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Bioremediation of 2-chlorophenol containing wastewater by aerobic granules-kinetics and toxicity

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ABSTRACT

2-Chlorophenol (2-CP) degrading aerobic granules were cultivated in a sequencing batch reactor (SBR) in presence of glucose. The organic loading rate (OLR) was increased from 6.9 to 9.7 kg COD m^{-3} d^{-1} (1150–1617 mg L^{-1}COD per cycle) during the experiment. The alkalinity (1000 mg L^{-1} as $CaCO_3$) was maintained throughout the experiment. The specific cell growth rate was found to be 0.013 d^{-1}. A COD removal efficiency of 94% was achieved after steady state at 8 h HRT (hydraulic retention time). FTIR, UV, GC, GC/MS studies confirmed that the biodegradation of 2-CP occurs via chlorocatechol (modified *ortho*-cleavage) pathway. Biodegradation kinetics followed the Haldane model with kinetic parameters: V_{max} = 840 mg2-CP gMLVSS^{-1} d^{-1}, K_s = 24.61 mg L^{-1}, K_i = 315.02 mg L^{-1}. Abiotic losses of 2-CP due to volatilization and photo degradation by sunlight were less than 3% and the results of genotoxicity showed that the degradation products are eco-friendly.

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1. Introduction

Chlorophenols are xenobiotic pollutants that enter the environment through various industrial operations such as pulp bleaching, pesticides production, chlorination of water, etc. Chlorophenols are generally recognized for their toxicity and recalcitrant nature [1]. They are used as herbicides, insecticides, fungicides, wood preservatives and resins [2]. 2-Chlorophenol (2-CP) is on the list of 129 water-related priority organic pollutants [3] and formed as a result of breakdown of pesticides and chlorinated aromatic compounds [4]. It is highly toxic and persistent and cause gastrointestinal problems, skin irritation and carcinogenicity and in some cases poses a serious ecological threat as environmental pollutant [5]. EC_{50} of 2-CP with *Daphnia magna* was found to be 6.20 mg L^{-1} over a period of 48 h [6].

Several decontamination techniques including adsorption, solvent extraction, and chemical oxidation are available for removing chlorophenols. However, each of these techniques suffers from high cost and possibility of secondary pollution [7]. Biological treatment of chlorophenol containing wastewaters provides more specific conversions, is relatively inexpensive and usually results in complete mineralization [8,9]. In most of the bioremediation processes Gram-negative bacteria, e.g. *Pseudomonads* are used for degradation [10]. Chlorinated aromatics are usually degraded via *ortho*-cleavage pathway. However, in some cases successful ring cleavage via chlorocatechol or catechol using *meta*-cleavage pathway has also been reported [11]. Intermediates formed during biodegradation of chlorinated phenols may be phenol and/or catechol (dehalogenation before ring cleavage) or chlorocatechol (dehalogenation after ring cleavage) [12]. Hence, catechols are the main intermediates during biodegradation of a wide range of chlorinated aromatics.

In conventional biological treatment systems, the microorganisms are easily inhibited by the toxic chlorophenols to be treated and the biomass is easily lost from the system thus greatly reducing the treatment efficiency [13,14]. Utilization of immobilized cells or biofilms reactors was reported to improve chlorophenols removal due to high biomass retention [15].

Among the available immobilization techniques, aerobic granulation is attracting increasing interest [16]. Aerobic granulation is a novel environmental biotechnique, which has been extensively reported in sequencing batch reactors (SBR) [17]. Microorganisms

Abbreviations: 2-CP, 2-Chlorophenol; S, critical substrate concentration at which maximum reaction rate occur; COD, chemical oxygen demand; EPS, extracellular polymeric substance; H/D, height to Diameter ratio of SBR; HRT, hydraulic retention time; K_i, inhibition constant; K_s, half saturation constant; MLVSS, mixed liquor volatile suspended solids; SBR, sequential batch reactor; SEM, scanning electron microscopy; SRT, sludge retention time; SVI, sludge volume index; V, specific degradation rate; V_{max}, maximum theoretical specific substrate degradation rate; β, inhibitory parameter.

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under high shear force conditions would promote the formation of compact granules to resist damage of suspended cells at high substrate concentrations [18]. Microbial aggregation into compact aerobic granules offers additional benefits such as protection against predation and resistance to chemical toxicity [19]. Aerobic granulation offers better effluent quality, higher treatment efficiency [20] and the better tolerance to higher organic loading rates. Compared to conventional process, the main feature of SBR is its cycle operation, where each cycle consists of filling, aeration, settling and discharging. The objective of this study is to characterize the effluent coming out of the reactor (biodegradation pathway) and to evaluate the toxicity removal and kinetics of 2-CP biodegradation in a SBR. A feed concentration of 2-CP in the range 20–140 mg L^{-1} was utilized in SBR operating at 8 h HRT with 50% volumetric exchange ratio.

2. Material and methods

2.1. Chemicals

2-Chlorophenol, a xenobiotic compound was a G.R. Product of Fisher Scientific, India and was used as received. All chemicals were used in analytical reagent grade and supplied by Fisher Scientific India.

2.2. Biomasses and basal medium

Mixed microbial culture with a MLVSS of 1.5 g L^{-1} and SVI of 120 mL g^{-1} was taken as a source of aerobic sludge from secondary clarifier of Star Paper Mills, Saharanpur, India. It was brown initially with a loose, fluffy and irregular morphology. Its colour was then changed to white and finally to yellow during the course of experiment.

