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Degradation of cationic surfactants using immobilized bacteria: its effect on adsorption to activated sludge

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Highlights

- Removal of cationic surfactants from industrial activated sludge.
- Adsorption of cationic surfactants to activated sludge depends on its hydrophobicity.
- QACs desorption from sludge is inversely proportional to the extent of adsorption.
- An immobilized consortium of QACs-degrading bacteria avoid its adsorption to sludge.

Abstract

Adsorption of cationic surfactants (QACs) Br-tetradecyltrimethylammonium (TTAB), Cl-tetradecylbenzyldimethylammonium (C_{14}BDMA) and Cl-hexadecylbenzyldimethylammonium (C_{16}BDMA) to activated sludge from a wastewater treatment plant was tested. Adsorption equilibrium was reached after 2 h, and for initial 200 mg L^{-1} 81%, 90% and 98% of TTAB, C_{14}BDMA and C_{16}BDMA were respectively adsorbed. After six successive desorption cycles, 21% of TTAB and 12.7% of C_{14}BDMA were desorbed from the sludge. In agreement with the percentage of QACs pre-adsorbed, the more hydrophobic the compound, the lesser the extent of desorption. Wastewater samples with activated sludge were supplemented with TTAB 200 mg L^{-1} and Ca-alginate beads containing the QACs-degrading

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microorganisms *Pseudomonas putida* A (ATCC 12633) and *Aeromonas hydrophila* MFB03. After 24 h, 10 mg L^{-1} of TTAB were detected in the liquid phase and 6-8 mg L^{-1} adsorbed to the sludge. Since without Ca-alginate beads or with empty beads total TTAB amount (phase solid and liquid) did not change, the 90% reduction of the initial 200 mg L^{-1} after treatment with immobilized cells was attributed to the bacterial consortium's capacity to biodegrade QACs. The results show the advantages of using immobilized bacteria to achieve complete QACs elimination from wastewater systems, thus preventing them from reaching the environment.

Keywords Cationic surfactants; Adsorption; Activated sludge; Biodegradation.

1. Introduction

Quaternary ammonium compounds (QACs), cationic surfactants among them, are commercial chemicals widely used in numerous industrial items such as sanitizers, antistatic agents and food preservatives, as well as in the formulation of pharmaceutical, cosmetic and cleaning household products (McDonnell and Russell, 1999; Gilbert and Moore, 2005; Zhao and Sun, 2007; Takenaka et al., 2007). The most used QACs are benzalkonium chloride (BAC) and cetrimonium chloride/bromide (Cetrimide). Commercially, BAC and Cetrimide are a mixture of alkylbenzyldimethylammonium chlorides and alkyltrimethylammonium bromide, respectively, with different alkyl chain lengths. BAC with a chain length of C12 to C14 is the predominant QAC in pharmaceutical products and industrial sanitizers. The bromine salts of cetrimonium with alkyl chain between 14 and 16 carbons long, are commonly used as active ingredient in personal care products (shampoos and cosmetics) (Merianos, 2001; Xue et al., 2004; Gilbert and Moore, 2005; Hegstad et al., 2010). Global demand for surfactants (including nonionic, anionic, cationic and amphoteric surfactants) in 2010 was more than 12 million tons and an increase of 4.5% per year until 2018 is anticipated (Brycki et al., 2014). Of the total demand for surfactants, QACs represent over 10% and are therefore classified as high production volume chemicals (Tezel et al., 2012; Tezel and Pavlostathis, 2015). After use, residual cationic surfactants are discharged into sewage treatment plants or directly into the environment at levels that endanger the environmental systems. In industrial treatment plants, the wastewater containing QACs are combined with other wastewater streams and subsequently treated in an activated sludge system, process that allows obtaining high-quality effluent

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(Ismail et al., 2010; Renet al., 2011; Brycki et al., 2014; Zhang et al., 2015). However, also the cationic surfactants have negative influences on the biochemical reactions of activated sludge, my decrease the microbial communities due to the toxicity of these compounds on the microorganisms, and accordingly decrease the system treatment efficiency and even cause system failure (Tezel et al., 2008; Hajaya et al., 2011). Several studies demonstrate QAC toxicity in microbial communities of natural systems and wastewater treatment systems. For instance, Zhang et al. (2011) found that BAC (C12–C16) causes inhibition of respiratory enzymes in an activated sludge system with EC50 values of 0.12 to 3.60 mg L^{-1}. Another study showed that 10-15 mg L^{-1} of BAC were inhibitory to an activated sludge nitrifying microbial community (Yang et al., 2015).

