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Biodegradation of *p*-Chloro Meta Xylenol (PCMX) and modelling of degradation kinetic analysis using *Pandoraea sp.*

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p-Chloro Meta Xylenol (PCMX) is resistant to biodegradation and may cause health hazards. Removal of this toxic material from waste waters is required to protect the environment and human health. Two phenol degrading bacteria e.g. BT102 and BT201 having accession numbers JQ80423 and JQ595990 respectively were isolated and used for conducting bioremediation study of PCMX. These bacterial strains could resist high concentration of PCMX (1566 mg L^{-1}). Biomass was harvested from the growth medium and freeze dried followed by encapsulation in biocompatible calcium alginate beads. These beads were used for bioremediation of PCMX in spiked water samples containing 10 mg L^{-1} to 100 mg L^{-1} of PCMX. A removal efficiency of 99% was achieved in this process. Degradation kinetics of PCMX was studied with initial PCMX concentration ranging from 1 mg L^{-1} to 100 mg L^{-1}. The degradation kinetics followed Haldane's model showing higher substrate affinity constant value of 0.723 mg L^{-1} with R^2 = 0.9508. The bacterial species (BT102 and BT201) proved to be useful in remediating PCMX from water when used in alginate beads and the degradation rate could be predicted by a simple kinetic model.

Keywords: PCMX, biodegradation, alginate encapsulation, degradation kinetics.

Introduction

Compounds containing phenolic functional groups are highly toxic and carcinogenic for humans[1]. Para Chloro Meta Xylenol (PCMX) or 4-chloro-3,5-dimethylphenol, is a halogenated phenolic compound and supposed to be the oldest antimicrobial worldwide[2]. It is utilized as a major constituent of some disinfectant formulations like hand cleaners viz. Dettol (4.8% w/v), surgical cleanser, pre-operative skin sanitizing composition, and restorative topical products and is likewise employed as a topical and urinary antiseptic, preservative in various pharmaceutical products[3–6]. Though it is useful to mankind, the same when present in waste water, manufacturing unit effluent poses threat to useful microbes by causing spillage of cell components[7]. Due to the poor biodegradability and high lethality, phenolic compounds are highly toxic and its presence in drinking water and irrigation water causes serious health hazards to living beings and microorganisms[8]. As per the notification updated on 26th March 2019 given by Ministry of Environment, Forest and Climate change under Central Pollution Control Board (CPCB), India[9], the limiting value for phenolic compounds in inland surface water is 1 mg L^{-1} and on land for irrigation is 1 mg L^{-1}. The discharge of phenolic compounds into the environment from factory-made sources is of extreme concern and is highly poisonous to fish[10] even at moderately low level from 5–25 mg L^{-1}. It has been reported that the phenol released in wastewater by various industrial processes is within 13–88 mg L^{-1} for petroleum refinery, 180 mg L^{-1} for steel industry and 70 mg L^{-1} for resin industry[11]. The water purity standard, however, should be less than 1 μg L^{-1} in surface water as per Environmental Protection Agency (EPA)[12]. Several case studies have been reported regarding probable routes of human exposure as well as human health effects because of PCMX in U.S. National Library of Medicine recorded on Toxicology Data Network[13].

Thus, it is very important to remove PCMX from waste water, and also from drinking water. One of the best methods could be biodegradation process which itself is an eco-friendly as well as cost-effective process and the process can achieve complete removal of phenol. Extensive studies

have been carried out to evaluate the potential of phenol biodegradation by microbes isolated from the environment[14]. Biological conversion of the harmful compounds will help in establishing techniques to limit their presence in the environment[15]. Several processes like thermal decomposition, adsorption, chemical coagulation, ion exchange etc. have been used for removal of phenolic compounds but these techniques are associated with high operational costs, formation of hazardous by-products and poor removal at high concentration range[16]. Several reports are available on adsorption of phenolic compounds such as chlorophenols using nanocomposites[17–22] but only a few used bacteria. The biodegradation process using selected bacteria in the present study could show a pathway for remediation of even low concentration of PCMX in waste water. Since PCMX is a source of carbon and nitrogen, micro-organisms can uptake these sources for their growth and hence there is a possibility of full degradation of PCMX. First objective of the present study was to check the bioremediation potential of BT102, Gram-negative, round shaped *Pandoraea sp.* (accession No. JQ80423) and BT201, Gram-negative, round shaped *Delftia sp.* (accession No. JQ595990) and to observe the uptake of PCMX from wastewater. The second aim of this study was to investigate the degradation kinetics of PCMX.

