Perchlorate removal using two component biodegradable carriers in particle-fixed biofilm reactor
Fang He, Fusheng Li, Haihong Zhou, Lingling Niu and Liguo Wang

ABSTRACT
In this research, biocompounds designed out of two polymers having different degradability was investigated for use as the sole carbon source and biofilm carrier to remove perchlorate in particle-fixed biofilm reactors. Both laboratory batch and column experiments were conducted with perchlorate contaminated groundwater. Batch experiments demonstrated clearly that ClO₄⁻ was removed from the aqueous phase readily and the degradation rate constants (k) changed in the range of 0.23–0.37 mg/L h as ClO₄⁻ concentration increased from 2 to 8 mg/L. Simultaneous perchlorate and nitrate degradation occurred in the polymer bioreactor. Effluent concentrations of perchlorate varied positively with temperature and fitted the Arrhenius equation expression as $k = k_{20} \cdot 10^{0.0316(T-20)}$ over the range of 13–30°C. No perchlorate was detected in the effluent of polymer columns after 20 days’ startup. Complete perchlorate removal was observed at a hydraulic loading rate doubled to 1.8 mL/min. Images prove the concept of the pore and filament structure within the biocompounds, which provide both a heterotrophic biofilm and carbon source. Denaturing gradient gel electrophoresis analysis and partial sequencing of 16S rRNA genes indicated that formerly reported perchlorate-reducing bacteria were present in the polymer particle-fixed biofilm reactors.

Key words | biocompound, biodegradation, biofilm, carbon source, perchlorate

INTRODUCTION
Perchlorate salts, highly soluble compounds, have raised public health concerns due to its potential toxicity. It inhibits the transfer of iodide from the blood to the thyroid gland, which is required for the gland to produce hormones essential for metabolism and growth. Long-term disruption in thyroid hormones may result in hypothyroidism and related changes in metabolism, decreased mental performance, and altered development (Wolff 1998; Blount et al. 2009; Xiao et al. 2010; Zhao et al. 2011). Perchlorate salts, especially ammonium perchlorate, are known to be most widely used as oxidant in solid rocket fuel propellants, explosives, pyrotechnics, and as chemical reagents. Perchlorate salts are also commonly used in the manufacture of many commercial products ranging from electronics to pharmaceuticals (Srinivasan & Sorial 2009). Perchlorate contamination has been found in groundwater, surface water, and soil in North America and Europe. Perchlorate has also been detected in plants, food products, cow’s milk, and human breast milk (Baidas et al. 2011).

Technologies applicable for treating perchlorate contamination in drinking water include ion exchange, membrane technologies (electrodialysis and reverse osmosis), activated carbon adsorption, and bioremediation (Wallace et al. 1998; Herman & Frankenberger 1999; Coates & Anderson 2000; Coates & Achenbach 2004; Srinivasan & Viraraghavan 2009; Xu et al. 2013). Among these technologies, the most promising techniques appear to involve use of bacteria that respire and degrade perchlorate to chloride and water. Another attractive feature of biological treatment is its potential to simultaneously remove multiple contaminants, such as chromate, selenate, and dichloromethane (Nerenberg & Rittmann 2004).
As interest in perchlorate bioremediation has increased, so have reports of isolation of perchlorate-degrading bacteria (Coates et al. 1999; Waller et al. 2004; Nozawa-Inoue et al. 2005; Zhang et al. 2005; Bardiya & Bae 2008; Choi et al. 2008). Bacterial perchlorate reduction can be quite rapid and laboratory studies with both pure-strain and mixed-culture perchlorate-degrading bacteria have shown perchlorate degradation half-lives on the order of hours (Rikken et al. 1996; Coates et al. 1999; Giblin & Frankenberger 2000). It is known that organic carbon is needed as the electron donor in the process of reduction of perchlorate and chlorate transformation to chloride. However, electron donors are extremely insufficient in perchlorate-contaminated groundwater. Therefore, an exogenous electron donor must often be added in significant quantities (at significant cost). The traditional technique is to add an organic carbon source (such as ethanol or acetic acid, etc.) into a perchlorate treatment reactor (Shrout et al. 2006; Li et al. 2010; Baidas et al. 2011; Ghosh et al. 2011). The disadvantage of this treatment process is that it requires sophisticated, costly process control and knowledge of the biological system and presents the risk of overdosing. In addition, few studies have addressed the capabilities of these organisms to respire perchlorate under conditions likely to be found at perchlorate bioremediation sites. Optimal conditions are rarely observed in situ. The challenge of in situ perchlorate remediation is to provide an effective electron donor source for bacteria that respiring perchlorate.