The basal medium used in this experiment contained (g L^{-1}):

Macro nutrients: $C_6H_{12}O_6$ (1.0), NH_4Cl (0.28), K_2HPO_4 (1.65), $MgSO_4.7H_2O$ (0.13), KH_2PO_4 (1.35) [21]. The conversion factor used for commonly used glucose (co-substrate) in wastewater treatment was 1065 mg COD g^{-1} glucose.
Micro nutrients: H_3BO_3 (0.05), $FeCl_2{\cdot}4H_2O$ (0.05), $ZnCl_2$ (0.05), $MnCl_2{\cdot}4H_2O$ (0.05), $CuCl_2{\cdot}2H_2O$ (0.03), $NH_4SeO_3{\cdot}5H_2O$ (0.05), $AlCl_3{\cdot}6H_2O$ (0.05), $NiCl{\cdot}6H_2O$ (0.05), $NaSeO_3{\cdot}5H_2O$ (0.1). [21].

2.3. Experimental Set up

A column type cylindrical reactor made of transparent Perspex-glass (height 150 cm and diameter 5 cm) was used with a working volume of 1.4 L. The reactor was maintained at room temperature and started up with 1.4 L of aerobic sludge. Fine bubble aerator was fixed at the bottom for supplying air at a superficial gas velocity above 1.2 cm s^{-1}. A port was fitted at 70 cm along the height of the SBR and used for collecting effluent samples.

All the experiments were performed at room temperature and the pH of the system was maintained at around 8 throughout the study. In the present study, the aerobic sludge was taken from a paper mill and since the effluents from paper mills contain phenolic compounds, so the microbes present in such waste are already acclimatized towards phenols and chlorophenols through natural selection, which shortens the acclimatization period in the present case. However, for getting more specific microorganism for 2-chlorophenol removal activated sludge was initially conditioned over a 15 day period to allow the biomass to adapt to 2-CP before inoculating into SBR. Fifteen percent of the sludge was wasted every third day, which gives a constant sludge retention time (SRT) of 20 days.

Table 1
Various operating parameters of the SBR.

Parameter	Value	Unit
HRT	8	h
SRT	20	days
Biomass concentration (MLVSS)	2.56	g L^{-1}
Influent 2-CP	20–140 per cycle	mg L^{-1}
Glucose (Co-substrate)	1.0	g L^{-1}
Glucose COD	1065	mg L^{-1} g^{-1} glucose
Organic load	6.90–9.70	kg COD m^{-3} d^{-1}
DO	4–5	mg L^{-1}
Alkalinity	1000	mg L^{-1} as $CaCO_3$
Working volume	1.4	L
Residual volume	0.7	L

2.4. SBR operation

The reactor was operated sequentially in 4 h cycle with 50% volumetric exchange ratio giving an HRT of 8 h. Each cycle consists of: 5 min influent addition, 195–223 min aeration, 30–2 min settling, 5 min effluent withdrawal and 5 min idle (no stirring). Various operational parameters of the SBR are shown in Table 1.

For measuring abiotic losses, a controlled reactor was used having same dimensions as experimental SBR but without aerobic sludge. It was observed that abiotic losses due to volatilization and photo degradation by sunlight were less than 3%.

2.5. Seed sludge and wastewater

Aerobic sludge with a MLVSS concentration of 1.5 g L^{-1} was used as seed sludge for cultivating aerobic microbial granules. Initially, the sludge was acclimated in an aeration tank for a period of 15 days. During acclimation period, it was fed with 5–20 mg L^{-1} of 2-CP in mineral salt medium. Later on, the acclimated sludge was inoculated in to SBR.

A mother solution of 2-CP was prepared with a strength of 1000 mg L^{-1} in double distilled water. Required concentrations of 2-CP were obtained by proportionate dilution with double distilled water.

The SBR was started with 20 mg L^{-1} of 2-CP in mineral salt medium (basal medium) and its concentration was gradually increased to 140 mg L^{-1} (Fig. 1) in mineral salt medium whose composition is given in Section 2.2.

2.6. Analytical methods

Chemical oxygen demand (COD), pH, sludge volume index (SVI), mixed liquor volatile suspended solids (MLVSS), and alkalinity were evaluated according to the standard methods for the examination of water and wastewater [22]. Growth of the culture was monitored in terms of optical density at 600 nm using UV–visible spectrophotometer (Shimadzu 1601) according to the method given by [23]. The specific growth rate was estimated from the slope of the exponential phase of growth curve (optical density curve). All the analyses were performed in triplicate.

UV–visible spectral studies were done using Shimadzu 1601 UV–visible spectrophotometer in the wavelength range 190–700 nm [22]. The spectra of the influent samples were recorded after required dilution in order to confine the absorbance value below 2.0. However, the effluent samples were first filtered using 0.22 μm pore size filter paper and then the spectra were taken directly using double distilled water as blank. A calibration plot (absorbance versus concentration of 2-CP) was also drawn and used for estimating the concentration of unknown 2-CP solutions. The plot was linear (R^2 = 0.98) between 0 and 150 mg L^{-1} 2-CP solution. The test samples drawn from experiments with higher concen-

