CHEMBIOEN-2020 Special Issue

J. Indian Chem. Soc.,
Vol. 97, March 2020, pp. 332-338

Biodegradation and kinetic analysis of phenol using low-density polyethylene immobilized *Bacillus flexus* GS1 IIT (BHU) in a packed bed bioreactor

Ganesh Swain, R. K. Sonwani, Pankaj Nagar, B. S. Giri, R. P. Jaiswal, R. S. Singh and B. N. Rai*

Department of Chemical Engineering and Technology, Indian Institute of Technology (BHU), Varanasi-221 005, Uttar Pradesh, India

E-mail: bnrai.che@itbhu.ac.in

Manuscript received online 07 December 2019, revised and accepted 03 January 2020

Phenol is considered as a precarious pollutant due to its carcinogenic, mutagenic nature even at low concentration. The present study emphasizes the degradation kinetics and behavior of a packed bed bioreactor (PBBR) for biodegradation of phenol using bacterial strain *Bacillus flexus* GS1 IIT (BHU). Low density polyethylene (LDPE) was used as packing material in a laboratory scale PBBR. Process variables such as pH, temperature were optimized and found to be 7.0 and 30°C, respectively. Phenol biodegradation efficiency in PBBR was analyzed at optimum condition by varying substrate concentration between 25–700 mg/L. At optimum conditions, the maximum removal of phenol was observed to be 99.45% at 300 mg/L of initial phenol concentration. The kinetic constants were calculated from Monod and Andrew-Haldane model and found to be μ_{max}: 0.215 h^{-1}; K_s: 80 mg/L and μ_{max}: 0.27 h^{-1}; K_s: 66 mg/L; K_i: 300 mg/L, respectively.

Keywords: PBBR, phenol, kinetic variables, *Bacillus flexus*, COD removal.

Introduction

Wastewater is now a primary concern to the environmentalists due to the scarcity of fresh water sources in India. The noxious organic wastes need to be removed from the wastewater discharged from the heavy metal, resin, oil, and petrochemical industries since it impacts toxic effects on both aquatic animals and human beings. Phenol, an aromatic compound found in the wastewater, has the potential to inhibit the growth, spawning, and metabolic activity of the aquatic animals. The various sources of phenol include coke oven industries, steel plants, dye manufacturing, pesticides[1–3]. Also, the acute exposure to phenolic compounds causes abnormality in the digestive tract, skin irritation, DNA mutation, damage of the internal organs[3]. So, the Central Pollution Control Board has restricted the limit of phenolic compounds discharged by the industries into inland surface water, and public sewers should be 1 and 5 mg/L, respectively[4].

Bioremediation gains more popularity due to its various advantages includes less sludge generation, low cost, high removal efficiency, complete mineralization of pollutants[1]. But, the degradation of phenol becomes a challenging task due to its toxicity and growth inhibition tendency for bacteria[1,2]. Therefore, to enhance biodegradability, robust and sustainable techniques should be developed.

Packed bed bioreactor (PBBR) has been proven to be low cost, easy to fabricate, operate at high organic loading rate, and can accumulate higher biomass within a specific time[1,5,6]. Further, different packing materials such as polyvinyl alcohol gel beads biochar, liapor clay, and gravels, are used in the bioreactors. Among these, low-density polyethylene (LDPE) has been used as packing material in PBBR for aromatic compound degradation. LDPE which is an inert and porous substance with an average density of 0.930 g/cm^3, stable below 90°C, and is generally used in packaging for electronic devices.

From the literature survey, it is found that no work has been reported on the biodegradation of phenol using *Bacillus flexus* GS1 IIT (BHU) immobilized on LDPE in PBBR. The main objective of this study is as follows: (1) to evaluate the efficacy of phenol degrading bacteria using Monod and Andrew-Haldane model, (2) operation of a PBBR at a wide range of phenol concentrations.