Recent reports in the literature have suggested the potential use of biodegradable polymers (BDPs) as solid carbon source to remove nitrate in recirculating aquaculture systems with good efficiency (Hamlin et al. 2008). Zhou et al. (2009) and Chu & Wang (2011) had successfully studied the denitrification of drinking water using synthetic BDP materials such as PCL and biodegradable meal box. BDPs were used as both the carrier and carbon source for denitrification, which solved the problem of carbon sources dosage insufficient or excessive. Therefore, there is great potential for success in perchlorate degradation using BDPs, but using BDPs as electron donors for perchlorate degradation bacteria has not been directly investigated (Rikken et al. 1996; Herman & Frankenberger 1999; Giblin & Frankenberger 2001; Choi et al. 2008). Additionally, as studies have shown the ability of using BDPs as carbon source for denitrification, the use of BDPs with bacteria could lead to a synergistic removal of perchlorate and co-contaminated nitrate in the affected groundwater (Tan et al. 2004; Van-Ginkel et al. 2008; Xiao et al. 2010).

In the current study, a novel type of biodegradable carrier was applied for treatment of drinking water polluted by perchlorate. It was composed of two different polymers, which were combined in an extruder process, an easily degradable starch and a less easily degradable poly (butylene succinate) (PBS). It is hypothesized that with the degradation of the easily degradable component by microbes, small pores or channels can develop on the carrier surface. As a result, the protected surface area available for slow-growing microorganisms, which are susceptible to detachment from the outer surfaces, becomes greater. Therefore, in addition to acting as an extra carbon source for microorganisms, the biocompound carriers provide microniches for biofilm growth. In particular, those pores will provide micro niches for bacterial species with different requirements concerning substrates and oxygen concentration, since concentration gradients will occur within the pores due to turnover and limited mass transfer. This might allow for perchlorate removal.

The specific objective of this work was to evaluate the feasibility of starch/PBS polymer as the carbon source and the only physical support for perchlorate reduction by using batch and column tests.

MATERIALS AND METHODS

Batch tests were first conducted to evaluate the efficiency of the selected biodegradable substrate. These investigations were followed by a series of column tests. In addition, biofilm morphology and microbiology community composition on the carrier, and changes in the surface morphology of the starch/PBS polymer material were also evaluated.

Materials

The fresh synthetic groundwater (culture medium) designed according to the composition determined for a real
groundwater was a neutral (adjusted to pH 7.0 by 1.0 M NaOH) and anoxic solution (purged with oxygen-free nitrogen), containing, per liter: 43.87 mg KH2PO4, 6.90 mg K2CO3, 17.75 mg Na2SO4, 289.18 mg NaHCO3, 2.46 mg NaClO4 (i.e. 2 mg ClO4–), 10 mL trace metal solution. The trace metal solution contained, per liter: 1.5 g CaCl2 · 2H2O, 0.55 g MnCl2 · 4H2O, 0.12 g NiCl2 · 6H2O, 85 mg CuCl2 · 2H2O, 2.5 g MgSO4 · 7H2O, 0.28 g FeSO4 · 7H2O, 67.8 mg ZnCl2, 0.6 mg H3BO3. All chemicals used were of ACS grade.

The carriers are produced by an extrusion process combining two substances with different properties concerning their biodegradability, that is (1) an easily degradable starch and (2) a less easily degradable PBS. Carrier structure can be characterized as a lumped mixture of both components, thus, pores will develop during the cultivation, which provide niches for microbes growth under anaerobic and anoxic conditions.

The mechanical stability of the carriers remains rather high, so that increasing porosity will not lead to mechanical dispersion at moderate cultivation conditions. Size shrinkage can mainly be ascribed to microbiological consumption of carrier material. The particles are of cubic to cylindrical shape of 3.5 mm edge length. The main characteristics of the carriers are given in Table 1.

In the bench-scale testing of this paper, especially in the batch experiments, inoculum from activated sludge was introduced to quickly develop strong biofilm with perchlorate reducing bacteria (PRB). The biofilm could be used to evaluate the feasibility of the biodegradable polymers bioreactor concept for removal of perchlorate in a short period of time. Activated sludge used in the present study was sampled from the biological reaction basin of an urban wastewater treatment plant (WWTP) in Jinan, China. The sampling point was set at the outlet of the aeration basin; it was thus inferred that in the sampled sludge liquor there was almost no remaining organic carbon that originally existed in the influent and could be easily degraded by microbes populated in the activated sludge. The sludge was cultured for 3 d in a liquid medium (KCl 2 g/L; K2PO4 0.5 g/L; MgSO4 7H2O 0.2 g/L; C6H2KNaO4 4H2O 20 g/L) and then used as inoculum seeding in the perchlorate removal reactor.

