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THE EFFECT OF OXYGEN ON PERCHLORATE REDUCTION IN A BIOFILM REACTOR

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Abstract : The purpose of this research was to investigate the effects of low concentration of oxygen on reduction of perchlorate, especially low perchlorate influent concentrations in a biofilm reactor, as well as the effect of flow pattern in a biofilm reactor. Dissolved oxygen averaging 1 mg/L did not inhibit reduction of influent perchlorate from 23 to 426 μg/L in the biofilm reactors when sufficient acetate was added, probably due to limitation of oxygen diffusion into the biofilm. Influent perchlorate ranging from 23 to 426 μg/L was reduced to below detection level (4 μg/L) in the presence of 1 mg/L dissolved oxygen (DO). Chloride was produced in a ratio of $0.37gCl^-/gClO_4^-$ and $0.35gCl^-/gClO_4^-$ in plug flow and recirculation biofilm reactor which is similar to stoichiometric amount ($0.36gCl^-/gClO_4^-$) indicating complete perchlorate reduction at 426 μg/L of ClO_4^- feeding. At 23 μg/L influent perchlorate, total biomass solids were 3.18 g and 2.81 g in the plug flow and recirculation biofilm reactors. The most probable number (MPN) analysis for perchlorate-reducing bacteria showed 10^4 to 10^5 cells/cm^2 in both biofilm reactors throughout the experiments. The effluent perchlorate concentrations were not significantly different in the two different flow regimes, plug flow and recirculation biofilm reactors.

Key Words : Biofilm reactor, Oxygen, Perchlorate

INTRODUCTION

Perchlorate contamination of groundwater in areas of the US is primarily a result of release of ammonium perchlorate salts from rocket and missile propellants used in military and aerospace applications onto soils. The highly soluble perchlorate ion is transported in groundwater with almost no retardation.[1)] According to the report of National Academy of Sciences (NAS) in 2005, perchlorate at concentrations above 4 μg/L has been detected in public water supplies serving over 11 million people through US.[2)] Recently, the US Food and Drug Administration (USFDA) reported that perchlorate also has been found in foods such as milk and lettuce in 15 states using a more sensitive LC-MS-MS method.[3)]

The primary human health effect of perchlorate has been reported as the interference with iodine uptake by the thyroid grand. Perchlorate is also a suspected endocrine disruptor.[1,4)] Health risks at low concentrations of perchlorate are not well quantified, so a Federal drinking water standard is still being debated. The California Department of Health Services (CDHS) established a drinking water action level of 18 μg/L for perchlorate in 1997, which was lowered to 4 μg/L in 2002. In 2004, the Office of Environmental Health Hazard Assessment (OEHHA) established 6 μg/L of perchlorate as a public health goal (PHG). Based on this PHG of OEHHA, CDHS revised its action level to 6 μg/L in 2004.[5)]

Bacteria reduce perchlorate (ClO_4^-) to chloride

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(Cl^-) in diverse environments.[6,7] It has been suggested that perchlorate is produced in the atmosphere by oxidation of chloride ions transported from the ocean with perchlorate then deposited on land by precipitation.[8] This cycle would contribute to widespread bacteria populations with the capability to reduce perchlorate during respiration. Most perchlorate reducing bacteria are heterotrophic facultative anaerobes that use many organic compounds as electron donors in a process that has similarities to denitrification.[9-11]

Biofilm treatment processes have been primarily considered for perchlorate reduction because suspended culture process such as activated sludge cannot easily maintained with dilute influent. However, groundwater contaminated with low concentration of perchlorate above the drinking water standard may be difficult to treat in a biological reduction process even with addition of a carbon/energy substrate because of transport of substrate to biofilm and inability to sustain even an attached biomass. In addition, there have been few studies on the effect of biofilm reactor flow and transport conditions on competitive inhibition of perchlorate respiration by bacteria. As reported by Choi and Silverstein, recirculation appears to result in improved reduction of perchlorate in a biofilm reactor under sudden increasing perchlorate loading condition, possibly due to reduced transport resistance and more uniform biomass distribution.[12]