Cationic surfactants are biodegradable under aerobic conditions, and diverse bacterial species with the ability to metabolize these compounds have been identified, including different strains of *Pseudomonas*, *Aeromonas*, *Achromobacter*, *Xanthomonas* and *Stenotrophomonas* (Patrauchan and Oriel, 2003; Takenaka et al., 2007; Liffourrena et al., 2008; Tezel et al., 2012; Oh et al., 2013; Bergero et al., 2017). The removal of QACs in wastewater treatment plants is affected on a large extent, by the biodegradation of these compounds. QACs biodegradability depends on two factors: their structure (it generally decreases with the increase of alkyl chain and by the presence of benzyl group (Ying, 2006)), and also on the presence of microorganisms with the capacity to metabolize them. Previous studies carried out in our laboratory have shown that free-cells and Ca-alginate immobilized cells of *Pseudomonas putida* A (ATCC 12633) were able to effectively degrade the cationic surfactant tetradecyltrimethylammonium bromide (TTAB) and offers promising opportunities for the efficient biological removal of this or similar QACs (Liffourrena et al., 2008; Lucchesi et al., 2010; Bergero and Lucchesi, 2015). In this bacterium, the TTAB degradation is initiated by N-dealkylation catalyzed by a TTAB monooxygenase activity resulting in the formation of tetradecylalkanal and trimethylamine (TMA) (Liffourrena et al., 2008; 2009). The next step involved the oxidation of tetradecylalkanal to tetradecanoic acid, which would be further metabolized via β oxidation. The second product, TMA, is metabolized to NH_3 through oxidation and demethylation (Liffourrena et al., 2010).

On the other hand, in wastewater treatment plants QACs are rapidly and extensively adsorbed to sludge (Tezel et al., 2006; Clara et al., 2007; Zhang et al., 2011). This decreases their bioavailability and hence reduces the rate and extent of their biodegradation and consequently, the fate of the QAC in the environment (Xia et al., 2005; Ismail et al., 2010). The mechanism by which QACs are adsorbed to

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sludge is complex. It depends on temperature and QAC structure and includes both hydrophobic and ionic interactions (García et al., 2006; Ismail et al., 2010; Ren et al., 2011). The high QACs adsorption to sludge leads to these compounds are accumulated into treatment plants and reach the environment through the effluent discharge. In soils, the QACs play an important role in the sorption and desorption behaviors of many organic contaminants and consequently, influences in their degradations (Jones-Hughes and Turner, 2005). For instance, sorption of toluene and naphthalene on soils was enhanced in the presence of the cationic surfactant cetyltrimethylammonium bromide (CTAB) (Zhang and Zen, 2006). Also has been described that the sorption of the CTAB to the sediment increased the sorption of the contaminant perfluorooctanesulfonate (Pan et al., 2009). Contrary to what has been described for some non-ionic surfactants, the adsorption of cationic surfactants onto soil particles decrease the solubility of hydrophobic organic contaminants such as petroleum hydrocarbons, polycyclic aromatic hydrocarbons and polychlorobiphenyls, causing a significant limitation to the remediation of soils contaminated with these compounds (Cheng et al., 2017 and cites included). While adsorption of cationic surfactants has been investigated in numerous adsorbents like fly ash (Banerjee et al., 2006), clays (Mykola et al., 2016), bentonites (Zhu et al., 2010), montmorillonite (Kozaka and Domka, 2004), activated carbon (Choi et al., 2009) and municipal sludge (Ismail et al., 2010), there are no studies on how to avoid the QACs adsorption to industrial sludge in treatment plants in order to prevent them from reaching the environment. Our objective was to evaluate how QACs of differing molecular structure were adsorbed to activated sludge obtained from the industrial wastewater, both in absence and presence of Ca-alginate beads containing QAC-degrading microorganisms. The results indicate that cationic surfactants are rapidly adsorbed to industrial sludge and that they are desorbed, to a greater or lesser degree, according to their structure. Moreover, we aimed to demonstrate the advantages of using immobilized bacteria to both reduce the amount of QACs adsorbed to the sludge and to achieve complete surfactant elimination in wastewater systems, thus preventing them from reaching the environment via effluent and sludge and application.