### Table 1 | Main characteristics of the starch/PBS polymer carriers

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Characteristics and value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Material</td>
<td>Starch 60%: PBS 40% composite</td>
</tr>
<tr>
<td>Color</td>
<td>Light yellow</td>
</tr>
<tr>
<td>Diameter</td>
<td>3.5 mm</td>
</tr>
<tr>
<td>Height</td>
<td>3.5 mm</td>
</tr>
<tr>
<td>Density</td>
<td>1.25 kg/m³</td>
</tr>
<tr>
<td>Draw intensity</td>
<td>≥15 MPa</td>
</tr>
<tr>
<td>Specific surface area</td>
<td>1,725 m²/m³</td>
</tr>
</tbody>
</table>
and nitrate’s spiking concentrations, for which six runs were carried out using the starch/PBS polymer biofilm reactor spiked with ClO$_4^-$ and NO$_3^-$ at 2, 4 and 8 mg/L, 20, 30 and 50 mg/L, respectively. The second series, comprising three runs under three liquor temperatures (15, 20 and 30°C), was devised for examining the likely impacts of water temperature. The bottles were shaken (80 rpm) in the dark throughout the experiment. Samples were taken periodically for nitrate and perchlorate analyses.

**Column experiments**

PVC column used as reactors were 35 cm high with an inner diameter of 30 mm, and with sampling ports every 10 cm on the side. Polymer granules were placed into the reactor. A thin layer of glass wool was placed at both ends of the packing material. After the granules were packed, medium and inoculum were dosed into the column. The inoculated reactor was allowed to stand for 3 d before flow was initiated. The flow created an up-flow through the column with a rate regulated by a peristaltic pump (BT01-100). All experiments were conducted in light-tight conditions by cover of aluminium paper (see Figure 1), and under the simulation condition of water treatment field. Temperature of the bioreactor system was in the range of 15–20°C. No pH and dissolved oxygen (DO) control throughout the experimental period, pH was 7.5 ± 0.5 and DO was 1.8 ± 1 mg/L, respectively. Samples were taken from side ports and effluent periodically for perchlorate analyses.

The inlet concentration of perchlorate was kept at about 2 mg/L. During the startup period, the columns were operated at a water hydraulic loading rate of 0.9 mL/min (HRT = 4 h). Flow rate was increased to match a hydraulic loading rate of 1.8 mL/min (HRT = 2 h), and later 3.6 mL/min depending on the treatment performance. Effluent samples were taken periodically for perchlorate analysis. Random effluent samples were selected for dissolved organic carbon (DOC) measurements.

**Analytical methods**

For each sampling, about 6 mL of the mixed suspension was taken into a 10 mL centrifuge glass tube and centrifuged immediately at 3,500 rpm for 1 min, followed by prompt filtration of the supernatant through a pre-washed 0.45 μm membrane filter. The dissolved organic carbon from starch/PBS polymer in the filtrates were removed through C18 pillars and finally through a 0.2 μm membrane filter before being subjected to analysis. The concentrations of ClO$_4^-$ was determined using an ion chromatograph (Dionex, ICs2000) equipped with a suppressed conductivity detector, an AS20 column, and an AG20 guard column.

The analysis of ClO$_4^-$ in the filtrate (the samples were first passed through Ag Dionex filters to remove Cl$^-$) was made using a mobile phase of 35 mM of NaOH (flow rate 1 mL/min). Under these conditions, the detection limit was 4 μg/L because it was necessary to inject a smaller sample volume (250 μL) due to the presence of other anions in the synthetic groundwater, which interfere with the perchlorate analysis by overlapping the perchlorate peak. For the determination of NO$_3^-$, the mobile phase (flow rate 1 mL/min) was a 5 mM solution of NaOH. The detection limit was 0.1 mg/L (20 μg/L NO$_3^-$).