The effect of competing anions on bacterial perchlorate reduction is important because nitrate from anthropogenic sources is a common co-contaminant of perchlorate in groundwater, and both groundwater and surface water with perchlorate contain variable amounts of dissolved oxygen. Both perchlorate reduction and denitrification are inhibited by oxygen, a more energetically-favorable electron acceptor.[11,13,14] However, the relation between perchlorate and nitrate reduction, particularly, is still a matter of controversy in mixed cultures. In addition, the effect of the relative concentration of competing electron acceptors on perchlorate reduction has not been well investigated. Therefore, the objective of this research was to investigate the effect of flow recirculation on the inhibition of perchlorate reduction by oxygen in a fixed biofilm reactor.

MATERIALS AND METHODS

Two identical biofilm reactors packed with high-porosity plastic media were operated with an 8-hour empty bed contact time (EBCT). Influent to one column was treated in a dispersed plug flow regime, while the second reactor had internal recirculation at 20:1 of recirculation:influent flow rate.

Bench Scale Biofilm Reactors

Biofilm reactors were constructed with acrylic columns (69 cm long, 15.24 cm ID) packed with cylindrical plastic pall rings (Jaeger Products, Inc.) as biofilm support media. Anoxic conditions in the bioreactors were maintained by sealing the top plate with a rubber gasket during experiment. A plastic screen was positioned at 5.1 cm from the column bottom to prohibit biofilm clogging at the feeding inlet. Plastic media were packed on the top of this screen. After finishing all experiments 1,572 rings and 1,620 rings were in plug-flow and recirculation reactors. The experimental system parameters are summarized in Table 1 with the physical properties of plastic media (rings). Both reactors were operated upflow mode and influent flow rates were 37.5 L/day based on empty bed hydraulic residence time of 8 hour. Internal recirculation flow rate was 750 L/day that means 20 times greater than influent flow rate.

Figure 1 is a schematic of the bench-scale biofilm reactors. There were three sampling ports (base, middle, and top) to investigate transport effect in the reactor. These sampling ports consisted of thread fittings with rubber septa. Liquid and attached biofilm (plastic rings) samples were collected from the sampling ports.

A synthetic groundwater containing perchlorate was made with deionized water. Perchlorate as $NaClO_4 \cdot H_2O$ was added to set 426, 84, and 23 μg/L of ClO_4^-. Acetate, nitrogen and phosphorous

Table 1. Experimental system parameters in biofilm reactors

Empty bed reactor volume	12.5 L
Empty bed contact time (EBCT)	8 hours
Recirculation rate	20 times faster than feeding
Media characteristics (Jaeger rings)	
diameter	1.6 cm
surface area	354 m^2/m^3
specific gravity	0.9
void space	86%