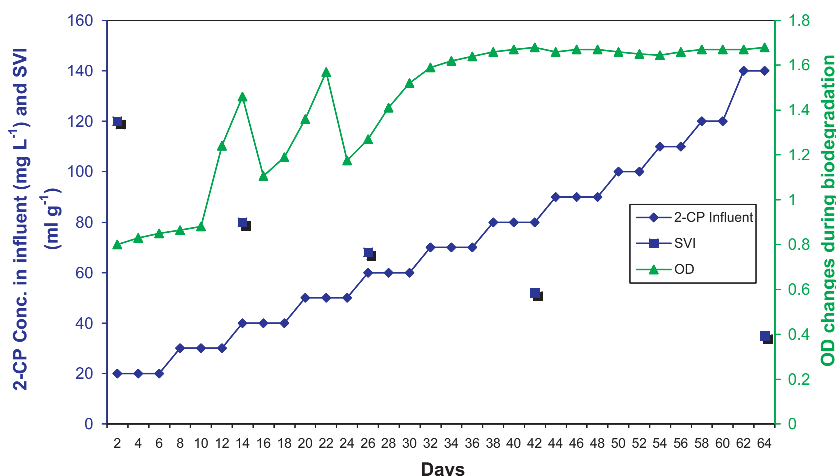


Fig. 1. Variation of influent 2-CP concentration, SVI and OD during granulation.

trations of 2-CP were adequately diluted prior to the absorbance measurement.

Morphology and surface characteristics of mature aerobic granules were studied using scanning electron microscopy (ZEISS EVO Series EVO 50 Microscope). Granules were prepared for SEM image according to the method described by [24]. Briefly, granules were prepared for SEM image by washing with a phosphate buffer and fixing with 2% glutaraldehyde overnight at 4 °C. Fixed granules were washed with 0.10 M sodium cacodylate buffer, dehydrated by successive passages through 25, 50, 75, 80, 90, 95 and 100% ethanol and dried with a CO_2 Critical Point Dryer. FTIR of the samples (influent, effluent and EPS) were taken using Interspec 2020 Spectrolab (UK).

GC (Gas chromatography) and GC/MS (Gas chromatography–mass spectrometry) analysis of effluent samples were done in order to identify the number of products formed during aerobic biodegradation of 2-CP. The reaction end products were analyzed on a GC system (Buck Scientific 910 Model, USA equipped with a DB-5, 30 m by 0.25 mm capillary column, RESTEK, USA) and PERKIN ELMER CLARUS 500 Gas Chromatograph–Mass Spectrometer, respectively. Samples were extracted and derivatized by a previously described method [25]. Briefly, the reaction solutions were acidified to pH 4 (10 μL of concentrated HCl per mL of reaction mixture), and aromatic compounds were extracted into ethyl acetate (1:1 v/v).

The ability of granules to degrade 2-CP was evaluated by studying the batch kinetics of biodegradation in 250 mL serum vials containing seven different concentrations of 2-CP (ranging from 20 to 140 mg L^{-1}) in mineral salt medium. 100 mL of granular sludge was added to each serum vials and the content was kept on rotatory shaker for 4 h and studied periodically (after every 30 min till the steady state).

Escherichia coli K-12 strain was used to check the toxicity of degradation product. This assay was carried out as described by Rahman et al. [26]. Briefly, 0.5 μg of covalently closed circular pBR322 DNA (0.5 μg) was treated with the influent TCP, control as well as treated 2-CP water samples in a total volume of 20 μL for 1 h. After the treatment, 8 μL of 5× tracking dye (40 mM EDTA, 0.05% bromophenol blue and 50% (v/v) glycerol) was added and loaded on 1% agarose gel. The gel was run at 50 mA for 2 h and stained with ethidium bromide (0.5 μg L^{-1}) for 30 min at room temperature. After washing, the gel was visualized on fotodyne UV transilluminator (USA) and photographed.

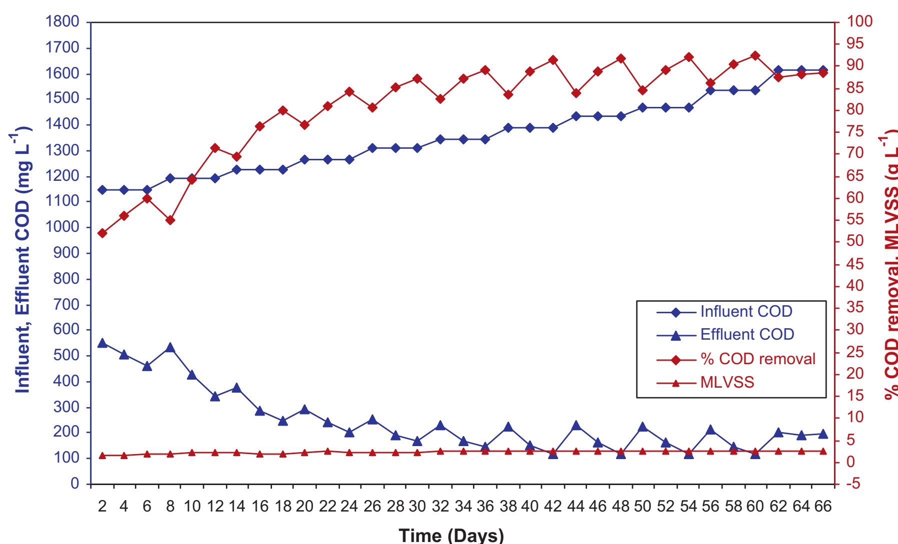


Fig. 2. COD, MLVSS and reactor performance (% removal efficiency).