Experimental

Material and methods:

Chemicals and bacterial culture:

The chemicals used in the experiment were purchased from Sigma Aldrich and Himedia. The doubled distilled water was used for the preparation of mineral salt media (MSM). Phenol (99% purity) was purchased from Himedia, India. HPLC grade chemicals (water and methanol) were purchased from Thermo Fisher Scientific Pvt. Ltd., India. A desirable quantity of phenol was added to MSM for the preparation of wastewater. The composition of MSM was described as follows (g/L): $MgSO_4$ (0.01); KH_2PO_4 (0.5); K_2HPO_4 (0.8); $(NH_4)_2SO_4$ (0.5); $CaCl_2$ (0.02); NaCl (0.5); $CuSO_4$ (0.02); $MnSO_4$ (0.02); $FeSO_4$ (0.02); Na_2MoO_4 (0.02); H_3BO_3 (0.005). The pH of the salt media was adjusted to the desired value by adding 0.1 *N* NaOH or HCl.

The petroleum-contaminated soil samples were collected in a sterile steel container from IOCL Refinery, Mathura (UP), India and stored at 4°C for further use. 10 g soil sample was added in a conical flask (250 mL) containing 100 mL MSM and phenol (50 mg/L) to enrich the bacterial culture. This flask was incubated at 35°C and 120 rpm for seven days. The incubation period was repeated by transferring 10 mL aliquot sample from the previous batch to a 250 mL flask containing 100 mL MSM with phenol concentration (100 mg/L). Further, this process was repeated four times by gradually increasing the phenol concentration for the enrichment of bacteria culture in phenolic environments. The pure culture was obtained by serial dilution method. The obtained pure species were stored in nutrient broth at 4°C for further used in phenol biodegradation study.

Process optimization, PBBR set up and operation:

In this experiment, the process parameters like pH and temperature were optimized in the free cell system. 100 mL autoclaved MSM were taken in Erlenmeyer flasks containing phenol (150 mg/L) as sole carbon source. A wide range of pH (5–10) and temperature (25–40°C) was chosen for the optimization study. Aliquots were taken intermittently for the determination of substrate removal.

The PBBR was made up of borosilicate glass (60 cm in length and 5.5 cm in the internal diameter), having a working volume of 1 L (total volume of 1425 mL) (Fig. 1). For the biodegradation study, LDPE sheets were procured from a local market near IIT, BHU, Varanasi, India. The sheets were