The morphology of the biofilm attached to the carriers and the raw and used starch/PBS polymer carriers in column after being washed with deionized water were examined by a scanning electron microscope (SEM, JSM-6700F, JEOL, Japan). Biomass samples from the polymer carriers using denaturing gradient gel electrophoresis (DGGE) analysis and partial sequencing of 16S rRNA genes analysis to study the microbiology community composition after perchlorate exposure.
RESULTS AND DISCUSSION

Concentration behavior of perchlorate in starch/PBS polymer batch bioreactor

Effect of spiking ClO₄⁻ concentration

The microcosm degradation curves of ClO₄⁻ spiked with varying concentrations in polymer batch bioreactors are illustrated in Figure 2.

The starch/PBS polymer biofilm reactor, which was treated at the initial ClO₄⁻ concentration of 2 mg/L, corresponding to a typical perchlorate-contaminated site and indicating the degradation of ClO₄⁻, took place in a manner much faster. After running for 5 h, the residual ClO₄⁻ in the reactor fell to only about 500 μg/L. Complete disappearance of the spiked 2 mg/L ClO₄⁻ was noticed in association with the data point after running almost for 9 h. In addition, the results from Figure 2 indicate that the higher the perchlorate concentration, the faster the perchlorate removal rate (from 0.23 to 0.37 mg/L/h), and this was confirmed at all three ClO₄⁻ concentration levels. This represents a rise in the removal rate by about 37% as the ClO₄⁻ concentration was promoted from 2 to 8 mg/L.

The results shown above resembled the result reported by Son et al. (2006) that iron-supported mixed cultures completely removed 65 mg/L of perchlorate in batch reactors in 8 days. The removal rate was similar to that observed with hydrogen gas (5%) and acetate (173 mg/L) as electron donors (Shrout et al. 2006). The removals observed in the latter study were almost equal compared to the removals observed in the present study. Although the reason behind the removal differences is still unclear, the differences in the bacterial population densities, types and activeness, which were in turn affected by such parameters as the geographic location, operation condition and influent water characteristics (including inorganic nutrients and organic substrates, etc.) of the treatment reactors were probably responsible.

Effect of nitrate presence

Nitrate is often a co-contaminant with ClO₄⁻ due to fertilizer application or explosives, and many sites contaminated with ClO₄⁻ also contain high nitrate levels. However, conflicting results have been reported on the effect of nitrate on ClO₄⁻ degradation. The concentration profiles of NO₃-N conducted under the constant ClO₄⁻ concentration (2 mg/L) spiked with varying NO₃-N concentrations (0, 20, 30 and 50 mg/L) in starch/PBS polymer batch bioreactors are displayed in Figure 3.

From Figure 3, it can be seen that perchlorate removal under the initial concentration of 20 mg/L of NO₃-N was almost matched to the absence of NO₃-N in the reactor, which indicated that simultaneous perchlorate and nitrate degradation were occurring in the polymer reactor if perchlorate and nitrate concentration corresponded to a typical perchlorate-contaminated site. Effluent ClO₄⁻ increased from below the detection limit to 233 and 571 μg/L after 9 h from the spiked concentrations of 2,000 μg/L under the variable initial concentrations of 20, 30 and 50 mg/L of NO₃-N, respectively. The results showed that reduction of 2 mg/L perchlorate decreased slightly with the addition of 20–50 mg/L NO₃-N when sufficient organic carbon was supplied even after complete denitrification. However, increasing the influent nitrate concentration did result in relatively higher effluent perchlorate despite being not significant. The fact that the profiles for the two electron acceptors virtually overlay one another suggests that when sufficient substrate is present, these reduction processes can occur in parallel. Complete denitrification was observed in the starch/PBS polymer reactors with nitrate and perchlorate coexisting in the aqueous solution, and no nitrite was detected in any samples, indicating that induction of denitrification was very rapid in the mixed culture biofilm. Other researchers observed transient nitrate
accumulation after nitrate was added to perchlorate cultures grown on perchlorate (Herman & Frankenberger 1999).

**Effect of temperatures**

Seasonal temperature fluctuations in ground water, if present at all, are mild compared to those observed in many surface waters. Given the number of surface water perchlorate detections, an investigation of temperature impacts on biological treatment would be a useful addition to the existing body of knowledge on perchlorate treatment. Influent temperatures varied from 13 to 30 °C and were examined to study the effect on removing efficiency of perchlorate in starch/PBS polymer batch bioreactors and the results are displayed in Figure 4.