Experimental

Materials: All chemicals were of analytical grade and used as received.

Beef extract, sodium chloride, agar, ammonium chloride, 4-aminoantipyrene, calcium chloride and sodium hydroxide were procured from Merck. PCMX was obtained from Sigma Aldrich (Germany). Sodium alginate and peptone were purchased from Loba Chemie, Mumbai, India. Potassium ferricyanide was obtained from Central Drug House, New Delhi, India and Dettol was purchased from Reckitt Benckiser, Mysore, India. Previously isolated microbes in the laboratory viz. BT102, *Pandoraea sp.* (accession No. JQ80423) and BT201, *Delftia sp.* (accession No. JQ595990) have been used for the biodegradation study of PCMX. The culture medium was prepared using deionized water (18.2 MΩ) (Sartorius) and sterilized by autoclaving at 121°C, under 105 K Pa pressure for 15 min.

Composition of nutrient media:

The nutrient medium for maintenance and growth of the bacteria was prepared using peptone (5 g L^{-1}), beef extract (3 g L^{-1}) and sodium chloride (2 g L^{-1}) and maintained at pH 7 at room temperature and then autoclaved and cooled down for use. In the bacterial growth experiment, 2% (v/v) bacterial inoculum of the main culture was poured into 100 ml nutrient broth under biosafety cabinet and incubated in a shaker incubator for 24 h at 37°C at 120 rpm. A PCMX induced inoculum for each experiment was also prepared. Plate cultures were also completed to preserve the bacteria. In the case of plate culture, agar (18 g L^{-1}) was added to the nutrient broth at pH 7.

Analytical methods: Standard curve for PCMX:

The concentration of PCMX was determined by the 4-aminoantipyrene (AAP) assay[23]. In this method, 5 ml of filtrate containing PCMX was made up to 100 ml with ultrapure water in a volumetric flask. This 100 ml diluted sample was transferred into a beaker and 2 ml ammonium chloride (50 mM) was added followed by pH adjustment to 10±0.2. To this, 2 ml of each of 4-aminoantipyrene (50 mM) and potassium ferricyanide (50 mM) were added. The intensity of the developed colour was measured by spectrophotometer at 510 nm (Fig. 1).

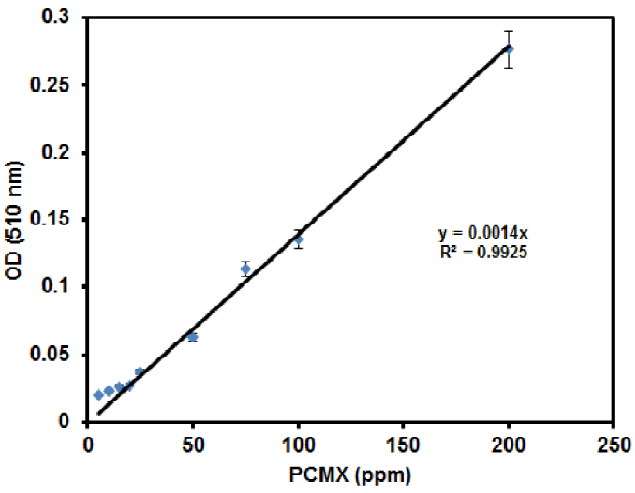

Fig. 1. Standard curve for PCMX at 510 nm using 4 AAP assay.

Preparation of calcium alginate beads with whole cell bacteria:

BT102 and BT201 bacteria were previously isolated from a crude source and were characterized and identified[24]. The present batch study of bioremediation was carried out using

whole cells of BT102 and BT201 strains. These strains were immobilized in sodium alginate beads. The biomass of the strains was collected after 24 h incubation in the broth and preserved at –20°C in 0.89% NaCl for use in bioremediation process. Before using the bacterial mass, it was washed twice with ultra-pure water (18.2 mW) to remove NaCl followed by centrifugation at 120 rpm at a temperature of 27°C. The collected mass (4%, w/v) was then mixed with sodium alginate solution (3% w/v). The same was dispensed drop wise to 2% (w/v) $CaCl_2$ solution and was continuously stirred (250 rpm)[25]. Beads thus produced were kept in the saturated $CaCl_2$ solution overnight and were washed three times with reverse osmosis (RO) water and excess water was removed using filter paper. These modified alginate beads of both strains were used to remediate PCMX in water.