2. Materials and methods

2.1. Determination of quaternary ammonium compounds (QACs)

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Quantifications of tetradecyltrimethylammonium bromide (TTAB), tetradecylbenzyldimethylammonium chloride (C_{14}BDMA) and hexadecylbenzyldimethylammonium chloride (C_{16}BDMA), were performed in whole and centrifuged wastewater samples by colorimetric method based on the reaction of QACs with bromothymol blue (Cross,1970). According to this method, an anionic dye-QAC ion pair is formed, which is then solvent extracted, and the color intensity is measured spectrophotometrically at 420 nm. The concentration of each QAC was calculated by calibration graphs previously constructed. The detection limit, calculated as three times the standard deviation of the blank divided by the absolute value of the slope, was 0.05 mg L^{-1}. The limit of quantification, calculated as ten times the standard deviation of the blank divided by the absolute value of the slope, was found to be 0.17 mg L^{-1}. In this method the interference of amines (nonquaternary species) with the quantification of QACs is insignificant because the extraction of amine-bromothymol blue complexes with chloroform is reduced at pH 7.4 (Gupta and Herman, 1973).

2.2. Industrial wastewater

The study was conducted using sludge collected from a tank of wastewater treatment plant of a poultry industry of Río Cuarto, Córdoba, Argentina. pH value measured in wastewater samples was 7.2, QACs content 2 mg L^{-1} and total suspended solids (activated sludge) 3.5 g L^{-1} (Gravimetric Method, APHA 2540-D). For the experiments, the content of the activated sludge (consisting of microorganisms, non-living organic matter and inorganic materials) was adjusted to 1 g L^{-1} using tap water. For the adsorption/desorption experiments without alginate beads, samples were supplemented with sodium azide 200 mg L^{-1} to rule out any biological activity. For the same experiments with Ca-alginate beads, wastewater samples were previously autoclaved at 1 atm during 15 minutes. All determinations were made in triplicate and the average values ± SD were reported.

2.3. Adsorption kinetic

Wastewater samples containing 1 g L^{-1} of activated sludge were supplemented with 200 mg L^{-1} of TTAB, C_{14}BDMA or C_{16}BDMA and incubated at room temperature (20-22 ºC) with agitation (150 rpm) in an orbital shaker. At different time intervals, samples were collected and centrifuged at 10,000 rpm during 10 min. The supernatants obtained were used to QAC quantification (Cross, 1970). As control and in order to determine that there was no loss of QACs by adsorption to other surfaces, wastewater samples

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were centrifuged (10,000 rpm for 15 min) to eliminate the activated sludge. The supernatants obtained were supplemented with 200 mg L^{-1} of QAC each and then manipulated in the same manner as described above.

2.4. Adsorption assays

Adsorption of different initial concentrations for each of the three QACs to the activated sludge was evaluated in batch essays. Wastewater samples containing 1 g L^{-1} of activated sludge were supplemented with 50, 100, 150, 200, 250 and 300 mg L^{-1} of TTAB, C_{14}BDMA or C_{16}BDMA, and incubated for 5 h at 22 ºC with 150 rpm agitation. The adsorbed amount of QAC to the sludge was determined by the difference between the QAC values quantified in the supernatants obtained by centrifugation (10,000 rpm for 10 min) at the initial and final times. To describe adsorption equilibrium of QACs to the sludge, the Freundlich isotherm model was used (Freundlich, 1906). This isotherm model is described by the following equation: $q_e=K_F C_e^{1/n_F}$ where q_e is mg QAC adsorbed per g of adsorbent at equilibrium and C_e is mg QAC in the liquid phase at equilibrium (mg QAC/L). The adsorption capacity (K_F) and adsorption intensity ($1/n_F$) were estimated from the intercept and slope of the plots of log q_e versus log C_e.

2.5. Desorption assays

To analyze desorption, we used 20 ml of wastewater sample containing 1 g L^{-1} of activated sludge that had been previously equilibrated in the adsorption process with 200 mg L^{-1} TTAB or C_{14}BDMA for 5 h. The liquid phase was removed from the solid phase by centrifugation (10,000 rpm for 15 min), and 20 ml of distilled water with sodium azide (200 mg L^{-1}), were added. In order to desorb QACs from the activated sludge, flasks were stirred and then incubated for 24 h in a shaker (150 rpm) at 22-23 ºC. Finally, the solid phase was separated from the liquid by centrifugation and concentration of desorbed QACs was determined in the supernatant by colorimetric method (Cross, 1970). This operation was repeated in six successive desorption cycles.