At all three temperature levels, the lower the temperature the higher the residual ClO$_4^-$ concentrations, thus indicating a significant impact of liquor temperatures on the biodegradation of ClO$_4^-$ over the studied range of 15–30 °C. The spiked ClO$_4^-$ (2 mg/L) decreased to 1.1, 0.7 and 0.2 after 5 h at 13, 20 and 30 °C, respectively, with the residual concentration at 13 °C being about two- and five-fold larger than that at 20 and 30 °C. This represents a reduction in the removal by about 20% (from 65 to 45%) as the temperature was lowered from 20 to 15 °C; and a further reduction by about 45% (from 90 to 45%) as the temperature was lowered from 30 to 13 °C. After 5.5 h, the spiked ClO$_4^-$ disappeared from the aqueous phase at 30 °C; at 20 and 13 °C, however, its residuals were still detected even if the levels were relatively low.

![Figure 3](https://iwaponline.com/wqrj/article-pdf/49/3/234/379413/234.pdf)  
**Figure 3** | Concentration profiles of NO$_3$-N (left) and ClO$_4^-$ (right) with time at variable initial concentrations (temperature = 25 °C).

![Figure 4](https://iwaponline.com/wqrj/article-pdf/49/3/234/379413/234.pdf)  
**Figure 4** | Effect of temperature on the reduction behavior of perchlorate.
Moreover, according to the Arrhenius equation given below:

\[ k = k_{20} \times 10^{K_t (t - 20)} \]  

(1)

the following expression can be inferred:

\[ \log \left( \frac{k}{k_{20}} \right) = K_t \times (t - 20) \]  

(2)

where \( k_{20} \) is the rate constants (\( k \)) of perchlorate removal at 20 °C, \( K_t \) is the temperature coefficient, \( t \) is the time. From Figure 5, the inferred Arrhenius equation was given as below:

\[ k = k_{20} \times 10^{0.0316(t - 20)} \]  

(3)

This expression indicated that a statistically significant temperature effect on the degradation rate of \( \text{ClO}_4^- \) was achieved, a distinct trend, showing that the higher the temperature, the larger the magnitude of \( k \).

Several studies have also reported maximum perchlorate degradation at 30 °C for enriched mixed consortium (Ghosh et al. 2011). On the contrary, Wu et al. (2008) reported that a slight increase in the reaction temperature from 20 to 40 °C had a stimulatory effect on the rate of perchlorate reduction by an indigenous mixed culture using acetate as sole carbon source. Based on the findings obtained in the present study, it is also concluded that, to achieve higher \( \text{ClO}_4^- \) removals throughout a year by compensating for the adversary impacts under low temperatures, some operational measures that could bring about an increase in the microbes concentration level or the HRT may become necessary for treatment plants operating in cold areas or during the winter season of warm areas.

**Flowthrough column experiments**

With an inlet perchlorate concentration of 2,200 ± 200 μg/L and a flow rate of 0.9 mL/min (HRT = 4 h), perchlorate concentration in the effluent in treatment reached the detection limit of 4 μg/L within 20 days, and remained below this level for the remainder of the experiment (data not shown). Liquid samples were also taken from the side sampling ports on selected days to evaluate perchlorate penetration along the flow direction. The concentration profiles (Figure 5) showed that 70–85% of perchlorate removal was achieved in the first 10 cm of polymer column, and that removal to non-detect concentrations was achieved at the 20 cm mark.

Given the good treatment performance, the hydraulic loading rate was doubled to 1.8 mL/min on day 40 (Figure 6). After several days of fluctuation, complete removal of perchlorate was achieved in polymer reactor column, and the effluent perchlorate concentration was again below the detection limit of 4 μg/L. In addition, at the beginning of this operation stage, the effluent DOC concentration was as low
as 4–8 mg/L. This could be attributed to the change of flow rate on the 40th day, which led to the amount of perchlorate flowing into the bioreactor per hour increasing quickly. Therefore, small organic molecules degrading from starch/PBS polymer could be consumed rapidly and DOC level in the reactor decreased sharply. After the effluent perchlorate changed steadily, the effluent DOC level became relatively stable, that is, around 10 mg/L. DOC in the effluent was slightly higher than that of around 3 mg/L in the influent (synthetic groundwater), which could be attributed to the microbes metabolism and decomposition of the polymer. So a subsequent research topic needs detailed investigations of post-treatment in drinking water using biocompounds carriers for field-scale perchlorate removal in the future.

However, after increasing the hydraulic loading rate to 3.6 mL/min, there was only 40% removal in influent perchlorate. Therefore, the flow rate was reduced to 1.8 mL/min to determine whether the system would return to the initial performance. Low removal was observed in the first 10 cm of polymer column, and almost complete removal was observed in the 20 and 30 cm mark in the first few days after the adjustment. Then, perchlorate in effluent remained non-detectable until the end of the test. Perchlorate concentration profiles in polymer columns were not affected by time, indicating that treatment performance was stable over the duration of the experiment.

