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Bioremediation of chlorpyrifos contaminated soil by two phase bioslurry reactor: Processes evaluation and optimization by Taguchi's design of experimental (DOE) methodology

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ABSTRACT

Two phase bioreactor was constructed, designed and developed to evaluate the chlorpyrifos remediation. Six biotic and abiotic factors (substrate-loading rate, slurry phase pH, slurry phase dissolved oxygen (DO), soil water ratio, temperature and soil micro flora load) were evaluated by design of experimental (DOE) methodology employing Taguchi's orthogonal array (OA). The selected six factors were considered at two levels L-8 array (2ˆ7, 15 experiments) in the experimental design. The optimum operating conditions obtained from the methodology showed enhanced chlorpyrifos degradation from 283.86 µg/g to 955.364 µg/g by overall 70.34% of enhancement. In the present study, with the help of few well defined experimental parameters a mathematical model was constructed to understand the complex bioremediation process and optimize the approximate parameters upto great accuracy.

1. Introduction

Contamination through anthropogenic activities are now widely recognized a potential threat to environment, which has led to international efforts to restore contaminated soils and aquifers (Smith, 1988; Hrudey, 1993; Woo et al., 2001). Chlorpyrifos [O,O-diethyl O-(3,5,6-trichloro-2-pyridyl) phosphorothioate] an organophosphorus insecticides used world over to control a broad-spectrum of insects of economically important crops and also to control mosquitoes, flies, crop and household pests and aquatic larvae (Bicker et al., 2005; Goel et al., 2005). Chlorpyrifos and other insecticides such as diazinon, malathion has been reported to be potential chemical mutagens (Bolognesi and Morasso, 2000), which has contaminated a wide range of water and terrestrial ecosystems (Singh and Seth, 1989). Chlorpyrifos in soil is reported to have half life ranging from 60 and 120 days and in favorable condition upto 1 year (Howard, 1991) and thus calls for sustainable degradation/remediation. Current methods rely on chemical, physical (landfills) and incineration which are not that much effective as compared to bioremediation, which a cost effective and environmentally sound process is where microbes can degrade wide range of aliphatic, aromatic and heterocyclic chemicals.

Bioslurry phase reactor offers best way to remediate chlorpyrifos contaminated soil by utilizing the existing soil microbes, thus reducing the time for inoculums to acclimatize to the conditions. It is advantageous as there is consortia of different microbes already present in soil that utilizes the chlorpyrifos due to their varying metabolic pathways and also provide the ease of controlling different operational parameters such as mixing ratio (soil: water), pH, temperature (°C), nutrients and other specialized amendments such as surfactants (Troy, 1994; Venkata Mohan et al., 2004, 2006a, 2006b) to enhance degradation. However the problem arises with controlling the operating parameters such as oxygen requirement, oxygen transfer rate, soil water ratio (soil loading rate), physical and chemical properties of soil, reactor design and operating conditions, nature of inoculum, contaminant nature etc., may affect the bioslurry reactor performance. Further conventional optimization of parameters such as keeping one parameter variable and all other constant is much more time consuming and requires large datasets generated by series of experiments at large scale, is not feasible. Design of experiments (DOE) is one approach, which helps to gain information's about the optimized levels, by taking large number of variables at a given time (Taguchi, 1987).

Taguchi DOE is method is developed by Genichi Taguchi to improve the quality of manufactured goods and more recently it is been applied to engineering (Rosa et al., 2009), biotechnology (Rao et al., 2008, 2004), advertising and marketing (Selden, 1997) etc. This statistical methodology developed by Taguchi study the system by a set of independent variables (factors), which can be both controllable and uncontrollable (dynamic/noise), over a specific region of interest (levels)

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(Mitra, 2001). Taguchi's experimental design works by extracting useful information from main factors (variables) having considerable effect on design parameters, from minimum number of experiments. Further the conclusions drawn from the small-scale experiments are valid over the entire experimental region spanned by control factors and their setting levels (Phadke, 1989). It also helps to understand the influence of individual factors and establishes the relationship between factors and operational conditions and finally the performance at the optimum levels are obtained. The use of this methodology has been reported in myco-synthesis of nano-silver endophytic *Trichoderma harzianum* SYA.F4; Optimization of laccase production by *Pleurotus ostreatus;* processes optimization: Catalytic degradation of polypropylene, Optimization and evaluation of fermentative hydrogen production and wastewater treatment processes, process optimization for bioremediation of Hg (II) by *Pseudomonas aeruginosa,* Improving the surface roughness of lathe facing operation, optimization of physicochemical parameters for biodegradation of 4-chlorophenol by *Candida tropicalis* PHB5 and statistical optimization: Fermentation process parameters to improved bioethanol production by Shahira et al. (2017), Rathinasamy and Thayumanavan (2010), Achyut and Singh, (2013), Venkata Mohan et al. (2009); Tupe et al. (2006), Athreya and Venkatesh (2012), Basak et al. (2013) and Das et al. (2014) respectively.