2.6. Adsorption assays in presence of bacterial immobilized cells

The batch experiments involving TTAB adsorption in presence of encapsulated cells were carried out with a bacterial consortium formed by *P. putida* A (ATCC 12633) and a strain isolated from effluents and identified as *A. hydrophila* MFB03. The cells were encapsulated in calcium alginate (Ca-alginate)

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following Bergero et al. (2017). The final Ca-alginate concentration to prepare the beads was 2.7% w/v and the encapsulated population was approximately 10^8 cfu mL^{-1} beads for each bacterial member. For the adsorption experiments, 50 ml of wastewater samples with 1 g L^{-1} of activated sludge were supplemented with TTAB 200 mg L^{-1} and 1.2 g (wet weight) of beads (equivalent to approximately 100 beads) and incubated in a shaker at 22-23 ºC and 150 rpm. TTAB concentration was determined both in whole and centrifuged (10,000 rpm, 10 min) wastewater samples to obtain the total and liquid phase TTAB concentration. The amount adsorbed on the sludge was calculated by the difference between total and liquid phase concentration**.** As control, the samples were added with Ca-alginate beads without microorganisms and then processed in the same manner as described above.

For the determination of TTAB-monooxygenase activity, twenty alginate beads were removed of the wastewater samples, washed with a 0.9% NaCl solution and suspended in 900 µl of 0.1 M phosphate buffer, pH 7.4, to achieve dissolution of the alginate. The cells were disrupted by sonication (20,000 Hz, 10 times for 10 s each time). The whole cells and cell debris were removed by centrifugation (14,000x*g* for 15 min) and the supernatant was used for TTAB monooxygenase determination by fluorescence, as described (Liffourrena et al, 2009). Protein concentrations were measured by the Bradford method (1976) with bovine serum albumin (Sigma Chemical Co., SL, USA) as a standard.

3. Results and discussion

3.1. Adsorption and desorption of QACs to activated sludge

Fig. 1 shows the results of the adsorption kinetic assays for each cationic surfactant to activated sludge. For the three detergents tested, TTAB, C_{14}BDMA and C_{16}BDMA, adsorption to the activated sludge followed a biphasic pattern with rapid adsorption over the first 30 min. After this time, adsorption rate decreased and reached equilibrium within 2 h. Because QAC levels in the liquid phase did not change when the activated sludge was eliminated from the samples (not shown), decrease in QAC concentrations detected in the liquid phase is consistent with the adsorption to activated sludge. The time our study determined to be necessary for QACs to reach adsorption equilibrium to activated sludge was similar to that reported in other studies (Garcia et al., 2004; Ismail et al., 2010). The initially rapid adsorption of each QAC to activated sludge is likely due to relative high number of binding sites present in the

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adsorbent. On the other hand, slowdown in adsorption with increasing contact time could be due to decrease in the QAC concentration gradient (Caner et al., 2009).

For 200 mg L^{-1} of each QAC, approximately 81% of TTAB, 90% of C_{14}BDMA and 98% of C_{16}BDMA were adsorbed to the activated sludge after 2 h. It is likely that the increase in the alkyl chain length enhances the hydrophobicity of the compound, thus leading to higher affinity adsorption for C_{16}BDMA. In addition, the results indicate that QAC adsorption to activated sludge also increases with the replacement of a methyl group for a benzyl group, as has been previously reported by other authors (García et al., 2006; Tezel et al., 2006; Ismail et al., 2010).