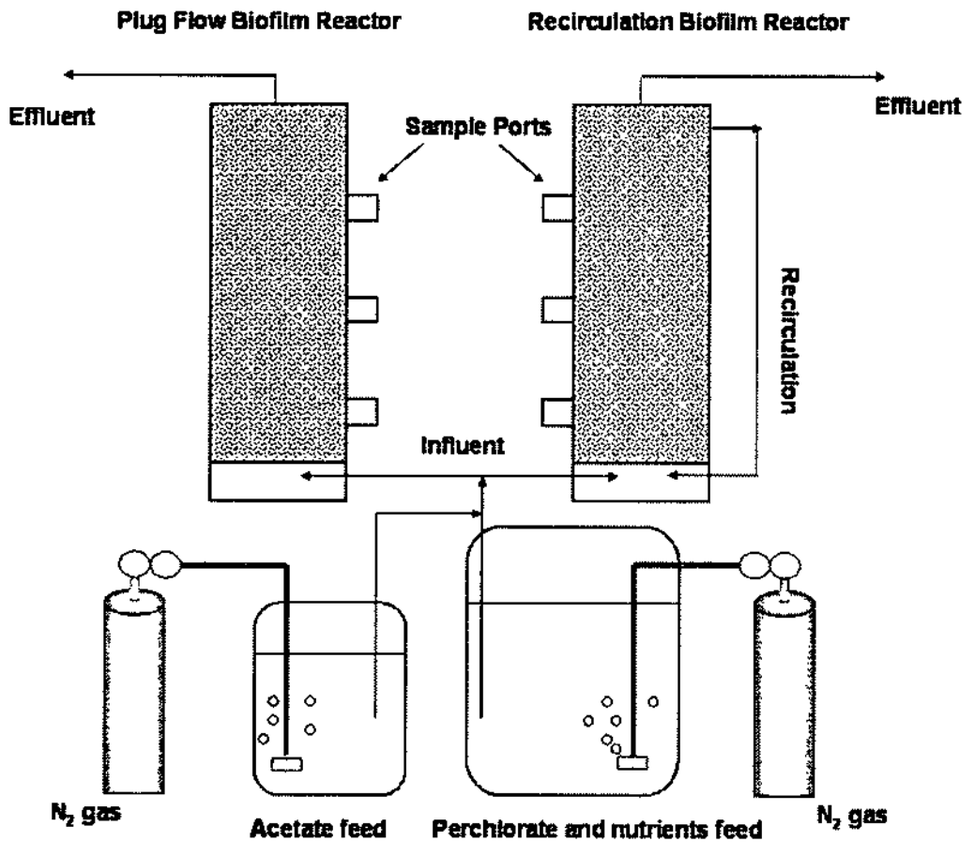

Figure 1. Schematic diagram of bench scale biofilm reactors.

were added as food vinegar (CH_3COOH), $(NH_4)_2SO_4$, and K_2HPO_4. Ammonium and phosphate (nutrients) were to maintain the C:N:P ratio of 100:12:3. $NaHCO_3$ was used to achieve alkalinity of 100 mg/L as $CaCO_3$. In addition, 51.25 mg/L of $MgSO_4 \cdot 7H_2O$ and 43.0 mg/L of $CaSO_4 \cdot 2H_2O$ (5 mg/L of Mg^{2+}, and 10 mg/L of Ca^{2+}) were added as trace nutrients. Fe^{2+} and other trace minerals required for biofilm growth were not added because acclimated returned activated sludge was expected to have sufficient trace nutrients.

The biofilm reactor columns were inoculated with returned activated sludge that had been acclimated to perchlorate reduction in the SBR under anoxic conditions for several months. Five liters of acclimated culture was transferred from the SBR into each of the biofilm reactors. The reactors were filled to the top with tap water. The system sat for 2 days before feeding and operation started to encourage attachment of bacteria.

To prohibit growth of bacteria in the feeding tanks, two separate resealable plastic tanks (220 L and 120 L) were used as feeding reservoirs for a synthetic perchlorate/nutrients/mineral water and carbon source. Synthetic water was fed into the reactors by peristaltic pumps (Cole-Parmer, Chicago, IL). Nitrogen gas was blown directly into the feeding tanks maintain 1 mg/L of dissolved oxygen (DO) controlled by flowmeters (Cole-Parmer).

Analytical Methods

Liquid was sampled through each sampling port in the biofilm reactor columns by using 6 inch (15.24 cm) long septum penetration needles. All samples were filtered with a 0.2 μm syringe filter and kept in refrigerator (4°C) before analysis. All anions (ClO_4^-, Cl^- and CH_3COO^-) were measured by using an ion chromatograph (Dionex model DX300, CA) with IonPac AS11 analytical and AG11 guard column. Perchlorate was measured with 100 mM NaOH eluent and a 1,000 μL sample loop. Chloride and acetate concentrations were measured using 5 mM NaOH eluent and a 10 μL sample loop. The perchlorate detection limit was 4 μg/L with this protocol.