Gillham & Cherry (1978) reported that denitrification could produce in DO of 2 mg/L within groundwater environment. In the current study, mixed cultures within the biofilms on polymer were mostly composed of perchlorate reducing bacteria and included few aerobic bacteria, which could assimilate DO, on the surface of biofilm. Results from column tests showed that a small presence of DO in a groundwater environment could not have a significant effect on the perchlorate biodegradation due to the limited DO mass transfer.
Most importantly, the above results suggest that starch/PBS polymer is effective in water and wastewater treatment as the sole chemical and physical substrate for the microorganism for removing perchlorate. Moreover, starch/PBS polymer granules are a safer substrate when compared with traditional, liquid carbon sources. The synthetic organic substrate described here not only avoids the use of expensive carbon sources, but also offers an easily handled and sustainable method for water and wastewater treatment.

**Biofilm development and changes of starch/PBS polymer structure**

Examination of the carriers indicated that microbes gradually adhered to the surface of the polymer granules and thoroughly covered the surface of the granules within a week. Thin microbial films then formed and became gradually thicker, changing color from pale yellow to brown and finally dark brown. The thickness of the formed microbial film reached ca. 1 mm in the hydrated state. Figures 7(a) and 7(b) show that biofilm coverage on the outer surfaces of the carriers was dense, with a good layered structure and primarily composed of bacillus and coccus.

DGGE analysis and partial sequencing of 16S rRNA genes recovered from these films on carriers inside the column reactor revealed sequences of organisms known to reduce perchlorate (Figure 8). Results showed that changes of species and quantity of microbes in biofilm were observed with the increase of column height. Band P1 was closely related to the sequences of *Dechloromonas/Dechlorosoma*, two β-Proteobacteria, the group which many perchlorate-reducing bacteria have been isolated from wastewater treatment systems (Bruce et al. 1999; Logan et al. 2001; Zhang et al. 2002; Waller et al. 2004). Band P2 was 100% identical to the sequence of *Dechlorospirillum sp.* strain W/D, isolated from a swine lagoon (Coates et al. 1999). The organism whose sequence was closely related to the sequence of band P3, *Bacillus cereus*, has also been reported to be a perchlorate reducer (Hackenthal et al. 1964).

Additionally to the macroscopic biofilm structure, the development of the carrier shape and size can be seen in Figures 7(c) and 7(d). Due to the subsequent degradation of the carrier material, the diameter decreased over the cultivation time. Especially during the experimental phase, the pore and filament structure on the carrier surface developed due to consumption of carbon source on the carrier surface by microorganisms, while the surface of the starch/PBS polymer raw material was smooth and had no pore and filament. The deepness of the pores reached several hundred micrometers and, thus, provided space for anoxic and anaerobic microbial activity. Inside the pores of the carrier large amounts of bacteria could be detected.

The results indicated that after biofilm formed, the microorganisms in biofilm decomposed starch/PBS polymer through metabolism and provided a carbon source for themselves by releasing small organic molecules, causing the changes of polymer surface morphology. The process could be assumed as follows: with the degradation of starch by microbes, small pores or channels develop on the carrier surface. As a result, the protected surface area available for slow-growing microorganisms, which are susceptible to detachment from the outer surfaces, becomes greater. Therefore, in addition to acting as an extra carbon source for microorganisms, the carriers provide microniches for biofilm growth. In particular, those pores will provide microniches for bacterial species with different requirements concerning...
perchlorate and oxygen concentration, since concentration gradients will occur within the pores due to turnover and limited mass transfer. This might allow for rapid perchlorate removal.

CONCLUSIONS

The results presented clearly show that the biocompound carriers allow for the removal of perchlorate from contaminated groundwater to below their recommended limits, without secondary contamination. Simultaneous perchlorate and nitrate degradation occurred with the starch/PBS polymer granules as carbon source and biofilm carriers. A statistically significant temperature effect on the degradation rate of \(\text{ClO}_4^-\) was achieved with a distinct trend showing that the higher the temperature, the larger the magnitude of \(k\). Complete perchlorate removal was observed at a suitable hydraulic loading rate. Images prove the concept of the pore and filament structure within the biocompounds, which provide both a heterotrophic biofilm and carbon source. DGGE analysis and partial sequencing of 16S rRNA genes indicated that formerly reported perchlorate-reducing bacteria were present in the polymer particle-fixed biofilm reactors.

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