Taking into account the results of the adsorption kinetic assays, we selected an equilibration period of 5 h with 50 to 300 mg L^{-1} of TTAB, C_{14}BDMA or C_{16}BDMA to determine Freundlich adsorption isotherms (Freundlich, 1906) (Fig. 2). This empirical model assumes that the adsorption occurs on a multilayer, onto heterogeneous adsorption surfaces and represent adsorption processes in which more than one adsorption mechanism are involved (Schwarzenbach et al., 2003). Table 1 shows the estimated values of Freundlich parameters K_F and $1/n_F$ and the regression coefficients (R^2). As has been described for different QACs (Garcia et al., 2006; Ismail et al., 2010; Ren et al., 2011), the Freundlich model fitted the adsorption data to the industrial activated sludge adequately with R^2 values greater than 95%. For the three QACs analyzed, TTAB, C_{14}BDMA and C_{16}BDMA, $1/n_F$ values were similar and less than unity, indicating favorable adsorption and heterogeneity of industrial activated sludge surface. The K_F values obtained are different for each QAC, suggesting that adsorption capacity depends mainly on compound structure. Thus, as shown in Table 1, adsorption capacity followed the order C_{16}BDMA>C_{14}BDMA>TTAB. It was enhanced by the presence of a benzyl group, but it increased further with the increase in length of the alkyl chain. This means that the adsorption intensity of the QACs to the sludge is closely related to the hydrophobicity of the compound. This result is in agreement with previous findings which indicate that at low concentrations, adsorption of C_{14}BDMA to activated sludge mainly occurs through ionic interactions, but at high concentration the hydrocarbon chain dominates the process (Gurses et al., 2009; Ren et al., 2011). Fig. 3 shows desorption of QACs from industrial activated sludge after 5 h adsorption equilibration with 200 mg L^{-1} of TDTMA or C_{14}BDMA. Each successive desorption assay lasted 24 h and after six successive cycles using distilled water, percentages of desorption for TTAB and C_{14}BDMA were 21% (36.1 mg L^{-1}) and 12.7% (22.85 mg L^{-1}), respectively. Thus, the percentage of QACs desorbed relative to the amount pre-adsorbed to sludge decreased as the alkyl chain length increased and also with

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the substitution of a methyl group for a benzyl group. Ismail et al. (2010) investigated C_{12}TMA and C_{16}BDMA desorption from a municipal primary sludge using distilled water. According to their results, about 30% of C_{12}TMA and 5% of C_{16}BDMA were desorbed after five successive desorption cycles. Therefore, our results are in agreement with the ones reported by Ismail et al. (2010) and indicate that the more hydrophobic the QAC, the lesser its extent of desorption.

Although activated sludge adsorbed QACs (Fig. 1), in each desorption cycle significant amounts of the adsorbed surfactants were released (Fig. 3). Consequently, if the desorbed QACs are not degraded they accumulate in wastewater and contribute to polluting the environment. For this reason, it is necessary to improve QAC removal in wastewater treatment plants to avoid their adsorption to the sludge before they are released into the environment. Taking into account that the biodegradation can be used as alternative method to remove QACs from the environment, we decided to evaluate the use of bacterial cells immobilized in Ca-alginate to achieve complete QAC removal from industrial activated sludge.

3.2. Adsorption of QACs to activated sludge in presence of Ca-alginate beads containing QAC-degrading microorganisms

The percent of QACs depletion measured in industrial activated sludge may result from the combination of degradation by immobilized microorganisms and QACs adsorbed to activated sludge. Previously, we demonstrated that beads of Ca-alginate with cells of *P. putida* A (ATCC 12633) and *A. hydrophila* MFB03 were able to achieve degradation of cationic surfactants TTAB and BAC, both in flasks and in a bioreactor (Bergero et al., 2017). Now, we evaluated if the presence of this immobilized bacterial consortium modify the adsorption of 200 mg L^{-1} of TTAB to activated sludge. In these tests, the wastewater samples were previously sterilized to nullify biological activity of native microorganisms. Fig. 4a shows TTAB levels detected in liquid phase across time. In the presence of beads with the immobilized bacterial consortium after a 24 h assay, 10 mg L^{-1} of TTAB were detected in the liquid phase. Taking into account that in *P. putida* A (ATCC 12633) the TTAB degradation is initiated by N-dealkylation catalyzed by a TTAB monooxygenase activity (Liffourrena et al. 2008, 2009), we corroborate that TTAB is metabolized by the immobilized bacterial consortium determining the specific activity of this enzyme. In cell-free extracts obtained from beads removed of the wastewater samples after1 h of assay, the specific activity of TTAB-monooxygenase was 1.22 ±0.10 (n = 3) nmol TMA min^{-1}

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mg protein^{-1}, indicating that the immobilized cells metabolized the TTAB once it entered the bead. The TTAB-monooxygenase activity remained with minimal variations after 24 h of assay (1.35 ±0.15 (n = 3) nmol TMA min^{-1} mg protein^{-1}) confirming the immobilized system capacity to degrade TTAB.

