Journal of Environmental Management 197 (2017) 373–383

Contents lists available at ScienceDirect

Journal of Environmental Management

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

Research article

Efficacy of free and encapsulated *Bacillus lichenformis* strain SL10 on degradation of phenol: A comparative study of degradation kinetics

S. Chris Felshia [a], N. Aswin Karthick [a], R. Thilagam [a], A. Chandralekha [b], K.S.M.S. Raghavarao [b], A. Gnanamani [a, *]

[a] CSIR-Central Leather Research Institute, Adyar, Chennai, India
[b] CSIR-Central Food Technological Research Institute, Mysore, India

ARTICLE INFO

Article history:
Received 19 March 2016
Received in revised form
10 February 2017
Accepted 2 April 2017
Available online 11 April 2017

Keywords:
Phenol
Bacillus licheniformis
Encapsulation
Degradation
Catechol 2, 3- dioxygenase

ABSTRACT

The present study exemplifies phenol degradation efficacy of the free and encapsulated bacterial isolate, explored the degradation kinetics and storage stability in detail. In brief, isolation, identification and phenol degradation potential of the bacterial made from wastewater treated sludge samples. The organism identified as *B. licheniformis* demonstrates phenol degradation at a concentration more than 1500 ppm. Optimization of environmental parameters reduces the time taken for degradation considerably. The organism has further been encapsulated using whey protein and the efficacy of encapsulated species suggested that encapsulation protects the cells from high concentration of phenol and at the same time expedite the degradation of the chosen pollutant at appreciable level. The encapsulated species effectively degrade 3000 ppm concentration of phenol within 96 h of incubation. Both pH and temperature stability observed in the encapsulated species suggests the effectiveness of the encapsulation. The encapsulated cells displayed storage stability for a four week period at 4 C and reusability up to three exposures. Degradation effected through intracellular catechol 2,3 dioxygenase. In conclusion, encapsulation of *B. licheniformis* (i) protects the cells from direct exposure to toxic pollutants; (ii) facilitates the field scale application and (iii) eliminate the practical difficulties in handling wet biomass in field application and assures the best possible way of remediating the phenol contaminated soil.

1. Introduction

The research on microbial degradation of carcinogenic/hazardous/toxic compounds for the past few decades infer that microbes can potentially serve as remediation agent. However, the efficiency of microbes to degrade toxic substances depends upon their ability to acclimate the toxic substance and produce degrading enzymes. The tolerance of the organism to high concentrations of such toxic substance in turn affects its degradation efficacy. Of toxic substances released into the environment, the concentration of phenol has not been much concerned. However, phenol and its derivatives even at low concentrations has devastating toxic effect on aquatic species and has been reported to cause adverse health effects in humans (Bull, 1982; Gallard and von Gunten, 2002; Michałowicz and Duda, 2007).

Degradation of phenol has been reported by various microbial species in aqueous and soil phase, especially by bacterial species. Table 1 summarizes the list of organisms reported for the degradation of phenol and their phenol tolerance level. It could be inferred that the maximum tolerance limit was 1500 ppm of phenol reported in *Bacillus* sp. (Chris et al., 2014). However, the concentration of phenol in industrial wastes and in the sludge/soil/sediments is > 1500 ppm, which necessitates intensive research to have organisms with increased degradation efficacy and tolerance towards phenol or to increase the efficacy through alternative means (Kirk and Othmer, 1980; Kurane, 1997).

Immobilization/encapsulation of microbial species has been reported to improve the efficiency of degrading toxic ingredients by microbial species (Giraud et al., 2002). However, the major drawbacks in encapsulation reside with the selection of matrices (Sarma and Wright, 1997). The matrices available for encapsulation are reported with low storage stability, which makes great concern on cell viability and survival. This drastically affects the exploitation of encapsulated organisms at the field scale level.

Encapsulation of cells in whey protein enhances the cell survival and viability and has high storage stability (Doherty et al., 2010).

* Corresponding author. Microbiology Division, CSIR-CLRI, Adyar, Chennai, 20, Tamil Nadu, India.
E-mail address: gnanamani3@gmail.com (A. Gnanamani).

http://dx.doi.org/10.1016/j.jenvman.2017.04.005
0301-4797/

Table 1
Microbial isolates reported till date for the maximum concentration of phenol degradation.

Source	Strain	Phenol concentration	Reference
Fungi	*Candida tropicalis*	2000 mg/l	Yan et al., (2005)
Bacteria	*Pseudomonas putida*	100 mg/l	Monteiro et al., (2000)
	Bacillus sp.	1400 mg/l	Ajaz et al. (2004)
	Bacillus cereus	1000 mg/l	Banerjee and Ghoshal, (2010)
	Halomonas sp.	1100 mg/l	Haddadi and Shavandi, (2013)
	***Rhodococcus* sp.**	1500 mg/l	Arif et al., (2013)
	Stenotrophomonas sp.	200 mg/l	Gunasundari and Muthukumar, (2013)
	Acinetobacter sp.	600 mg/l	Paisio et al., (2013)
	Pseudomonas putida MTCC 1194	1000 mg/l	Kumar et al., (2013)
	Acinetobacter sp.	1500 mg/l	Adav et al. (2007)
	Bacillus brevis sp.	700 mg/l	Arutchelvan et al., (2006)
	Pseudomonas sp.	250 mg/l	Shah, (2014)
	Bacillus pumilus MCG 03	2000 mg/l	Chris et al. (2014)