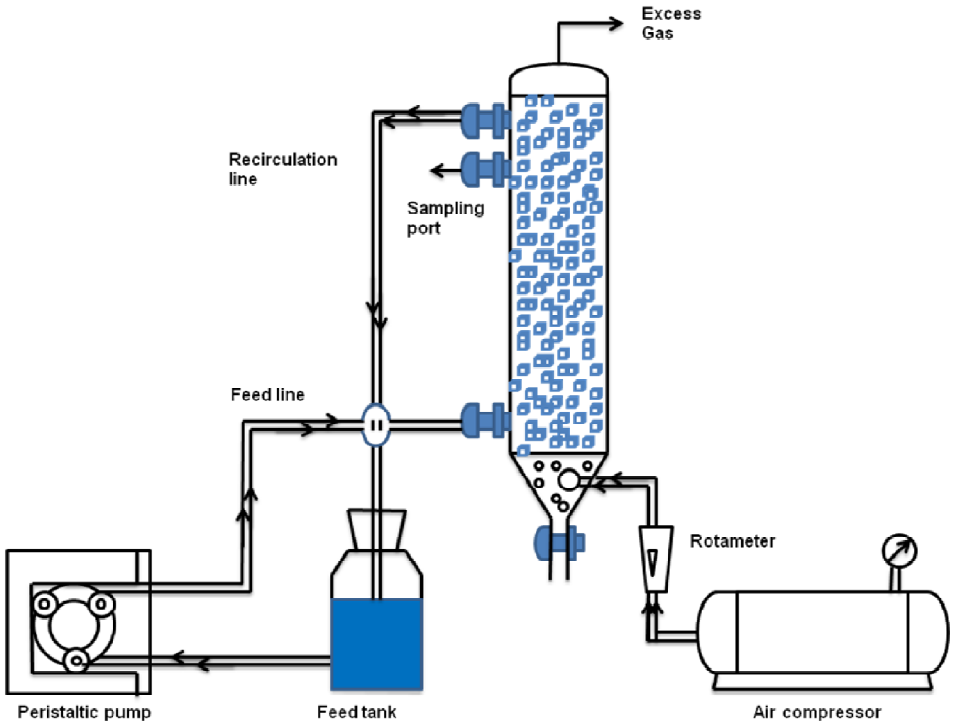

Fig. 1. Schematic representation of PBBR for phenol biodegradation.

cut into the cubical size of 1 cm and washed with 70% ethanol. Then the cubes were washed and dried in an oven at 40°C. The dried packing materials were stored at room temperature (30±3°C) for further immobilization purpose. The immobilization of *Bacillus flexus* species on LDPE was carried out, as reported by Sonwani *et al.*[6]. 6 g of LDPE cubes were packed in the PBBR. Then 1000 mL of sterilized MSM was recirculated inside the PBBR with addition of glucose (1 g/L), yeast extract (0.1 g/L), peptone (0.1 g/L), and bacterial inoculums (3% v/v). At the initial phase, the PBBR was operated in the presence of glucose (1 g/L) and bacterial inoculum for 15 days for the growth of *Bacillus flexus* on the porous LDPE. After the confirmation of biofilm by SEM analysis, the PBBR was fed with the phenol as an only carbon source at a feed rate of 10 mL/min. Then, the PBBR was operated at optimized conditions at a constant influent flow rate of 10 mL/min. The efficacy of PBBR was investigated by varying the initial phenol concentration from 25–700 mg/L. The air was supplied by a compressor at a flow rate of 0.3 LPM into the PBBR.

The liquid sample was collected from the system intermittently, centrifuged at 5000 rpm for 10 min and filtered through a 0.45 μm Whatman filter paper. The dried cell mass was determined by taking the weight difference of the filter paper after drying in an oven at 105°C for 24 h[7].

Growth kinetics study for phenol biodegradation:

To analyze the bacterial growth kinetics in phenol biodegradation, two models, such as Monod and Andrew-Haldane, were successfully used. The Monod equation can be expressed as eq. (1):

$$\mu = \frac{1}{x}\frac{dx}{dt} = \frac{\mu_{max} S}{K_s + S} \tag{1}$$

where, μ, x, μ_{max}, S and K_s represent specific growth rate (h^{-1}), biomass concentration (mg/L), maximum specific growth rate (h^{-1}), phenol concentration (mg/L), and half- saturation constant (mg/L), respectively. Again, the specific growth rate (μ) value can be found out by evaluating the slope of the curve plotted between logarithm value of biomass ratio and time duration as given by eq. (2) (Hussain *et al.*[4]):

$$\mu = \frac{\log\left(\frac{x_2}{x_1}\right)}{t_2 - t_1} \tag{2}$$

where, the terms x_1 and x_2 represent the value of cell concentration at time t_1 and t_2, respectively.

The microbial growth under inhibitory condition can be expressed by eq. (3) (Zhang *et al.*[7]):

$$\mu = \frac{\mu_{max} S}{K_s + S + \frac{S^2}{K_i}} \tag{3}$$

where, K_i refers to the inhibition constant (mg/L). The kinetic parameters involved in the Haldane model were obtained by a nonlinear least-squares regression method.

Analytical techniques:

Morphological characteristics and bacterial identification:

The morphological structure of biomass attached to LDPE was analyzed by scanning electron microscopy (SEM) analysis (EVO-SEM MA15/18).