The plastic media attached biofilm taken from each sample port were dried at 104°C and then weighed according to the Standard Method.[15] The weight of the dried media and biomass was then compared to the clean media weight. Used rings were not sacrificed because biofilm growth on the new clean rings would require a long start-up time.

A modified most probable number (MPN) procedure[16] was used to determine the activity of a microbial population (perchlorate reducing

bacteria) in the reactor column. The MPN technique was performed at the end of each phase of experiments using 10-fold serial dilutions of eight replicates placed into 0.32 mL wells of sterile tissue culture plates (Falcon, Becton Dickinson Company). The MPN plates were incubated in the dark in anaerobic chamber filled with N_2 gas for 5 weeks at lab room temperature. After incubation, the wells were scored positive or negative by color change (from blue to pink) of redox dye (resazurin) added to the media. Resazurin was reported as a good indicator of microbial heterotrophic growth.[17] Therefore, resazurin was used as redox dye for viable perchlorate reducing bacteria.

RESULTS AND DISCUSSION

The effect of dissolved oxygen on perchlorate reduction was investigated in a series of experiments where influent perchlorate was 426, 84, and 23 μg/L with 1 mg/L influent dissolved oxygen (DO). The respective influent perchlorate loading rates were 1.38, 0.27, and 0.07 $g/m^3/day$.

With one exception, perchlorate was reduced to below detection level (< 4 μg/L) in the effluent in all these experiments in both the plug flow and recirculation biofilm reactors. In the plug flow column receiving 426 μg/L of perchlorate, the effluent averaged 6 μg/L. In this experiment, the ClO_4^- concentration at top part of plug flow column was below detection level, but the effluent concentration was higher, likely the result of short circuiting (Figure 2).

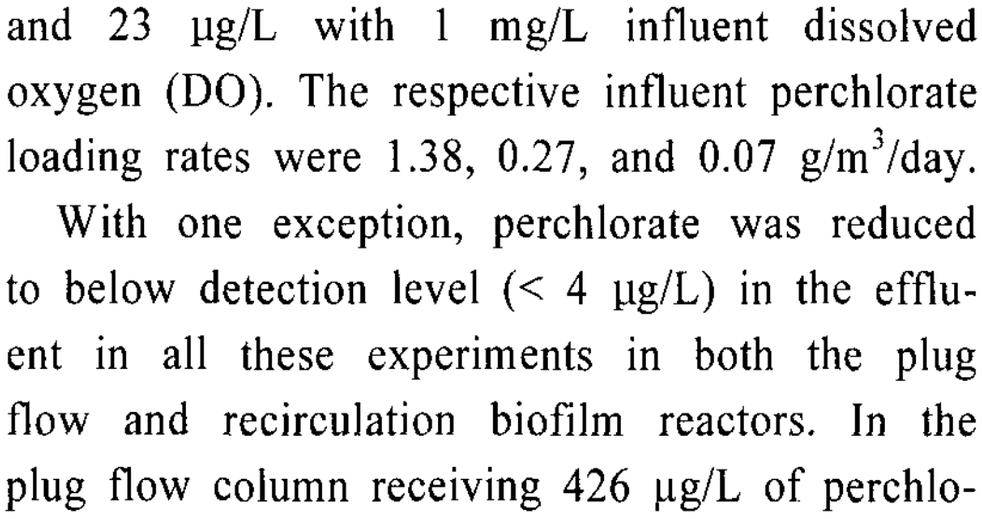

Figure 2. ClO_4^- profiles in biofilm reactors with 426 μg/L influent ClO_4^- and 1 mg/L influent DO. Error bars are ± one standard deviation.