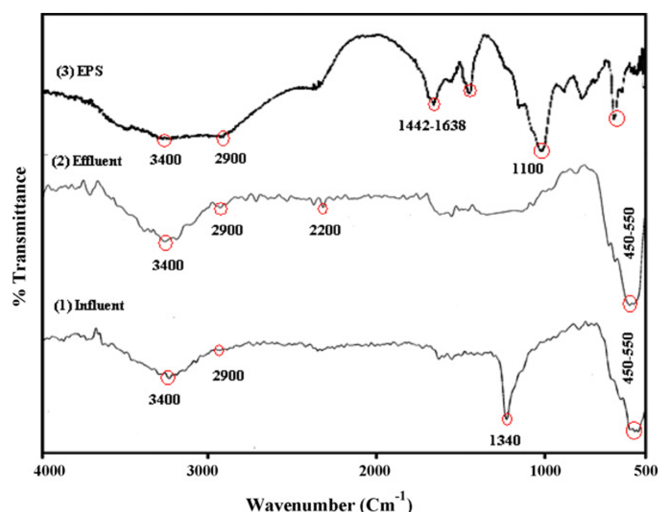


Fig. 3. FTIR spectra of 2-CP influent (1), effluent (2) and EPS (3) during 2-CP biodegradation.

3. Results and discussion

3.1. Formation of 2-CP aerobic degrading granules

The reactor was started up with acclimated activated sludge having SVI value of 120 mL g^{-1} (Fig. 1). A settling time of 30 min was given initially to avoid excess losses of sludge. In subsequent days, the settling time was reduced to 10 min (day 15) and finally to 5 min (day 22). Small granules rapidly grew in subsequent week while more floc like sludge was washed out due to reduction in settling time. After few weeks (5th week), the biomass was dominated by tough and stable granules. It was also observed during the study that as the fluffy biomass changed in to compact granules, the SVI value of the sludge decreases and the final SVI value of mature granules was 35 mL g^{-1} (Fig. 1.). Since, the aerobic granules are dense in structure with good settling characteristics, their SVI is quite low. The diameter of mature granules was around 1–2 mm. The average settling velocity of granules was in the range of 2.3–3.9 cm s^{-1}, which was much higher than that of activated sludge flocs reported by Campos et al. [27]. It was also observed that a 5 min settling causes granules to settle, leaving a clear supernatant.

Cell growth was monitored in terms of optical density (OD). Cell growth increases from an initial value of 0.9 to 1.7 (Fig. 1). However, cell growth show two sharp decrease on day 15 and 22 because of washout of poor settling flocs. The specific cell growth rate was found to be 0.013 d^{-1}. OD gives an idea about the gradual transference of microbial flocs to aerobic granules. Initially, when the system was dominated by loose microbial flocs, OD showed a variable behavior due to biomass losses but as soon as the system got stable and granules were formed (day 32) OD showed a constant behavior till the end (because of retention of biomass due to high settling velocities of aerobic granules). This behavior of OD reflects the gradual change of microbial flocs to aerobic granules (Fig. 1).

Fig. 1 also showed the variation of influent 2-CP concentration (20–140 mg L^{-1}) during the study. Increase in 2-CP concentration neither inhibits the growth of cells nor did it affect efficient granulation and reactor performance (Fig. 1). Hence, it was proved that aerobic granules in SBR can tolerate the toxic affects of 2-CP.

3.2. Characterization of granule

Scanning electron microscopic (SEM) observations revealed that the mature aerobic granules had a very compact microstructure in which cells were tightly linked together and round bacteria were predominant (Fig. 1S, Supplementary material). It is quite reflecting that aerobic granulation is a gradual process involving the progression from seed sludge to compact aggregates, further to granular sludge and finally to mature granules. The granules surface show folds, crevices and depressions, which allows greater mass transfer leading to better activity (Fig. 1S, Supplementary material).

3.3. Variation of COD, MLVSS and reactor performance (% removal efficiency)

Aerobic sludge with MLVSS of 1.5 g L^{-1} was inoculated into SBR. MLVSS of the sludge increases throughout the study to stabilize at 2.56 g L^{-1} as illustrated in Fig. 2. However, MLVSS also showed a sharp decrease on day 15 and 22 (similar to the trend shown by OD) due to reduction in settling time, which acts as a selection pressure for compact biomass and meanwhile favors granulation. A continuous increase in MLVSS after day 33 showed minimal biomass losses due to good settleability of granules and inturn shows the effectiveness of chosen operating strategy.

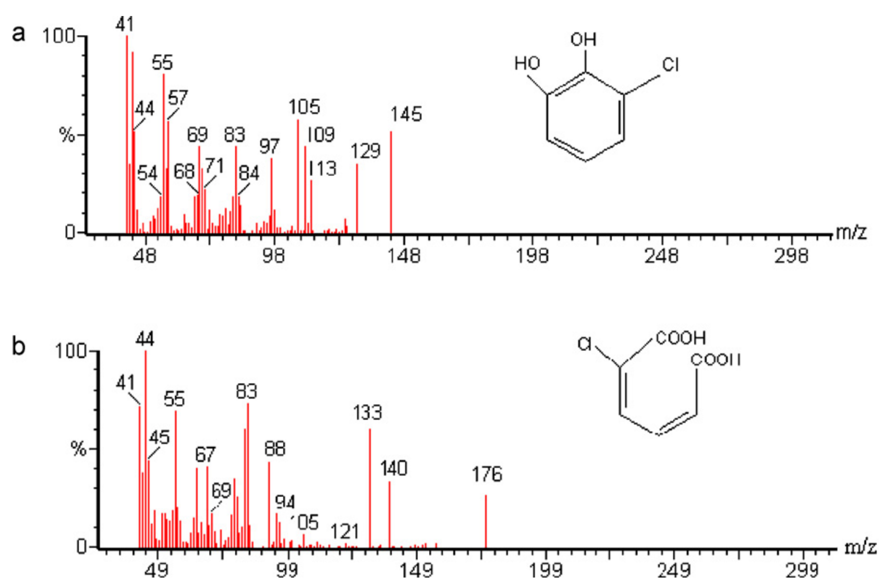


Fig. 4. Gas chromatographic–mass spectrometric investigations of the effluent (a and b).