The bacterial genomic DNA isolation and identification were carried out at Triyat Genomics, Nagpur, India, and a sequence was analyzed at the National Center for Biotechnology Information (NCBI). The 16S rRNA sequence of the specific microorganism was matched with an analogous rRNA sequence present in the database of NCBI. *Bacillus flexus* strain was the most matching species found from the Phylogenetic investigation using BLAST software. *Bacillus flexus* was most abundant species found having accession numbers MK850444.1. The revolutionary relationship of the potential species by the Neighbour-Joining technique is shown in Fig. 2. A similar kind of analysis for the *Bacillus flexus* strain was reported by other researchers[5,8] for the biodegradation of VOCs and pesticides.

Determination of phenol concentration:

The intermediate samples were collected, filtered through a 0.22 μm cellulose filter, and analyzed in HPLC for residual phenol concentration. The HPLC (UFLC Shimadzu, Japan) was associated with a C18 Shim-pack solar column (4.6 mm×250 mm; 5 μm particle size) and a PDA detector. The mobile phase was used a mixture of HPLC grade methanol and water (50:50; v/v), the flow rate of 0.8 mL/min, 20 μL of injection volume, and detector wavelength of 280 nm. The peak obtained for phenol at a retention time of 9.1 min. Then the phenol concentration was determined from the calibration curve obtained previously.

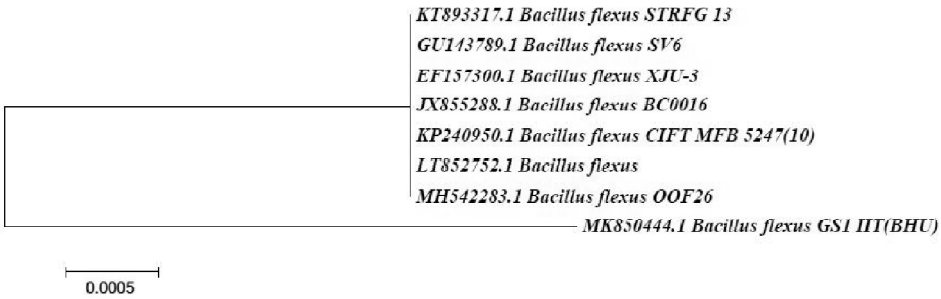

Fig. 2. Phylogenetic tree of isolated bacterial species *Bacillus flexus* GS1 IIT (BHU) (MK850444.1) for biodegradation of phenol.

Results and discussion

Process optimization in the batch process:

The biodegradation of phenol was carried out in 250 mL flask containing phenol (150 mg/L) along with MSM to evaluate the effect of pH and temperature. So, a wide range of pH (5–9) and temperature (25–40°C) were chosen for the optimization study. The microorganisms in the free suspension system are more sensitive towards pH and temperature change. Maximum phenol removal of 84.51% was obtained at a pH of 7.0, which was shown in Fig. 3(a). Then it decreased to 52.68% with an increase in pH up to 9.0. Biodegradation of phenol again decreased to 41.04% when the pH of the media decreased to 5.0. At the extreme condition of pH, the metabolic activity of the bacteria hindered, which results in depletion of the biodegradation of phenol[10]. The optimum removal rate of different organic pollutants at a pH of 7.0 was reported by various researchers[5,8] and reported to be 89%, and 81%, respectively. The effect of the temperature on the degradation of phenol was studied by varying temperatures 25–40°C at optimum pH conditions obtained from Fig. 3(a). During phenol biodegradation, temperature plays a crucial role as it directly impacts the enzymatic activity of the bacterial species. Significant phenol removal was observed at the temperature of 25°C and found to be 72.68%, as shown in Fig. 3(b). Optimum phenol removal was observed between the temperatures of 30–35°C and found to be 83.59%. It seems that the increase of temperature beyond 35°C may decrease the enzymatic activity during the phenol biodegradation, hence results in lower phenol removal efficiency. The optimum temperature for biodegradation of phenol by observed at 30°C was reported in the previous work[8] and found to be 92%.