Bacterial growth pattern in presence of PCMX and determination of MIC of isolates:

The phenol degrading bacteria, previously isolated in the lab viz. BT102 (JQ80423), BTUA (GU265556), BT201 (JQ595990) and BT302 (JQ782891) were used to study bacterial growth in presence of PCMX. Five (5) ml of PCMX in the concentration range of 1 to 10 mM in nutrient broth was poured in each of 15 ml test tubes and 100 µL of each bacterial culture was added inside the biosafety cabinet. The samples were then incubated for 24 h at 37°C in a shaker incubator at 110 rpm. The growth of the bacteria was monitored by measuring optical density at 600 nm at regular intervals.

Biodegradation kinetics of PCMX:

To study the degradation kinetics of PCMX in BT102, experiments were carried out in batch mode and the effect of PCMX for different concentrations (1 mg L^{-1}, 3 mg L^{-1}, 5 mg L^{-1}, 10 mg L^{-1}, 15 mg L^{-1}, 20 mg L^{-1}, 25 mg L^{-1}, 50 mg L^{-1} and 100 mg L^{-1}) and the growth of BT102 strain was investigated. Inoculum of 1 ml fresh culture of BT102 strain was added to the nutrient broth of 50 ml in each of 250 ml Erlenmeyer flasks containing the different strength of PCMX as indicated above. The samples were incubated in a shaker for 24 h at 37°C and 110 rpm. The growth of BT102 strain was monitored at regular intervals by measuring the absorbance using a UV-Vis spectrophotometer (Make: Perkin-Elmer, Model: Precisely Lambda 25 UV/Visible) for each concentration of PCMX at 600 nm (OD_{600}).

The experimental data of optical density with time were fitted in models viz. Monod and Haldane[26]. In a microbial mechanism, the growth rate of cell stops as hindrance may happen when the substrate or the product will inhibit the development of the microorganisms[26]. Carbon or nitrogen source is often considered as the growth limiting substrate[27,28]. PCMX in the media was treated as the source of carbon and nitrogen.

Specific degradation rate µ, is defined as the degradation in PCMX per unit time. The eq. (1) below defines the specific degradation rate, µ.

$$\mu = \frac{\ln (N_t/N_f)}{t} \quad (1)$$

where, $N_i = N_0$ = initial PCMX concentration (g L^{-1}) and $N_f = N_1$ = final PCMX concentration (g L^{-1}).

Thus, a plot between $\ln (N_0/N_1)$ versus t would give the specific degradation rate, µ.

Monod model: Monod model is the most principal model of growth kinetics. The Monod equation is as follows:

$$\mu = \frac{\mu_m S}{K_s + S} \quad (2)$$

where, µ = specific degradation rate, μ_m = maximum specific degradation rate, S = substrate concentration, K_s = substrate saturation constant (i.e. substrate concentration at half μ_m). At higher substrate concentrations, this model is not suitable in case of substrate inhibition. So, in order to overcome this disadvantage, Haldane model was used since it considers inhibition into account. The experimental data obtained from batch degradation experiments were fitted into Haldane Kinetics Model[29,30].

The Haldane model is as follows:

$$\mu = \frac{\mu_m S_o}{K_s + S_o + S_o^2/K_i} \quad (3)$$

where, µ is the degradation rate (/h), μ_m or μ_{max} is the maximum degradation rate (/h), S_o = initial substrate concentration (mg L^{-1}), K_s = substrate-affinity constant (mg L^{-1}), K_i = substrate-inhibition constant (mg L^{-1}).

From the plot of $\ln (S_o/S)$ vs time, the degradation rate (µ) was determined as the slope of the curve for each initial substrate concentration. The values of kinetic parameters, μ_m, K_s and K_i were obtained from the nonlinear regression

analysis using Matlab 7.12.0.635, R2011a software (Mathworks, Natick, MA, USA). A higher K_i value indicated that the strain was less sensitive to substrate inhibition. Degradation experiments for PCMX were carried out with the most effective strain BT102 for nine different initial substrate concentrations ranging from 1 mg L^{-1} to 100 mg L^{-1}.

