where; C_o and C_f are the *o*-cresol concentration (mg/l) in the influent and effluent streams respectively.