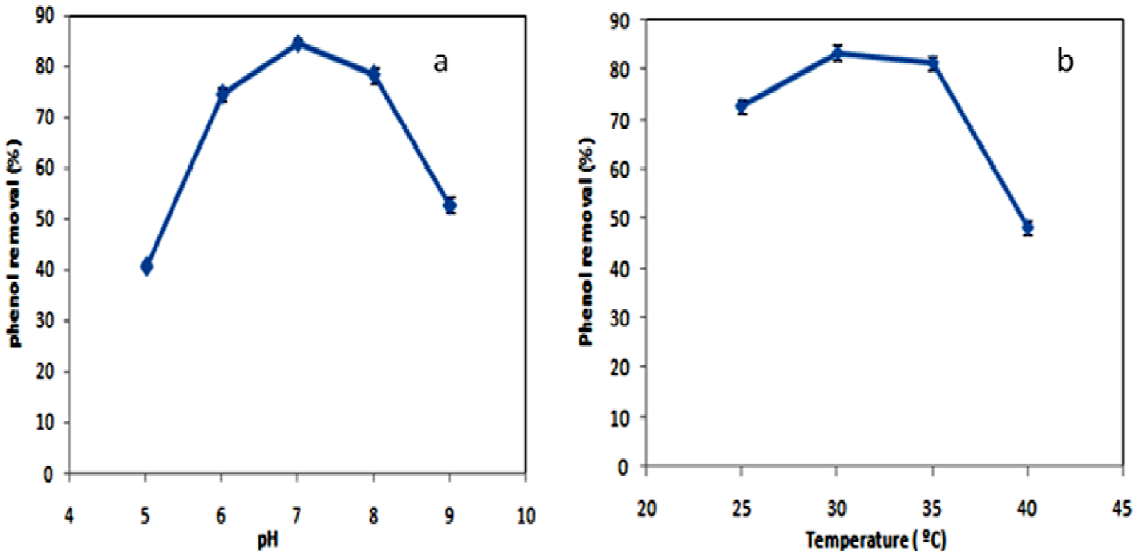

Fig. 3. Effect of (a) pH and (b) temperature on biodegradation of phenol.

Phenol biodegradation study in PBBR:

At the initial phase, the PBBR was operated at the batch mode in the presence of glucose (0.5 g/L) and bacterial inoculum for 15 days for the growth of *Bacillus flexus* on the porous LDPE. After the confirmation of biofilm by SEM analysis, the PBBR was fed with the phenol as an only carbon source at a feed rate of 10 mL/min. The phenol biodegradation study was carried out in PBBR by taking phenol concentrations of 25, 50, 100, 300, 500 and 700 mg/L, respectively. Intermediate samples were taken from the reactor outlet to determine phenol concentration of the effluent.

More than 85% of phenol was eliminated within 6 h of operation when initial phenol dosing was 50 mg/L. The time taken for complete removal of phenol was increasing with the increase of phenol dosing. Complete biodegradation of phenol resulted in 10, 18, and 27 h of operation at substrate concentrations of 100, 300, and 500 mg/L, respectively. More than 98% of phenol was completely mineralised due to the presence of high fixed biomass in immobilized LDPE. From the literature survey, it was found that the degradation efficiency of phenol by the immobilized cells is quite greater than the free cell[3,10]. Mohanty *et al.*[3] have investigated the efficacy of phenol removal in free cell study and reported that wastewater containing 500 mg/L of phenol was mineralized within 84 h. This is due to the fixed biomass attached to the packing support, which was capable of degrading each concentration of phenol within a specific time period[2]. Fig. 4 shows that almost more than 90% biodegradation of phenol was observed at concentrations of 700 mg/L at a reaction time of 43 h. Banerjee *et al.*[2] have reported that 500, and 700 mg/L of phenol was mineralized in PBBR using *Bacillus cerus* in 60, and 100 h of recirculation time, respectively. The time taken for the complete removal of phenol at the initial phenol concentration of 700 mg/L was quite less in our study compared to the above. This was may be due to the less availability of the substrate to the bio cells present inside the packing material because of the high mass transfer resistance offered by the polymer matrix[1,11]. Moreover, different packing support made up of gel matrix such as polyvinyl alcohol (PVA), calcium alginate, chitosan, and agar offers less permeability of phenol into the solid matrix due to cell entrapment[1]. Basak *et al.*[1] have reported that the high porous packing support (sugar cane bagasse) immobilized *Candida tropicalis* PHB5 can degrade phenol of concentration of 800 mg/L within 54 h of operation. Low-density polyethylene widely used for the packaging of various electronic devices, shopping bags, trays, and it is disposed of after one-time use, which ultimately leads to environmental pollution. Therefore, the use of LDPE immobilized *Bacillus flexus* as packing material for the biodegradation of phenol could be a better option to the environment as well as for bioremediation of toxic compounds[6].