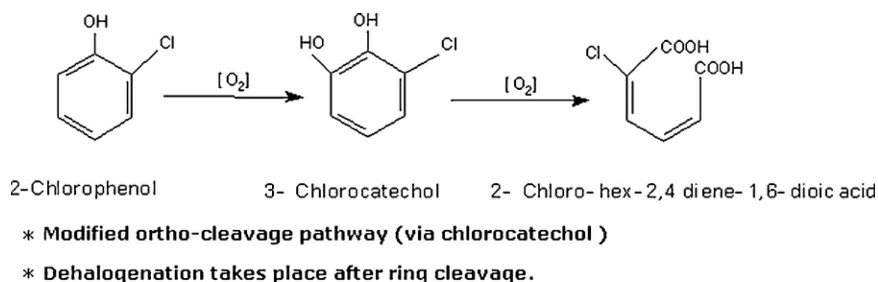


Fig. 5. Probable biodegradation pathway of 2-CP by aerobic granules in SBR.

COD of influent include of COD of 2-CP as well as glucose. It was observed that 4 h cycle time was sufficient for COD reduction from 1617 to 194 mg L^{-1}. For a given aeration period, it appeared that a starvation phase existed during SBR operation, which has also been reported to play a positive role in microbial granulation [24]. Initial COD values were around 1150 mg L^{-1} with corresponding removal efficiency of 60% because the system was unstable. However, after steady state (day 28), system was recovered to 70% and finally a COD removal efficiency of 94% was achieved (Fig. 2). It is quite reflecting that aerobic granules in SBR sustained the ill affects of variable COD from 1150 to 1617 mg L^{-1} COD corresponding to an organic load of 6.9–9.7 kg COD m^{-3} d^{-1}. Hence, the system can withstand higher organic load as well as fluctuation is organic load, which otherwise causes failure of conventional activated sludge process.

3.4. Characterization of products of biodegradation using different techniques

3.4.1. UV–visible study of the influent and effluent

UV spectra of influent (containing 2-CP in mineral salt medium) and effluent during biodegradation are shown in Fig. 2S (Supplementary material). The spectrum was taken in the wavelength range of 190–700 nm. Influent 2-CP spectrum shows a single prominent peak at λ_{max} of 270.0 nm corresponding to 2-CP (Fig. 2S, Supplementary material). However, no such prominent peak is visible in the effluent spectrum, showing the complete biodegradation of 2-CP was occurred by aerobic granules in SBR.

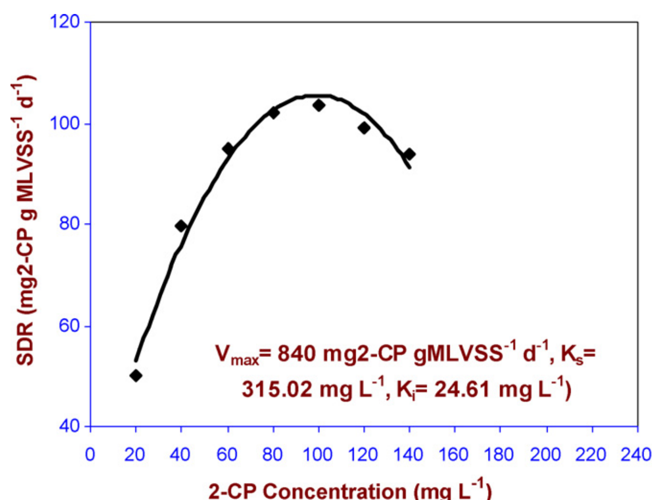
3.4.2. Gas chromatographic analysis

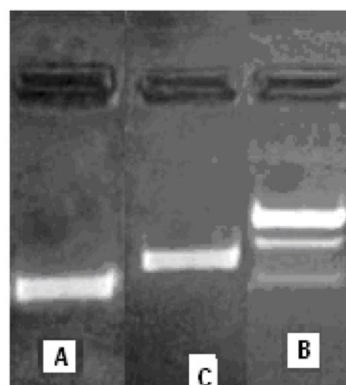
Gas chromatographic analysis was done in order to determine the number of products formed during biodegradation and the results are shown in Fig. 2S Supplementary material (inset). Three peaks were observed in the GC spectra of effluent samples extracted using ethyl acetate as described above. Largest peak corresponds to the solvent, i.e. ethyl acetate at a retention time of 10.325 min. In addition to ethyl acetate, two more peaks (at retention time of 4.575 min and 12.45 min) were observed showing the formation of two biodegradation products.

3.4.3. Characterization of influent, effluent and EPS during 2-CP biodegradation by FTIR

FTIR investigation was done to ascertain composition of influent, effluent and EPS secreted by bacteria during biodegradation of 2-CP. Fig. 3 indicates the frequencies of vibration and their corresponding functional groups. Fig. 3(1) and (2) shows the FTIR spectra of influent 2-CP and effluent. C—H and OH stretching are observed at 2900 and 3400 cm^{-1} [28]. A characteristic peak at 1340 cm^{-1} in Fig. 3(1) shows C=C stretching of benzenoid ring [29]. This peak occurs at a frequency lower than its expected value because of the interaction with metal ions present in tap water used in the influent. However, no such peak (around 1340 cm^{-1}) in effluent FTIR spectrum [Fig. 3(2)] shows the cleavage of ring during the SBR cycle by aerobic bacteria. A sharp peak at 2200 cm^{-1} in FTIR spectra of effluent corresponds to the presence of CO_2 formed during biodegradation. Peaks at 3300 cm^{-1} show the presence of —OH group. A hump towards the extreme right (450–550 cm^{-1}) is attributed to the presence of metal ions which can also be seen in Fig. 3(1).