On the other hand, 40 mg L^{-1} of TTAB were detected in liquid phase after 24 h but in the absence of Ca-alginate beads (Fig. 4a) or using empty beads without microorganisms (not shown). Fig. 4b shows the results of TTAB adsorbed to activated sludge. In the presence of Ca-alginate beads after a 24 h assay, approximately 6-8 mg L^{-1} of TTAB were detected in the solid phase whereas in the absence of beads after 24 h 160 mg L^{-1} were adsorbed. Thus, after 24 h total TTAB levels detected in presence of Ca-alginate beads containing QAC-degrading microorganisms were approximately 16 mg L^{-1}, indicating that more than 90% of the initial 200 mg L^{-1} were removed. Given that in absence of Ca-alginate beads or with empty beads without microorganisms the total TTAB amount (liquid and solid phase) did not change during the time of the assay (200 mg L^{-1}), reduction in QAC concentration detected using beads containing immobilized cells can be clearly attributed to the ability of the consortium to degrade QACs (Bergero et al., 2017).

Accordingly, our results support that the use of immobilized cells to remove QACs in wastewater treatment plants would be an appropriate and complementary method to prevent the QACs adsorbed to the sludge from being released and accumulated into the environment at levels that would finally lead to the contamination of soils and water resources.

4. Conclusions

Three commonly used QACs were rapidly adsorbed to industrial activated sludge. The adsorption capacity followed the order C_{16}BDMA>C_{14}BDMA>TTAB, indicating that it is closely related to compound hydrophobicity. Inversely proportional to the extent of adsorption, the QACs exhibited desorption from activated sludge. The use of QACs-degrading microorganisms immobilized in Ca-alginate beads considerably reduced the adsorption of surfactants to activated sludge. Accordingly, the results show the importance that implicates the QACs removal in wastewater treatment plants to avoid their adsorption to the sludge before they are released into the environment.

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Legends of Figures:

Fig. 1. Adsorption kinetic of different QACs to activated sludge. Wastewater samples containing 1 g L^{-1} of activated sludge were supplemented with 200 mg L^{-1} of TTAB (■), C_{14}BDMA (●) or C_{16}BDMA (▲) and incubated at 23 °C and 150 rpm. At different times, aliquots were taken and centrifuged and the supernatant was used for QAC determination by colorimetric method. Values are means ± SD (n = 3).