Monod and Andrew-Haldane kinetics for phenol biodegradation:

To evaluate the efficacy of phenol degrading bacteria, two kinetic models, i.e. Monod and Andrew-Haldane, were used under non-inhibition conditions. The specific growth rate (μ) of the microorganisms was calculated at different sub-

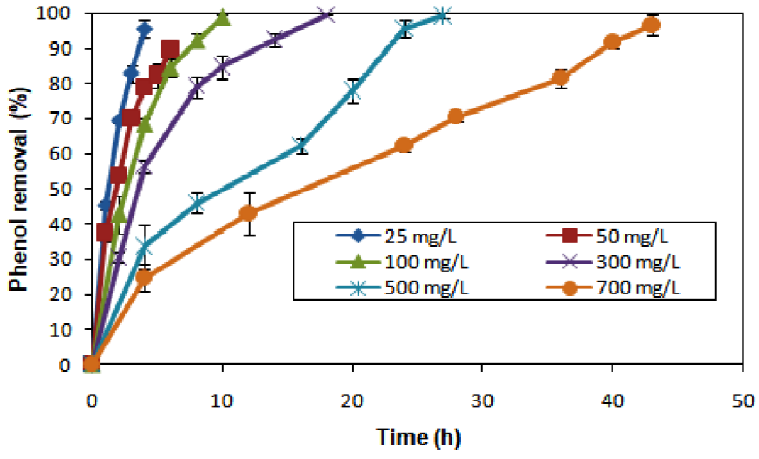

Fig. 4. Effect of phenol concentration on removal efficiency; Q = 10 mL/min, pH = 7.0, temperature = 30°C.

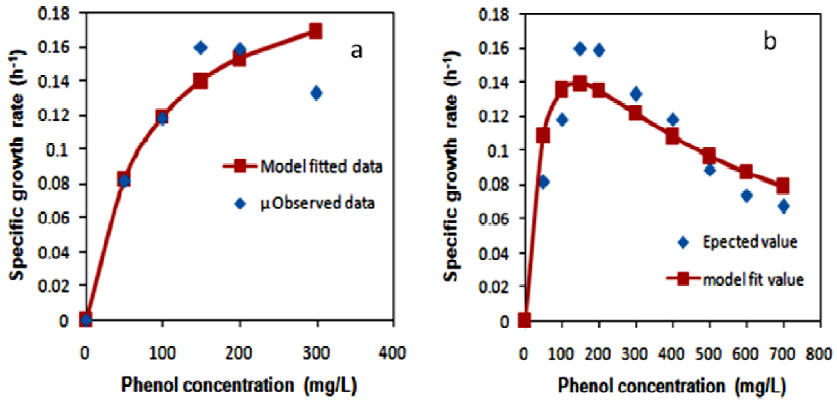

Fig. 5. Graph plotted between specific growth rates against phenol concentrations using (a) Monod model and (b) Andrew-Haldane model.

strate concentrations of range 0–700 mg/L and plotted against phenol concentration, as shown in Fig. 5. It seems that due to the toxic effect of phenol, the specific growth rate of *Bacillus flexus* increased up to 200 mg/L, and after that, it decreased continuously. At the substrate non-inhibition condition, Monod kinetics well described the efficacy of the microorganisms. The kinetic parameters i.e. μ_{max}, K_s, and $\frac{\mu_{max}}{K_s}$ were calculated and obtained to be 0.214 h^{-1}, 80 mg/L, and 0.00267 L mg^{-1} h^{-1}, respectively. For the biochemical reaction, high μ_{max} and low K_i values were always preferred, and the value of $\frac{\mu_{max}}{K_s}$ was taken to be an important parameter in evaluating the potential of substrate degradation capacity[5].