Characterization:

SEM analysis:

The morphological change of bacteria encapsulated calcium alginate beads in absence of PCMX as well as in presence of PCMX was analyzed and observed with the help of Scanning Electron Microscopy (FEI company quanta 200). The image of calcium alginate bead was also considered for comparison.

TEM analysis:

To observe the bioaccumulation, surface morphologies of bacteria in absence of PCMX and bacteria in presence of 10 mg L^{-1} PCMX were characterized by transmission electron microscopy (TEM) (Tecnai G2 Spirit BioTWIN, FEI Company, USA) in a scale bar of 200 nm.

Results and discussion

Analysis of bacterial growth pattern in presence of PCMX and determination of MIC of isolates:

The growth of bacteria in PCMX after 24 h is presented in Fig. 2. Both BT102 and BT201 bacteria exhibited a very high growth in the presence of PCMX. Hence, both these strains were considered for further study. To measure the maximum level of PCMX tolerance, minimum inhibitory concentrations (MIC) of BT102 and BT201 isolates were determined by spreading bacterial strain in agar plates in presence of 1 mM to 25 mM PCMX. In Fig. 3(a), it could be seen that BT102 strain could withstand up to 10 mM of PCMX since no inhibition zones could be seen at 10 mM concentration whereas in Fig. 3(b), inhibition zones could be observed in presence of 15 mM, 20 mM and 25 mM of PCMX. In Fig. 4(a) and (b) on the other hand, it is demonstrated that BT201 strain could withstand up to 20 mM of PCMX and no inhibition zones were observed below this concentration.

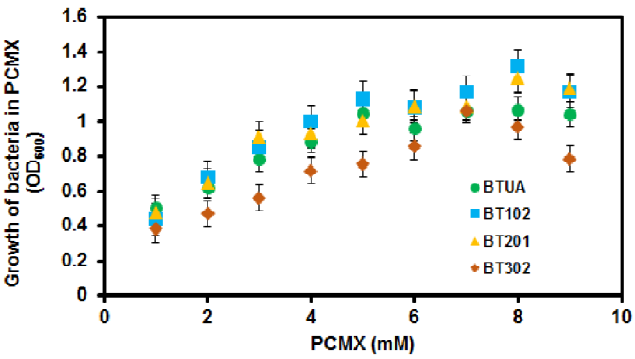

Fig. 2. Bacterial growth after 24 h in PCMX at 600 nm.

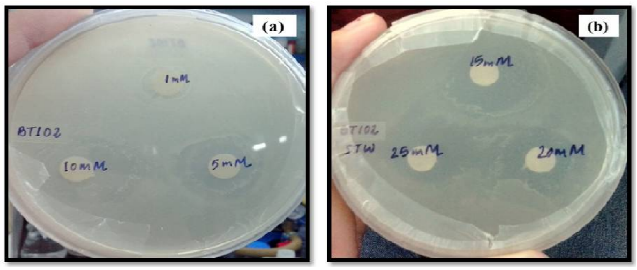

Fig. 3. MIC of BT102 strain (a) Growth of BT102 strain in 1 mM, 5 mM and 10 mM PCMX concentration and (b) Growth of BT102 strain in 15 mM, 20 mM and 25 mM of PCMX concentration.

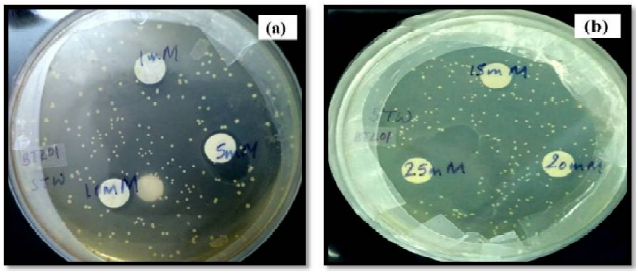

Fig. 4. MIC of BT201 strain (a) Growth of BT201 strain in 1 mM, 5 mM and 10 mM PCMX concentration and (b) Growth of BT201 strain in 15 mM, 20 mM and 25 mM of PCMX concentration.