The present study therefore, aims to investigate the methodological application of Taguchi OA experimental design (DOE) for optimizing the various process parameters and establishing the effect of different parameters with each other that are involved in the bioremediation of soil contaminated with chlorpyrifos in a two phase bioslurry reactor. Six abiotic and biotic factors viz., substrate concentration, slurry phase pH, slurry phase dissolved oxygen (DO), soil water ratio, operating temperature and soil microflora were considered in experimental design with an OA layout of L-8 array (2ˆ7).

2. Materials and methods

2.1. Taguchi's DOE methodology

The Taguchi's DOE methodology was used for designing the experiments and also quantifying the outcomes. The complete designed approach was broadly divided into four stages (with various steps) viz., designing, regulating, analysis, and validation as depicted in Fig. 1.

Each stage is interconnected to achieve the overall optimization process. In Taguchi method establishment of large number of experimental situations were used as OAs to enhance the efficiency and reproducibility of the laboratory experiments and reduce formidable errors. Taguchi loss function [L(y)] as deviation from the target value, as small deviation equals small loss, large deviation corresponds to large loss and no deviation equals no loss i.e., the working condition is optimized accordingly (Mitra, 2001), as represented by

$$L(y) = k(y - m)^2,$$

Where ‘k’ is proportionality constant, ‘m’ target value and ‘y’ is the experimental value obtained for each trial.

In case of ‘bigger is better’ quality characteristics depicted in Fig. 2, that are non-negative and have a ideal target value (i.e. larger values are better), the loss function modified as:

$$L(y) = k\frac{1}{y^2}$$

And, the expected loss function can be represented by

$$E[L(y)] = kE\left(\frac{1}{y^2}\right) = A\Delta^2 E\left(\frac{1}{y^2}\right)$$

where E $(1/y^2)$ can be estimated from a sample of ‘n’ items as

$$E\left(\frac{1}{y^2}\right) = \left(\sum_{i=1}^{n}\frac{1}{y_i^2}\right)/n$$

2.1.1. DOE methodology—designing (Stage I)

Six factors, which significantly influence the performance of the two phase bioslurry reactor were considered, viz., substrate-loading rate (SLR), slurry phase pH, slurry phase DO, soil water ratio, operating temperature and soil microflora (as CFU) (Table 1). These factors play an important role in determining the overall efficiency of the reactor, as variation in these factors has critical effect on the overall performance of the Bioslurry phase reactor operation and chlorpyrifos degradation efficiency. In present study, three levels of factor variation were considered and the size of the experiment was represented by symbolic arrays of experimental matrix [L8—15 experimental trails] with a layout of 2ˆ7. The total degree of freedom (DOF) for the studied experimental set is 14 (number of trails minus one). In the design OA, each column consisted of a number of conditions depending on the levels assigned to each factor. The diversity of factors was studied by crossing the OA of control factors as shown in Table 2.

2.1.2. Bioslurry phase biodegradation studies—regulating (stage II)

Bioremediation experiments were performed using two phase bioslurry reactor for chlorpyrifos degradation employing selected 15 experimental trails/ variations (Table 2) in combination of 6 factors at 2 levels (Table 1). Two phase slurry reactor (total volume—1.0 L; internal dia—5.3 and external dia- 5.7 cm; height—30.7 cm) was operated in sequencing batch mode under anoxic–aerobic–anoxic microenvironment with 96 h of total cycle period consisting of four phases (FILL—5.30 h; REACT—82.4 h; SETTLE—3 h; DECANT—5.30 h). The chlorpyrifos infused soil was mixed with tap water in the requisite soil water ratio (1:10, 1:20, 1:30) to prepare slurry. After loading the slurry was well mixed and aerated with sparger unit to get a homogenous mixture. To retain the microorganismic activity in log phase retarding the lag phase an inoculum of 10% of treated slurry was retained in the reactor in the DECANT phase at each cycle operation and the remaining 90% of treated soil was removed and filled with fresh untreated soil during the FILL phase. Common water based surfactant was used to avoid foam formation during the operation.

Chlorpyrifos supplies the necessary phosphorous and nitrogen source. Chlorpyrifos concentration was determined by gas chromatography (Shimadzu GC-2014 plus) using a FID detector (2 mm ID_120 cm long borosilicate glass column) by extraction into toluene. All the sets of experiments as per the assigned factors were conducted in a random fashion and the result obtained from each set of the 15 experiments is shown in Table 2, corresponding to chlorpyrifos degradation.

2.1.3. Data analysis (Stage III)

The data obtained from the biodegradation study was analyzed and processed using Qualitek-4 (Nutek Inc., Bloomfield Hills, MI) software to evaluate the multiple effects of selected factors and determination of optimum process parameters conditions for effective operation of two phase bioslurry reactor in treating chlorpyrifos contaminated soil and process performance at the obtained optimum conditions. The software uses L-4 to L-64 arrays along with a selection of 2–63 factors with two, three, and four levels to each factor. This software offers automatic design option to select the array used and assign factors to the appropriate columns. Software operation for optimization was performed at ‘bigger is better’ performance characteristics for all the cases. The data obtained using Qualitek-4 software was depicted in Tables 3–6.

2.1.4. Experimental validation (Stage IV)

Two phase bioslurry reactor experiments were validated and further studied by employing the established optimized process conditions