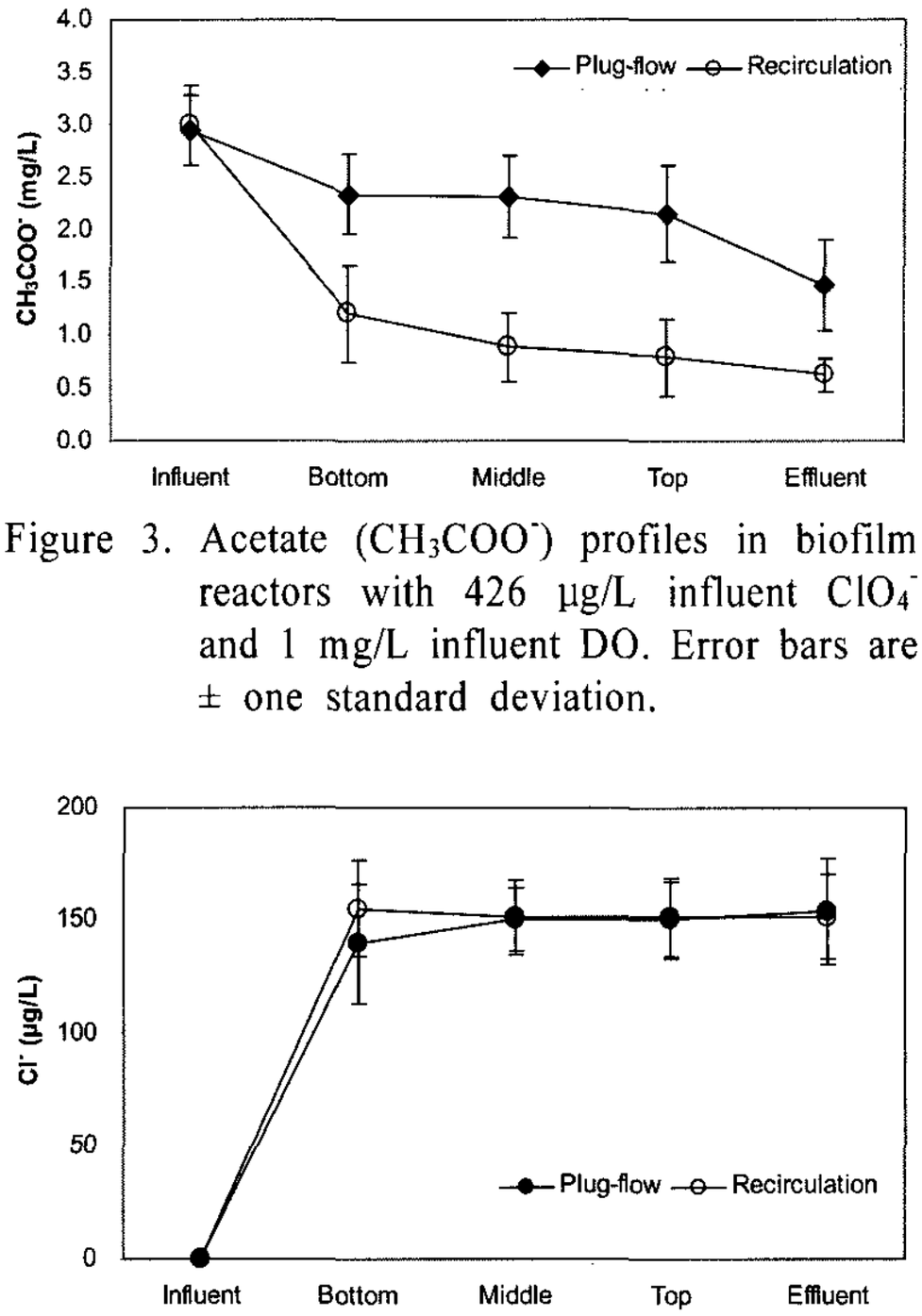

Figure 3. Acetate (CH_3COO^-) profiles in biofilm reactors with 426 μg/L influent ClO_4^- and 1 mg/L influent DO. Error bars are ± one standard deviation.

Figure 4. Cl^- profiles in biofilm reactors with 426 μg/L influent ClO_4^- and 1 mg/L influent DO. Error bars are ± one standard deviation.