Bioremediation studies of PCMX using bacteria BT102 and BT201:

The bioremediation of PCMX was very effective in bringing down the concentration to μg L^{-1} (ppb) level from a feed as high as 100 mg L^{-1} by the strains i.e. BT102 and BT201. From Fig. 5 (a) and (b), the bioremediation capacity could be seen for both the strains (BT102 and BT201) at 510 nm with respect to time. It could be observed that by increasing the initial PCMX concentration from 5 mg L^{-1} onwards, there was increase in the lag phase of bacterial growth which implied that the substrate inhibition at high initial substrate concentration of PCMX took place.

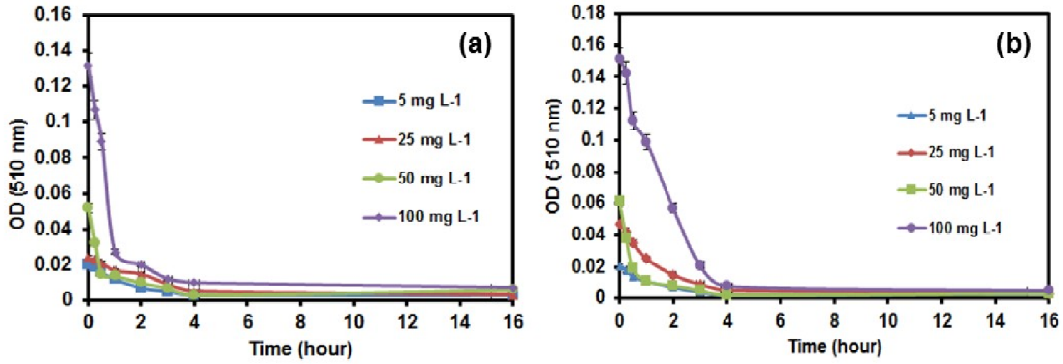

Fig. 5. Bioremediation of PCMX (a) with BT102 strain encapsulated in calcium alginate beads and (b) with BT201 strain encapsulated in calcium alginate beads.

For both the strains i.e. BT102 and BT201, there was no lag phase for initial 5 mg L^{-1} PCMX concentration and the bacteria degraded PCMX up to (85%) within first 4 h of incubation [Fig. 5(a) and (b)], after which rate of removal became comparatively slower up to 99% at 16 h. Thus, it could be concluded that both the strains had quite high bioremediation potential showing effective uptake of substrate rate (mg L^{-1} h^{-1}). It might be noted that no report in literature could show such a high uptake of PCMX using microbes within such a short span of time.

PCMX degradation kinetics and modelling:

Degradation kinetics:

In Fig. 6, the growth curve of BT102 and simultaneous uptake of 50 mg L^{-1} PCMX is shown. With the increase in the growth of BT102 strain, PCMX concentration decreased showing its depletion. The samples collected were used to measure PCMX by 4 AAP assay to monitor its degradation. The degradation efficiency (%) in Table 1 was calculated by the following[31]:

$$\text{Removal (\%)} = (1 - C/C_0)\times 100\% \quad (4)$$

where C_0 is the initial concentration of PCMX (g L^{-1}) and C is the PCMX concentration after 4 h of remediation.

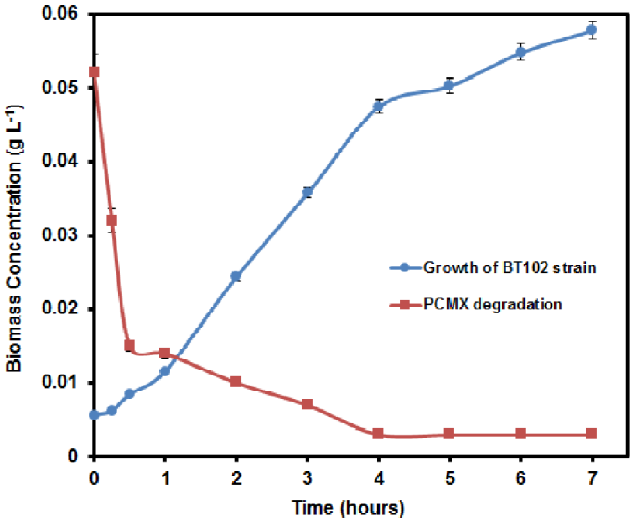

Fig. 6. Growth curve of BT102 and simultaneous uptake of 50 mg L^{-1} PCMX.