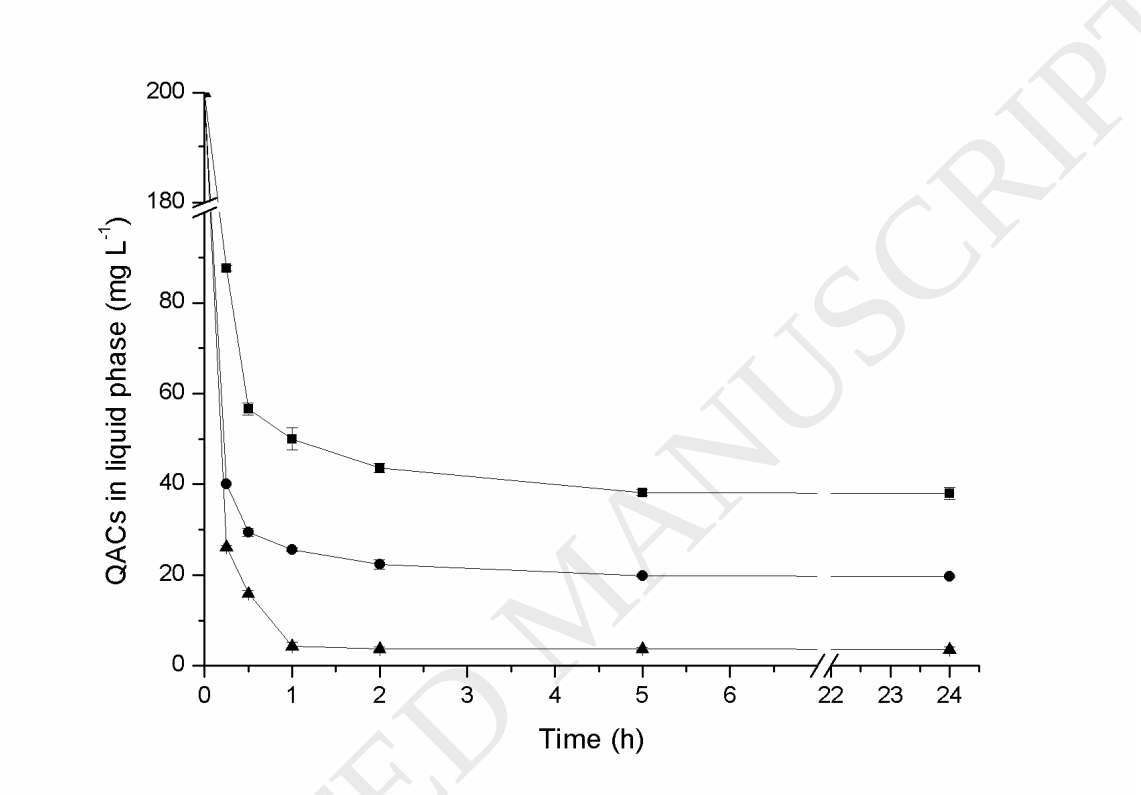

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Fig. 2. Freundlich adsorption isotherms for different QACs to industrial activated sludge after 5 h equilibration. Values are means ± SD (n = 3).

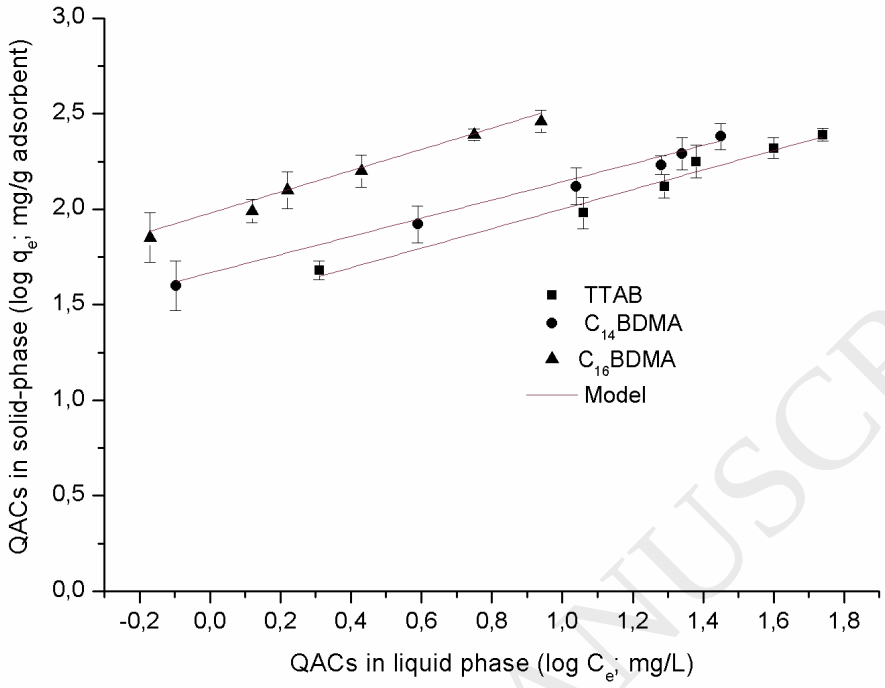

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Fig. 3. Successive desorption cycles of TTAB (■) and C_{14}BDMA (□) from the activated sludge. Wastewater samples with 1 g L^{-1} of activated sludge that had been previously equilibrated in the adsorption process with 200 mg L^{-1} TTAB or C_{14}BDMA were centrifuged. The liquid phase was removed and replaced by an equal volume of distilled water. After 24 h incubation at 150 rpm and 22-23 ºC, the samples were centrifuged and QACs desorbed were determined in the supernatant by colorimetric method. This operation was repeated in six successive desorption cycles. Values are means ± SD (n = 3).