Acetate consumption was higher with flow recirculation compared with the plug flow biofilm reactor (Figure 3), possibly due to entrainment of air during recirculation flow pumping. Chloride was produced in a ratio of $0.37gCl^-/gClO_4^-$ and $0.35gCl^-/gClO_4^-$ in plug flow and recirculation biofilm reactor which is similar to stoichiometric amount ($0.36gCl^-/gClO_4^-$) indicating complete perchlorate reduction at 426 μg/L of ClO_4^- feeding (Figure 4).

DO concentrations were significantly not changed along the column in both reactors during this experiment. In this experiment, higher DO concentrations were measured in the effluent than in the influent because oxygen may have been introduced from the atmosphere to effluent tubing by the peristaltic pump.

Results of these experiments showed that 1 mg/L of dissolved oxygen did not inhibit perchlorate reduction in either of the biofilm reactors provided sufficient acetate was added. Statistical

Table 2. Perchlorate profiles in the plug flow and recirculation bioreactors receiving 426 μg/L influent ClO_4^- in the presence of 1 mg/L DO

Location	Plug flow Perchlorate (μg/L)	Recirculation perchlorate (μg/L)	P (2-tailed)
Influent	420 ± 18	432 ± 19	
Base	43 ± 90	14 ± 4	
Middle	3 ± 9	11 ± 4	
Top	1 ± 2	8 ± 4	
Effluent	6 ± 14	1 ± 2	0.325

Criterion for significant difference of mean effluent ClO_4^- concentration in a two-tailed t-test was $p \leq 0.05$.

Table 3. Perchlorate profiles in the plug flow and recirculation bioreactors receiving 84 μg/L influent ClO_4^- in the presence of 1 mg/L DO

Location	Plug flow Perchlorate (μg/L)	Recirculation perchlorate (μg/L)	P (2-tailed)
Influent	84 ± 1	84 ± 2	
Base	13 ± 15	0 ± 0	
Middle	1 ± 3	0 ± 0	
Top	0 ± 0	0 ± 0	
Effluent	1 ± 2	0 ± 0	0.363

Criterion for significant difference of mean effluent ClO_4^- concentration in a two-tailed t-test was $p \leq 0.05$.

comparisons of effluent perchlorate by two tailed t-tests are shown in Table 2 and 3, and show no significant difference between the plug flow and recirculation operation.

An F-test of variance for the experiment with (nominally) 426 μg/L influent ClO_4^- showed significantly higher variability in the effluent from the plug flow column compared with recirculation indicating that recirculation improved the consistency of perchlorate removal (data not shown). This may have been due to flow irregularities in the plug flow column such as short circuiting. This is supported by the fact that average perchlorate at the middle and top sample ports were lower than in the effluent. Acetate consumption for reduction of 426 μg/L ClO_4^- with 1 mg/L DO in the plug flow reactor was 3.4 g-CH_3COO^-/g-ClO_4^- the same as measured in the low DO (0.2 mg/L) experiment in the Choi and Silverstein's report.[12] One might have expected higher acetate consumption with higher DO; however 1 mg/L was still relatively low dissolved oxygen (DO) and may not have stimulated acetate consumption in the plug flow biofilm reactor because the diffusion of DO into the biofilm was limited.

All experiments with dissolved oxygen addition were conducted within a five-month period and during these experiments, total biomass was ranging from 1.22 to 3.49 g as summarized in Table 4. The most probable number (MPN) analysis for perchlorate-reducing bacteria showed 10^4 to 10^5 cells/cm^2 in both biofilm reactors throughout the experiments. The MPN measurements during these experiments were not significantly different either in response to changing influent perchlorate concentration or reactor flow conditions.

Statistical analyses(t-test) comparing reduction of (nominal) 426 μg/L perchlorate in the biofilm reactors with and without influent DO are shown that a relatively low concentration of dissolved oxygen (1 mg/L) did not inhibit perchlorate reduction in either of the biofilm reactors when excess acetate was added (data not shown).