Table 1. Degradation efficiency chart for various concentrations of PCMX

PCMX concentration (mg L^{-1})	Degradation efficiency (%)
1	62.5
3	70
5	85
10	86.363
20	88.888
50	95.161
100	92.424

The results obtained from the experiments were used for degradation kinetics study using various non-inhibitory as well as substrate inhibitory models. To evaluate the degradation kinetics, ln (N_i/N_f) vs time was plotted for each of the initial concentrations of PCMX (viz. 1 mg L^{-1} to 100 mg L^{-1}) from which specific degradation rate, μ_{exp} was obtained. The experimental values for each of the initial concentrations of PCMX for specific degradation rate, μ_{exp} and R^2 are shown in Table 2. It can be observed from Table 2 that, with increase in PCMX concentration from 1 mg L^{-1} to 100 mg L^{-1}, the degradation rate gradually decreased. Within a very short

Table 2. The experimental value for each of the initial concentrations of PCMX for specific degradation rate, μ_{exp}, and R^2

Concentration of PCMX (mg L^{-1})	Specific degradation rate, μ_{exp} (h^{-1})	R^2
1	0.5936	0.9851
3	0.6281	0.9953
5	0.6117	0.9564
10	0.5317	0.9812
20	0.4902	0.9904
50	0.3839	0.9445
100	0.381	0.92

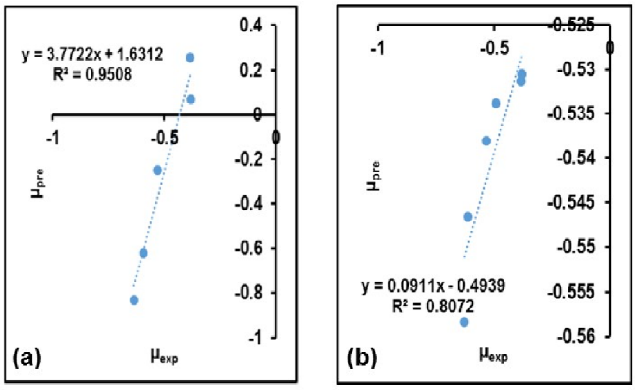

Fig.7. (a) Plot for μ_{exp} vs μ_{pre} using Haldane model and (b) Plot for μ_{exp} vs μ_{pre} using Monod model.

lag period (4–5 h), BT102 strain started degrading PCMX which showcased an effective as well as speedy degrading process. The results were fitted using Haldane and Monod models to predict the specific degradation rates after the calculations were obtained by non-linear regression analysis in Matlab. The predicted specific degradation rates obtained by Haldane and Monod models were compared with experimental values as shown in Fig. 7(a) and (b).

The results of rate constants and correlation efficiencies by eq. (2) and eq. (3) are summarized in Table 3. From this table, it is observed that Haldane model was best fitted as compared to Monod model. The substrate inhibition constant, K_i was found to be 31.735. The value of highest substrate affinity constant, K_s in Haldane model was found to be higher (0.723 mg L^{-1}) than the Monod model (0.154 mg L^{-1}) which confirmed a prominent biodegradation potential of PCMX by BT102 strain. The value of correlation coefficient (0.950) for the Haldane model as compared to that of Monod (0.807) clearly shows that the degradation kinetics followed Haldane model which is a substrate inhibitory one.

SEM analysis of sodium alginate beads with and without bacteria:

Fig. 8(a) gives the scanning electron microscopic image

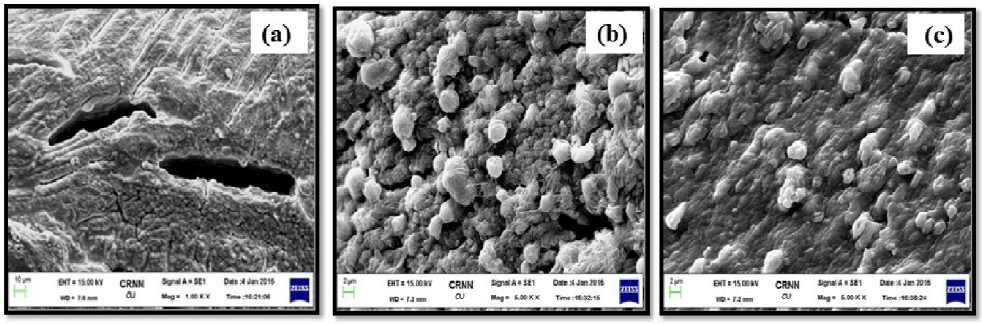

Fig. 8. (a) Calcium alginate bead, (b) calcium alginate bead encapsulated with BT102 bacteria (unused) and (c) calcium alginate bead encapsulated with BT102 bacteria (used).

Table 3. Degradation kinetic parameter values of Monod and Haldane model for biodegradation of PCMX using BT102 strain

Substrate	Monod's model			Haldane's model			
	μ_{max} (h^{-1})	K_s (mg L^{-1})	Correlation coefficient, R^2	μ_{max} (h^{-1})	K_s	K_i (mg L^{-1})	Correlation coefficient, R^2
PCMX	0.529	0.154	0.807	0.152	0.723	31.735	0.950

of virgin calcium alginate bead having porous surface. Fig. 8(b) shows the SEM images of calcium alginate beads encapsulated with the BT102 bacterium. In Fig. 8(c), SEM of used beads (after bioremediation) is shown. It could be seen that the beads were full of microbes in Fig. 8(b), whereas the surface morphology was very much changed when these were used for remediation of 50 mg L^{-1} concentration of PCMX as observed in Fig. 8(c).

TEM analysis of PCMX treated and untreated BT102 bacteria:

Fig. 9(a) shows the TEM image of an untreated single bacterium and the same is depicted after PCMX treatment in Fig. 9(b). It could be seen that the microbe treated with PCMX was dark in colour and the shape got distorted. It was also seen that the size of the bacterial strain shrinked in presence of PCMX. TEM results indicated the intracellular accumulation of PCMX within the bacterial cell.

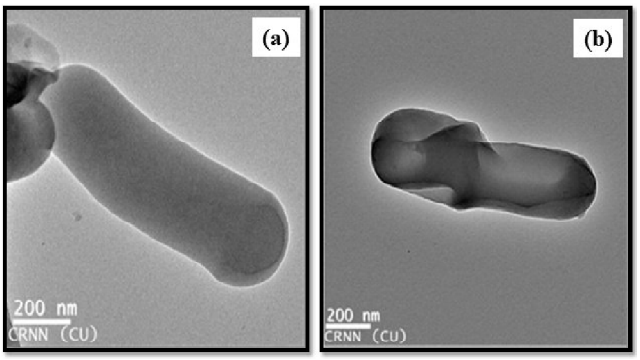

Fig. 9. (a) Controlled bacteria and (b) Dettol treated bacteria.

Conclusions

The two isolated strains BT102 and BT201 showed maximum tolerance of PCMX up to 1566 mg L^{-1} concentration. The encapsulated bacteria in calcium alginate beads exhibited high efficiency in bioremediation of PCMX in the batch operation by reducing PCMX from 10 mg L^{-1} to 100 μg L^{-1} (i.e. 99%). Unlike other biodegradation process, the degradation was fast (16 h) and could be used for semi-batch process. Degradation kinetics of PCMX showed substrate inhibition phenomenon. The experimental data could fit well with Haldane's model (R^2 = 0.9508) as compared to Monod's model (R^2 = 0.8072) for degradation kinetics of PCMX. This study proposed a cost-effective green technology using native bacterial strains for PCMX degradation from municipal waste water.