The Andrew-Haldane kinetic parameters, i.e. μ_{max}, K_s, and K_i, were calculated by non-linear regression analysis and found to be 0.270 h^{-1}, 66 mg/L, and 300 mg/L, respectively. The kinetic constant $\frac{\mu_{max}}{K_s}$ was calculated and found to be 0.004091 L mg^{-1} h^{-1}. The calculated values of kinetic parameters were shown in Table 1. The present study shows a high value of specific growth rate, as well as substrate inhibition constant compared to the previous studies[12] was may be due to the increase in acclimatization of the time period.

Table 1. Monod and Andrew-Haldane kinetic parameters for biodegradation of phenol

Phenol concentration (mg/L)	μ_{max} (h^{-1})	K_s (mg/L)	K_i (mg/L)	μ_{max}/K_s (L mg^{-1} h^{-1})	R^2
0–300 (Monod)	0.215	80	–	0.0027	0.96
0–700 (Andrew-Haldane)	0.27	66	300	0.0041	0.98

Conclusions

In this work, the PBBR was operated at optimized conditions with varying phenol concentrations from 25–700 mg/L. The phenol removal efficiency obtained from the research work states that the current PBBR can be able to eliminate phenol at high organic loading conditions. Also, the efficacy of bacterial species was studied using Monod and Haldane model. The present study demonstrates that high phenol removal efficiency can be achieved by using LDPE immobilized *Bacillus flexus* GS1 IIT (BHU) in a PBBR.

Acknowledgements

The author (GS) is gratefully acknowledged Ministry of Human Resource of Development, MHRD (India) for granting financial support and Indian Institute of Technology, BHU, Varanasi, India for providing laboratory facility to carry out the research work.

References

1. B. Basak, B. H. Jeon, M. B. Kurade, G. D. Saratale, B. Bhunia, P. K. Chatterjee and A. Dey, *Ecotoxicol. Environ. Saf.,* 2019, **180**, 317.
2. A. Banerjee and A. K. Ghoshal, *J. Environ. Chem. Eng.,* 2016, **4**, 1523.
3. S. S. Mohanty and H. M. Jena, *Brazilian J. Chem. Eng.,* 2017, **34**, 75.
4. A. Hussain, S. K. Dubey and K. Vinay, *Water Resour. Ind.*, 2015, **8**, 55.

5. S. R. Geed, M. K. Kureel, B. S. Giri, R. S. Singh and B. N. Rai, *Bioresour Technol*, 2017, **227**, 56.
6. R. K. Sonwani, B. S. Giri, T. Das, R. S. Singh and B. N. Rai, *Process Biochem.,* 2019, **8**, 55.
7. Y. Zhang, D. Lu, T. Ju, L. Wang, S. Lin, Y. Zhao, C. Wang, H. He and Y. Du, *Int. J. Electrochem. Sci*., 2013, **8**, 504.
8. M. K. Kureel, S. R. Geed, B. N. Rai and R. S. Singh, *Bioresour. Technol*., 2018, **8**, 55.
9. A. Banerjee and A. K. Ghoshal, *Int. Biodeterio. Biodegr*., 2011, **65**, 1052.
10. Z. Liu, W. Xie, D. Li, Y. Peng, Z. Li and S. Liu, *Int. J. Environ. Res. Public Health*, 2016, **115**, 74.
11. M. B. Kurade, T. R. Waghmode, J. Q. Xiong, S. P. Govindwar and B. H. Jeon, *J. Clean. Prod.,* 2019, **213**, 884.
12. A. Nuhoglu and B. Yalcin, *Process Biochem*., 2005, **40**, 1233.