The FTIR study of the EPS (3) could also provide valuable information about the product of biodegradation. The FTIR analysis of EPS confirms the presence of aromatic intramolecularly bonded hydroxyl (—OH) group, C—H stretching, disubstituted aromatic deformation (C—H), C=C stretching of aromatic ring in the EPS. The presence of these functional groups in the EPS spectrum arises the possibility of the formation of catechol or chlorocatechol





2-CP assay (A-Controlled, C-Effluent, B-Influent)

Fig. 6. Specific 2-CP degradation rate of aerobic granules at different concentrations (Haldane's Model) and genotoxicity assay.

during biodegradation of 2-CP. The absorption peak at frequency of 3400.14 cm^{-1} represents —OH stretching of two intramolecularly bonded —OH groups. Peak at 2900 cm^{-1} corresponds to C—H stretching and the peaks at 1442.22–1633.82 cm^{-1} are possibly due to C=C stretching of benzenoid and quinoid structure of aromatic ring [29]. A strong peak around 1100 cm^{-1} shows C—O absorption of —OH group of aromatic ring, i.e. phenols or catechol [30]. The peak at 686.74–763 cm^{-1} refers to out of plane C—H bending in a disubstituted aromatic system [31]. These absorption frequencies show a negative shift from the actual value for *ortho*-disubstituted aromatic ring due to interaction with metal ions. However the peaks at 572.27 and 438.36 cm^{-1} are due to metal ions present in influent tap water. FTIR spectra of EPS, influent 2-CP and effluent shows that the biodegradation of 2-CP occurred via catechol or chlorocatechol pathway.

3.4.4. Gas chromatographic and mass spectrometric studies

There may be several potential biodegradation products depending upon the type of pathway followed by mixed culture during biodegradation of a particular substrate [11,32]. Two catabolic pathways generally followed during chlorophenols biodegradation, which involves dehalogenation before ring cleavage and dehalogenation after ring cleavage [11]. Cleavage of the aromatic ring may preferentially occur using either the *meta*- or the *ortho*-pathway. Chlorinated phenols usually prefer *ortho*-cleavage since *meta*-cleavage of chlorophenols via 3-chlorocatechol results in the production of dead end metabolites [33]. A very few authors have reported the successful degradation of chlorinated aromatics (esp. chlorinated phenols) occurred through *meta*-cleavage pathway [34]. The probable potential intermediate of 2-CP biodegradation in the present study was 3-chlorocatechol (m/z 145, Fig. 4a) which then transformed into 2-chloro-hex-2,4-diene-1,6-dioic acid also known as 2-chloromuconic acid (m/z 176, Fig. 4b) an aliphatic compound via modified *ortho*-cleavage pathway and the dehalogenation takes place after ring cleavage as shown in (Fig. 5). Conversely, Farrell and Quilty [33] reported the production of 5-chloroformyl-2-hydroxy-penta-2,4-dieneoic acid via *meta*-cleavage of 3-chlorocatechol. Hence, the mixed culture is capable of degrading 2-CP via chlorocatechol pathway followed by a modified *ortho*-cleavage, which is also supported by FTIR results.

3.5. Biodegradation kinetics

Biodegradation kinetics of substrates is an important parameter for prediction of their fate and designs of the wastewater plants [35]. The sludge production and kinetics of aerobic granules under such a repeated operational condition are important to the process design and optimization [36,37]. The biodegradation rate of 2-CP by aerobic granules is often described by Haldane's model for inhibitory substrate (Fig. 6) and can be given as Eq. (1):

$$V = \frac{V_{\max} S}{K_s + S + S^2/K_i} \quad (1)$$

where V and $V_{\max}$ are the specific and the maximum theoretical specific substrate degradation rates (mg2-CP gMLVSS^{-1} d^{-1}), respectively, and S, K_s and K_i are the substrate concentration, half-saturation constant, and inhibition constant (mg L^{-1}), respectively. It was observed during the batch kinetic study that the specific degradation rate of 2-CP increases with initial concentration of 2-CP and peaked at 103.4 mg 2-CP gMLVSS^{-1} d^{-1} for 100 mg L^{-1} of 2-CP (among the 7 concentrations tested) and then decreases (Fig. 6).

The calculated kinetic parameters are ($V_{\max}$) 840 mg2-CP gMLVSS^{-1} d^{-1}, K_s = 315.02 mg L^{-1}, and K_i = 24.61 mg L^{-1}, with a correlation coefficient (R^2) of 0.9782. According to these values, the critical substrate concentration at which the maximum reaction rate is obtained is given by $S^*_{2-CP} = \sqrt{K_S K_I} = 88.06$ mgL^{-1} and β ($\sqrt{K_i/K_s}$) the inhibitory parameter that accounts for the extent of the inhibitory effects is equal to 0.28 (a smaller value of β gives a larger removal rate reduction at high-substrate concentration) [35].