However, the effect of encapsulation using whey protein on degradation efficiency of the organism has not explored well. Hence, an attempt was made to encapsulate the *Bacillus licheniformis* SL10 isolated from sludge samples in whey protein and evaluate the phenol degradation efficiency at higher concentrations of >1500 ppm. Further, compare the degradation efficacy of free and encapsulated cells on the rate of degradation, storage stability, enzyme production and inhibitory concentration levels.

2. Materials and methods

2.1. Chemicals

Whey protein was purchased from M/s. Pristine Organics Pvt Ltd, Bangalore. Growth media and minerals used in the preparation of minimal media were obtained from HiMedia Laboratories Pvt. Ltd. Mumbai. Compounds including phenol and catechol used in the present study were of HPLC grade and procured from Sigma Aldrich, Germany. Glasswares and materials were pre-sterilized at 121 °C for 15 min to avoid cross contamination.

2.2. Isolation and identification of organism

A bacterial strain was isolated from sludge samples of Common Effluent Treatment Plant (CETP), Pallavaram, Chennai, Tamil Nadu, India. Enrichment technique was followed for the isolation of bacterial species (Karigar et al., 2006). In brief, ten ml of the sludge sample was added to the mineral salt medium (MSM) containing (g/L) K_2HPO_4, 1.6; KH_2PO_4, 0.2; $(NH_4)_2SO_4$, 1.0; $MgSO_4 \cdot 7H_2O$, 0.2; NaCl, 0.1; $CaCl_2$. $2H_2O$, 0.02; $FeSO_4$. H_2O, 0.01; Na_2MoO_4. $2H_2O$, 0.05; $MnSO_4$. H_2O, 0.05; Na_2WO_4. $2H_2O$, 0.05; (pH 7.0) and 2000 ppm of phenol and incubated for 5 days at 30 °C. Samples were taken from the culture broth, serially diluted, spread plated on agar plates and incubated at 37 °C. Colonies grown after 1–2 days were sub-cultured to obtain pure cultures.

The bacterial strain exhibiting growth and degradation in the presence of phenol at concentration >1500 ppm was isolated and subjected to identification studies by biochemical assays, 16S rRNA sequencing and SEM analyses. Gram's staining followed by biochemical tests were performed using the standard Bacterial Identification Kit (KB 013, HiMedia). Genomic DNA was isolated from bacteria using Qiagen genomic DNA isolation kit (Zhu et al., 1993). It was amplified using the universal primers 8F:5′AGAGTTTG ATCCTGGCTCAG3′ and 492R:5′GGCTACCTTGTTACGACT 3′ as forward and reverse primers respectively (Turner et al., 1999). The PCR product was sequenced using Applied Biosystems Electropherogram (Model No: 3730xl/ABI3730XL-15104-208, Eurofins Genomics India Pvt Ltd., Banglore) and compared with GenBank databases.

2.3. Encapsulation of microorganisms

The microbial cell pellet obtained after 48 h of growth followed by centrifugation at 10000 RPM at 4 °C, was encapsulated using whey protein followed by spray drying as described below. Feed solution for spray drying was prepared by adding 5% (w/v) cell pellet along with 10% (w/v) of carrier material in 500 ml of double distilled water and the slurry was mixed thoroughly by using a table top with magnetic stirrer. Spray drying of the feed slurry was carried out in a pilot scale spray dryer (BE 1216, Bowen, UK; Diameter-0.76 m, height-0.72, cone height-0.74 m, 2-fluid nozzle type atomizer and 5 kg/h of water evaporation capacity). According to the cell powder yield and cell survival during the preliminary experiments, the operating conditions such as inlet air temperature (120 ± 1 °C), outlet air temperature (85 ± 1 °C), air flow rate (0.50 m^3/min), air pressure (2.5 psi), and feed flow rate (40 ± 1 ml/min) were suitably standardized. The yield of the spray dried powder was calculated as the ratio of the weight of the collected powder after spray drying to the total solid content in the feed as shown below:

$$\text{Dry cell powder yield } (\%,\ \text{w/w}) = \frac{\text{Weight of powder collected after spray drying}}{\text{Total solid content in the feed}} \times 100$$

The particle size of the powder was analyzed by using Microtrac Turbo trac dry powder dispersion system (BLUEWAVE, Pennsylvania, USA) attached with Particle Size Analyser (S3500 Series, Pennsylvania, USA). The analyser works on the principle of laser dispersion. For each measurement, 1 g of sample was loaded in the particle size analyser system. All the measurements were carried out in triplicates.

The dried microbial cell powder was then checked for cell survival by serially diluting and spread plate method. The microbial cell survival in the dried cell powder samples was calculated using the following equation (Reddy et al., 2009).