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References

1. S. S. Mohanty and H. M. Jena, *Brazilian J. Chem. Eng.*, 2017, **34**, 75.
2. M. K. Bruch, "Handbook of disinfectants and antiseptics", ed. J. M. Ascenzi, Marcel Dekker, New York, 1996, p. 265.
3. Y. Sun, J. Zhao, B. Zhang, J. Li, Y. Shi and Y. Zhang, *Chem. Eng. J.*, 2019, **368**, 553.
4. E. Capkin, T. Ozcelep, S. Kayis and I. Altinok, *Chemosphere*, 2017, **182**, 720.
5. T. Zhao and Q. Chen, "9-Halogenated phenols and polybiguanides as antimicrobial textile finishes", Elsevier Ltd., 2016, 141.
6. L. J. Yost, J. D. Rodricks, D. Turnbull, P. C. Deleo, J. F. Nash and A. Qui, *Regul. Toxicol. Pharmacol.*, 2016, **80**, 116.
7. J. C. Lear, J. Y. Maillard, P. W. Dettmar, P. A. Goddard and A. D. Russell, *Int. Biodeterior. Biodegrad.*, 2006, **57**, 51.
8. D. Gümüş and F. Akbal, *Process Saf. Environ. Prot.*, 2016, **103**, 252.
9. General Standards for Discharge of Environmental Pollutants Part-A : Effluents, 2019, 545. http://cpcb.nic.in/general-standards/, (accessed 6 May 2019).
10. Y. Li, J. Li, C. Wang and P. Wang, *Bioresour. Technol.*, 2010, **101**, 6740.
11. B. Das, G. Selvaraj and S. Patra, *Int. J. Environ. Sci. Technol.*, 2017, **16**, 711.
12. L. G. C. Villegas, N. Mashhadi, M. Chen, D. Mukherjee, K. E. Taylor and N. Biswas, *Curr. Pollut. Reports*, 2016, **2**, 157.
13. US National Library of Medicine, HSDB: CHLOROXYLENOL Human Health Effects : Human Toxicity Excerpts: Probable Routes of Human Exposure, 2019. https://toxnet.nlm.nih.gov/, (accessed 6 May 2019).
14. S. I. Ereqat, A. A. Abdelkader, A. F. Nasereddin, A. O. Al-Jawabreh, T. M. Zaid, I. Letnik and Z. A. Abdeen, *J. Environ. Sci. Heal., Part A.*, 2017, **53**, 39.
15. A. O. Olaniran and E. O. Igbinosa, *Chemosphere*, 2011, **83**, 1297.
16. M. Aryal and M. Liakopoulou-kyriakides, *J. Water Sustain.*, 2015, **5**, 45.
17. N. Ezzatahmadi, D. L. Marshall, K. Hou, G. Ayoko, G.

Millar and Y. Xi, *J. Environ. Chem. Eng.*, 2019, **7**, 102955.

18. A. Shojaie, M. Fattahi, S. Jorfi and B. Ghasemi, *J. Environ. Chem. Eng.*, 2017, **5**, 4564.
19. A. Moranchel T, J. A. Anderson, J. A. Casas, M. Faraldos and A. Bahamonde, *J. Environ. Chem. Eng.*, 2017, **5**, 4612.
20. A. Adewuyi, A. Göpfert, O. Anuoluwapo and T. Wolff, *J. Environ. Chem. Eng.*, 2016, **4**, 664.
21. S. Saha, U. Sarkar and S. Mondal, *Desalination and Water Treat.*, 2016, **37**, 3994.
22. R. Ali Khan Rao, S. Singh, B. R. Singh, W. Khan and A. H. Naqvi, *J. Environ. Chem. Eng.*, 2014, **2**, 199.
23. S. Sadhukhan, S. Singha and U. Sarkar, *Chem. Eng. J.*, 2009, **152**, 361.
24. T. Ghosh and P. Sarkar, *J. Biosci.,* 2014, **39**, 805.
25. N. Fiol, C. Escudero, J. Poch and I. Villaescusa, *React. Funct. Polym.*, 2006, **66**, 795.
26. S. Sadhukhan, R. Villa and U. Sarkar, *Biotechnol. Reports*, 2016, **10**, 84.
27. M. S. M. Annuar, I. K. P. Tan, S. Ibrahim and K. B. Ramachandran, *Brazilian J. Chem. Eng.*, 2008, **25**, 217.
28. P. Saravanan, K. Pakshirajan and P. Saha, *J. Hazard. Mater.*, 2009, **162**, 476.
29. J. B. S. Haldane, "Enzymes", MIT Press, Cambridge, Massachusetts, 1965.
30. S. P. Basak, P. Sarkar and P. Pal, *J. Environ. Sci. Heal., Part A*, 2014, **49**, 67.
31. L. Xu and J. Wang, *Environ. Eng. Sci.*, 2013, **30**, 294.