However, Basu and Oleszkeiwicz [38] reported a specific degradation rate of 95.04 mg2-CP gMLVSS^{-1} d^{-1} (3.96 mg2-CP gMLVSS^{-1} h^{-1}) at 30 mg L^{-1} of 2-CP in absence of any co-substrate. Zlateval et al. [39] reported the degradation rate of 2-CP to be 350 mg2-CP gMLVSS^{-1} d^{-1} and kinetic parameters K_s and K_i to be 150 and 50 mg L^{-1}, respectively.

3.6. Genotoxicity by plasmid nicking assay

The genotoxicity by plasmid nicking assay (Fig. 6) also supported the results for reduction in genotoxicity of 2-CP. 20 μL of 2-CP (influent) resulted in the conversion of the super coiled pBR322 DNA to the relaxed form (lane B) while 20 μL of treated 2-CP (effluent) resulted in maximum protection of plasmid from damage. The results show that aerobic granules were capable of reducing the genotoxicity of 2-CP.

4. Conclusions

The biodegradation approaches used in the present study provided useful descriptions of the aerobic granules, which were actively involved in treatment of wastewater containing high loading rate of 2-CP. Mature aerobic granules were compact with an SVI and MLVSS of 35 mL g^{-1} and 2.56 g L^{-1} respectively, giving a COD removal efficiency of 94%. Spectral studies showed that the biodegradation of 2-CP occurred via chlorocatechol pathway (modified *ortho*-cleavage) and genotoxic examination confirmed that the effluent was non-toxic. Biodegradation kinetics followed Haldane model for inhibitory substrate. Hence, aerobic granulation in SBR can be used for efficient bioremediation of 2-chlorophenol containing wastewater.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.jhazmat.2011.03.029.

References

[1] A. Carucci, S. Milia, G. Cappai, A. Muntoni, A direct comparison amongst different technologies (aerobic granular sludge, SBR and MBR) for the treatment of wastewater contaminated by 4-chlorophenol, J. Hazard. Mater. 177 (2010) 1119–1125.
[2] V.M. Monsalvo, A.F. Mohendano, J.A. Casas, J.J. Rodriguez, Cometabolic biodegradation of 4-chlorophenol by sequencing batch reactors at different temperatures, Bioresour. Technol. 100 (2009) 4572–4578.
[3] USEPA National Recommended Water Quality Criteria, United States Environment Protection Agency, EPA Report EPA-822-R-02-047, 2002.
[4] A.N.M. Fakhruddin, B. Quilty, The influence of glucose and fructose on the degradation of 2-chlorophenol by *Pseudomonas putida* CP1, World J. Microbiol. Biotechnol. 21 (2005) 1541–1548.
[5] X. Quan, H. Shi, J. Wang, Y. Qian, Removal of 2,4-dichlorophenol in a conventional activated sludge system through bioaugmentation, Process Biochem. 39 (2004) 1701–1707.
[6] T.L. Randall, P.V. Knopp, Detoxification of specific organic substances by wet oxidation, J. Water Pollut. Control Fed. 52 (1980) 2117–2130.
[7] P.K. Mondal, R. Ahmad, S.Q. Usmani, Anaerobic biodegradation of triphenylmethane dyes in a hybrid UASFB reactor for wastewater remediation, Biodegradation 21 (2010) 1041–1047.