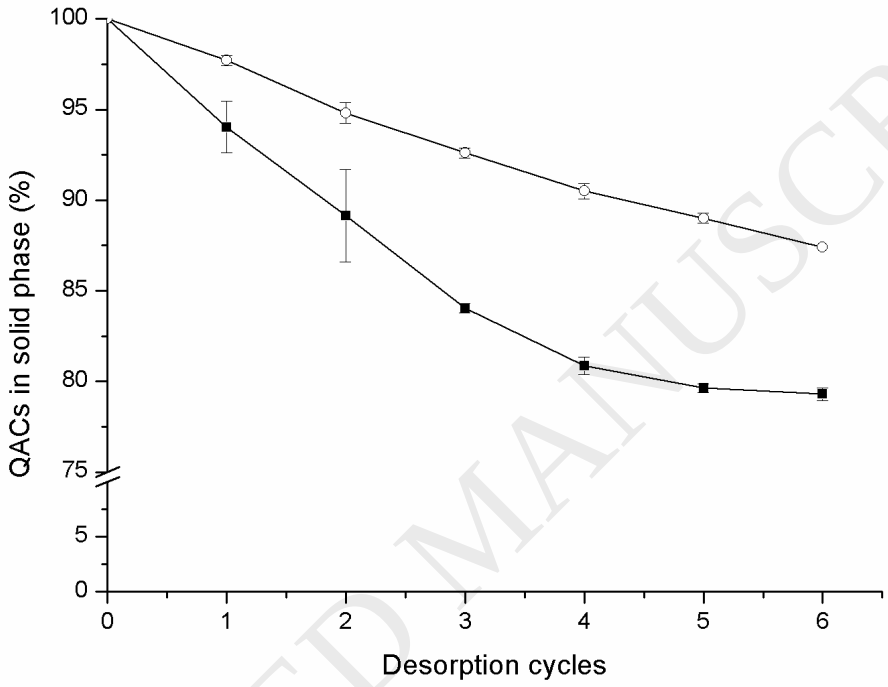

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Fig. 4. Adsorption of TTAB to activated sludge in absence (filled symbols) or in presence (empty symbols) of Ca-alginate beads containing a bacterial consortium. TTAB levels detected in liquid phase **(a)** and adsorbed to activated sludge **(b)** were determined at different times after centrifugation of wastewater samples with 1 g L^{-1} of activated sludge supplemented initially with TTAB 200 mg L^{-1}. Values are means ± SD (n = 3).

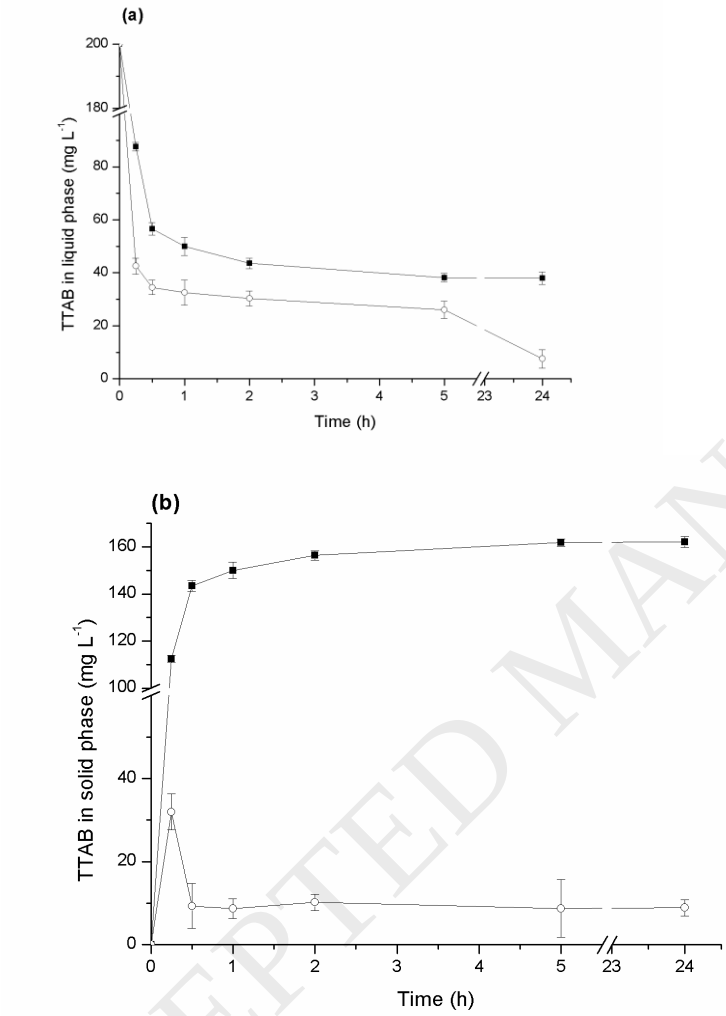

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Table 1 Freundlich adsorption parameters for adsorption of different QACs to industrial activated sludge

QACs	**K_F (mg/g adsorbent)(L/mg)$^{1/n_F}$**	**$1/n_F$**	**R^2**
TTAB	30.9±4.9	0.51±0.05	0.989
C_{14}BDMA	42.3±4.5	0.49±0.03	0.978
C_{16}BDMA	95.5±9.2	0.55±0.03	0.987

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