Competitive inhibition of perchlorate reduction by oxygen has been reported consistently, and while the results of these experiments appear to contradict that virtually universal observation, lack of inhibition was probably due to the combination of relatively low bulk water dissolved oxygen levels and limitation of oxygen diffusion in the biofilm.

The capacity to sustain perchlorate reduction at DO levels that are not uncommon in ground-

Table 4. Total solids and average MPN of perchlorate reducing bacteria in the plug flow and recirculation bioreactors during experiment with 1 mg/L dissolved oxygen in the influent

Condition: Influent ClO_4^-	Total Biomass (g)		ClO_4^- reducing bacteria (average MPN/cm^2)	
	Plug flow	Recirculation	Plug flow	Recirculation
426 µg/L	1.22	3.49	$1.02*10^5$ ($\pm$ $6.00*10^4$)	$2.41*10^5$ ($\pm$ $6.44*10^4$)
84 µg/L	3.43	2.12	$7.20*10^4$ ($\pm$ $5.29*10^4$)	$1.77*10^5$ ($\pm$ $5.59*10^4$)
23 µg/L	3.18	2.81	$8.27*10^4$ ($\pm$ $9.06*10^4$)	$4.04*10^4$ ($\pm$ $4.75*10^4$)

Table 5. Oxygen profiles in the biofilm reactors from mean and standard deviation of sample data

	Inf. 426 µg/L ClO_4^-		Inf. 84 µg/L ClO_4^-		Inf. 23 µg/L ClO_4^-	
	PF	Recir.	PF	Recir.	PF	Recir.
Influent	0.9 (±0.1)	1.0 (±0.2)	1.0 (±0.1)	0.9 (±0.1)	0.8 (±0.1)	0.9 (±0.1)
Base	1.1 (±0.1)	1.1 (±0.2)	1.1 (±0.1)	1.0 (±0.1)	0.9 (±0.1)	1.0 (±0.1)
Middle	1.0 (±0.1)	1.0 (±0.1)	1.0 (±0.1)	1.0 (±0.1)	0.9 (±0.1)	1.0 (±0.1)
Top	1.1 (±0.1)	1.1 (±0.1)	1.0 (±0.1)	1.1 (±0.1)	0.9 (±0.1)	1.0 (±0.1)

Inf.: Influent
PF: Plug flow bioreactor
Recir.: Recirculation bioreactor

water is a benefit of the fixed biofilm process, and provision of excess substrate might not even be required if DO levels remained relatively low. Table 5 shows the oxygen profiles in the both biofilm reactors during experiments in the presence of oxygen. Dissolved oxygen levels remained fairly constant through both bioreactor columns, indicating little aerobic growth.

As noted previously, maintenance of a stable biomass under low substrate conditions with competing inhibition is a significant problem for typical bioreactor. However, the results of this research indicate that fixed biofilm reactors are reliable for treatment of 23 µg/L of perchlorate influent with 1mg/L of DO.

CONCLUSIONS

1. 1.0 mg/L dissolved oxygen did not inhibit reduction of influent perchlorate from 23 to 426 µg/L in either the plug flow or recirculation biofilm reactors when sufficient substrate was added, probably due to limitation of oxygen diffusion into the biofilm.
2. The range of calculated total biomass was from 1.22 to 3.49 grams dry weight in plug flow and recirculation biofilm reactors at 23 to 426 µg/L of perchlorate influent with 1.0 mg/L of dissolved oxygen.
3. The most probable number (MPN) for perchlorate-reducing bacteria was between 10^4 to 10^5 cells/cm^2 in both biofilm reactors receiving 23 to 426 µg/L of perchlorate with 1.0 mg/L of dissolved oxygen.
4. Total biomass and populations of perchlorate reducing bacteria measured by MPN method did not differ significantly in the plug flow and recirculation biofilm reactors.

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