[8] M. Dilaver, F. Kargi, 2,4-Dichlorophenol (DCP) containing wastewater treatment using a hybrid-loop bioreactor, Bioresour. Technol. 100 (2009) 1459–1462.
[9] U. Bali, F. Sengul, Performance of a fed-batch reactor treating a wastewater containing 4-chlorophenol, Process Biochem. 37 (2002) 1317–1323.
[10] M. Ziagova, G. Kyriakou, M. Liakopoulou-Kyriakides, Co-metabolism of 2,4-dichlorophenol and 4-Cl-m-cresol in the presence of glucose as an easily assimilated carbon source by *Staphylococcus xylosus*, J. Hazard. Mater. 163 (2009) 383–390.
[11] C.J.P. Snyder, M. Asghar, J.M. Scharer, R.L. Legge, Biodegradation kinetics of 2,4,6-trichlorophenol by an acclimated mixed microbial culture under aerobic conditions, Biodegradation 17 (2006) 535–544.
[12] H. Gardin, J.M. Lebeault, A. Pauss, Degradation of 2,4,6-trichlorophenol by co-immobilization of anaerobic and aerobic microbial communities in an upflow reactor under air-limited condition, Appl. Microbiol. Biotechnol. 56 (2001) 524–530.
[13] C.C. Wang, C.M. Lee, C.H. Kuan, Removal of 2,4-dichlorophenol by suspended and immobilized *Bacillus insolitus*, Chemosphere 41 (2000) 447–452.
[14] S.G. Wang, X.W. Liu, H.Y. Zhang, W.X. Gong, X.F. Sun, B.Y. Gao, Aerobic granulation for 2,4-dichlorophenol biodegradation in a sequencing batch reactor, Chemosphere 69 (2007) 769–775.
[15] F. Kargi, S. Eker, Removal of 2,4-dichlorophenol and toxicity from synthetic wastewater in a rotating perforated tube biofilm reactor, Process Biochem. 40 (2005) 2105–2111.
[16] X.Y. Shi, G.P. Sheng, X.Y. Li, H.Q. Yu, Operation of a sequencing batch reactor for cultivating autotrophic nitrifying granules, Bioresour. Technol. 101 (2010) 2960–2964.
[17] S.G. Wang, X.W. Liu, W.X. Gong, B.Y. Gao, D.H. Zhang, H.Q. Yu, Aerobic granulation with brewery wastewater in a sequencing batch reactor, Bioresour. Technol. 98 (2007) 2142–2147.
[18] M. Belmonte, J.R. Vazquez-Padin, M. Figueroa, A. Franco, A. Mosquera-Corral, J.L. Campos, R. Mendez, Characteristics of nitrifying granules developed in an air pulsing SBR, Process Biochem. 44 (2009) 602–606.
[19] L. Jiang, J.H. Tay, A.M. Maszenan, S.T.L. Tay, Bacterial diversity and function of aerobic granules engineered in a sequencing batch reactor for phenol degradation, Appl. Environ. Microbiol. 70 (2004) 6767–6775.
[20] Y.Q. Liu, B. Moy, Y.H. Kong, J.H. Tay, Formation, physical characteristics and microbial community structure of aerobic granules in a pilot-scale sequencing batch reactor for real wastewater treatment, Enzyme Microb. Technol. 46 (2010) 520–525.
[21] R. Ahmad, P.K. Mondal, S.Q. Usmani, Hybrid UASFB-aerobic bioreactor for biodegradation of acid yellow-36 in wastewater, Bioresour. Technol. 101 (2010) 3787–3790.
[22] APHA, Standard Methods for the Examination of Water and Wastewater, 20th ed., American Public Health Association, Washington, D.C. USA, 2002.
[23] M. Ziagova, M. Liakopoulou-Kyriakides, Kinetics of 2,4-dichlorophenol and 4-Cl-m-cresol degradation by Pseudomonas species cultures in the presence of glucose, Chemosphere 68 (2007) 921–927.
[24] V. Ivanov, X.H. Wang, S.T.L. Tay, J.H. Tay, Bioaugmentation and enhanced formation of microbial granules used in aerobic wastewater treatment, Appl. Microbiol. Biotechnol. 70 (2006) 374–381.
[25] T.M. Louie, C.M. Webster, L. Xun, Genetic and biochemical characterization of a 2,4,6-trichlorophenol degradation pathway in *Ralstonia eutropha* JMP134, J. Bacteriol. 184 (2002) 3492–3500.
[26] M. Rahman, S. Connolly, W.C. Noble, B. Cookson, I. Phillips, Diversity of *staphylococci* exhibiting high-level resistance to mupirocin, J. Med. Microbiol. 33 (1990) 97–100.
[27] J.L. Campos, J.M. Garrido-Fernandez, R. Mendez, J.M. Lema, Nitrification at high ammonia loading rates in an activated sludge unit, Bioresour. Technol. 68 (1999) 141–148.
[28] R.M. Silverstein, G.C. Bassler, T.C. Morrill, Spectrometric Identification of Organic Compounds, John Wiley, New York, 1991, 109–130.
[29] J. Bandara, J.A. Mielczarski, J.I. Kiwi, Adsorption mechanism of chlorophenols on iron oxides, titanium oxide and aluminum oxide as detected by infrared spectroscopy, Appl. Catal. B: Environ. 34 (2001) 307–320.
[30] B. Bardacki, FTIR-ATR spectroscopic characterization of monochlorophenols and effects of symmetry on vibrational frequencies. *Çankaya Üniversitesi Fen Edebiyat Fakültesi*, J. Arts Sci. 7 (2007) 13–19.
[31] B. Pandit, U. Chudasama, Synthesis, characterization and application of an inorgano organic material: p-chlorophenol anchored onto zirconium tungstate, Bull. Mater. Sci. 24 (2001) 265–271.
[32] M. Fahmy, E. Heinzle, O.M. Kut, Treatment of bleaching effluents in aerobic/anaerobic fluidized biofilm systems, Water Sci. Technol. 24 (1994) 179–187.
[33] A. Farrell, B. Quilty, Degradation of mono-chlorophenols by a mixed microbial community via a *meta*-cleavage pathway, Biodegradation 10 (1999) 353–362.
[34] A.E. Mars, T. Kasberg, S.R. Kaschabek, M.H. van Agteren, D.B. Janssen, W. Reineke, Microbial degradation of chloroaromatics: use of the *meta*-cleavage pathway for mineralization of chlorobenzene, J. Bacteriol. 179 (1997) 4530–4537.
[35] M.C. Tomei, M.C. Annesini, S. Rita, A.J. Daugulis, Biodegradation of 4-nitrophenol in a two-phase sequencing batch reactor: concept demonstration, kinetics and modeling, Appl. Microbiol. Biotechnol. 80 (2008) 1105–1112.
[36] S. Yi, W.Q. Zhuang, B. Wu, S.T.L. Tay, J.H. Tay, Biodegradation of *p*-nitrophenol by aerobic granules in a sequencing batch reactor, Envion. Sci. Technol. 40 (2006) 2396–2401.
[37] Y. Chen, W. Jiang, D.T. Liang, J.H. Tay, Biodegradation and kinetics of aerobic granules under high organic loading rates in sequencing batch reactor, Appl. Microbiol. Biotechnol. 79 (2008) 301–308.
[38] S.K. Basu, J.A. Oleszkeiwicz, Factors affecting aerobic biodegradation of 2-chlorophenol in sequencing batch reactors, Environ. Technol. 16 (1995) 1135–1143.
[39] P.V. Zlateva, M.G. Gerginova, J.S. Manasiev, B.K. Atanasov, N.M. Peneva, N.D. Dimova, Z.M. Alexieva, Kinetic parameters determination of the phenolic derivatives assimilation by *trichosporon cutaneum* r57, Biotechnol. Biotechnol. Equip. 19 (2005) 93–97.