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ScienceDirect
Journal of Hazardous Materials 153 (2008) 663–669
Journal of Hazardous Materials
www.elsevier.com/locate/jhazmat

Kinetics of biological perchlorate reduction and pH effect

Chao Wang [a], Lee Lippincott [b], Xiaoguang Meng [a,*]

[a] *Center for Environmental Systems, Stevens Institute of Technology, Hoboken, NJ 07030, USA*
[b] *New Jersey Department of Environmental Protection, P.O. Box 409, Trenton, NJ 08625, USA*

Received 5 February 2007; received in revised form 30 August 2007; accepted 3 September 2007
Available online 6 September 2007

Abstract

Batch experiments were conducted to investigate the kinetics of perchlorate reduction by heterotrophic and mixed perchlorate-reducing bacteria. Substrate-utilizing and cellular maintenance models were employed to fit the experimental data for microbial perchlorate reduction. The half saturation constant, K_s, obtained in this study was below 0.1 mg/L, which indicated that perchlorate-reducing bacteria are effective at utilizing low concentrations of perchlorate. The effect of pH on the kinetics of microbial perchlorate reduction was also studied. Perchlorate reduction occurred throughout the pH range from 5.0 to 9.0. Nevertheless, the rates of perchlorate removal by a unit mass of bacteria were significantly different at various pHs with a maximum rate at pH 7.0. The variation of q_{max} with pH was described well with a Gaussian peak equation. This equation is expected to be applicable for practical purposes when pH effects need to be considered.
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Keywords: Biological reduction; Perchlorate; Kinetics; pH effect

1. Introduction

Perchlorate (ClO_4^-), a key ingredient in rocket fuel and explosives, has emerged as a significant new threat to drinking water supplies and the environment in the USA [1]. The majority of anthropogenic perchlorate contamination stems from historical disposal practices by the aerospace and ordnance industries, the military and chemical manufacturers. Minor natural sources were also found in the hyperarid Atacama Desert, in Chilean nitrates with their derived nitrate fertilizer, in southeastern California caliche nitrate deposits [2,3], and many rain and snow samples formed by a variety of simulated atmospheric processes [4]. Studies have also detected perchlorate in commercial samples of lettuce [5] and milk as well as in human breast milk [6]. Perchlorate affects thyroid hormone function by inhibiting the uptake of iodide anion into the thyroid gland and subsequent thyroid hormone synthesis [7,8]. To minimize the health risk, the EPA set a drinking-water-equivalent level of 24.5 μg/L in February 2005.

Because perchlorate is kinetically stable and inert at low concentration, most traditional physicochemical water and wastewater treatment processes are not applicable to the removal and decomposition of perchlorate ion [9]. Biological approaches for treating perchlorate have proven to be effective and economically attractive [1]. Perchlorate-reducing bacteria are ubiquitous, easily obtainable in the environment [10], and mostly have a wide range of metabolic capacities [11]. The kinetic parameters for biological perchlorate reduction are thus necessary for designing biological treatment systems, and predicting and evaluating their performance. Previous studies [12–16] have reported some parameters for both mixed and pure heterotrophic bacteria, but the effect of pH on the rate of microbial perchlorate reduction by a unit mass of bacteria was not systematically studied. The quantitative relationship between pH and the rate of microbial perchlorate reduction is important for practical applications. Besides, previous reports [12–16] showed that the range of the half saturation constant, K_s, for mixed or pure heterotrophic bacterium was 2.2–33 mg/L. These values conflicted with the general conclusion that terminal electron acceptors often have K_s values at the μg/L level when mass transport is not included in K_s and simple electron-donor substrates are considered [17].

The purpose of this study was to investigate the pH effect on the kinetics of microbial perchlorate reduction by mixed and heterotrophic bacteria. Based on the obtained data, a quantitative relationship between pH and the rate of perchlorate reduction

* Corresponding author. Tel.: +1 201 216 8014; fax: +1 201 216 8303.
E-mail address: xmeng@stevens.edu (X. Meng).

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doi:10.1016/j.jhazmat.2007.09.010

by a unit mass of bacteria was established. Special importance was also attached to the examination of the low value of K_s at the μg/L level by fitting the numerically calculated data to the experimental results.

2. Methods

2.1. Cultivation and enrichment of perchlorate-reducing mixed bacteria

Unless noted otherwise, the fresh culture medium was a neutral (adjusted to pH 7.0 by 1.0 M NaOH) and anoxic solution (purged with oxygen-free nitrogen), containing per liter: 1.44 g NaH_2PO_4, 0.1 g $(NH_4)_2SO_4$, 0.1 g $MgSO_4$, 4.0 mg $FeSO_4$, 0.6 mg $Na_2MoO_4 \cdot 2H_2O$, 1.0 mg $NaSeO_3$, and 0.6 mg H_3BO_3.

Anaerobic sludge collected from the wastewater treatment plant in Bergen County, NJ was washed with tap water to remove the coarse solid particles in the sludge. The gravity-settled sludge was subsequently collected and used as the seed of heterotrophic perchlorate-reducing bacteria. The seed was then cultivated for the enrichment of perchlorate-reducing bacteria in a sealed container.

During each cycle of batch cultivation, the seed was mixed with a culture medium, which was spiked with 1000 mg/L acetate (sodium salt) and 500 mg/L perchlorate (potassium, ammonium, or sodium salt). After about 23 h mixing in each cycle, the seed was allowed to settle for about an hour. Two thirds of the supernatant was decanted and was replaced by fresh culture medium. 1000 mg/L acetate and 500 mg/L perchlorate were spiked again before the start of the next cycle. Repeated batch cultivation was continuously performed for about 2 months at our lab. Effective microbial perchlorate reduction was observed after some cultivation cycles. Rapid and repeatable perchlorate reduction was achieved before the end of cultivation (data not shown).

2.2. Batch kinetic tests

2.2.1. The maximum observed microbial yield on perchlorate

Twenty-one BOD bottles containing the same amount of biomass each were filled with 300 ml of fresh culture medium spiked with 1200 mg/L perchlorate and 2000 mg/L acetate. The bottles were then sealed with stoppers and covered with Parafilm (Fisher Scientific). They were shaken in an orbital incubator at a rotation speed of 150 rpm (revolutions per minute). Triplicate samples were taken at different times to measure the concentrations of biomass and the remaining perchlorate.

2.2.2. Microbial perchlorate reduction kinetics

Three BOD bottles containing the same amount of biomass and 300 ml of culture medium each were spiked with 24.1 mg/L of perchlorate and 500 mg/L of acetate. The bottles were sealed and shaken by the same methods as above. Triplicate samples (500 μl for each) were taken from the 3 BOD bottles at different times. The bottles were then filled up with DI (deionized) water, sealed again, and shaken again in the incubator. All samples were analyzed to measure the residual perchlorate concentrations.

2.2.3. pH effect on the microbial perchlorate reduction kinetics

The medium spiked with about 25.0 mg/L perchlorate and 500 mg/L acetate was adjusted to a pH range from 5.0 to 9.0 with 1.0 M HCl and 1.0 M NaOH. Twenty-one BOD bottles containing a specific amount of biomass each were evenly divided into 7 groups and were filled up with 300 ml of the medium at different pH values. They were then sealed and shaken by the same methods. Triplicate samples (500 μl for each) from each group were taken at different times. The bottles were then filled up with DI water, sealed again, and shaken again in the incubator. All samples were analyzed to measure the remaining perchlorate concentrations.

2.3. Analytical methods

Perchlorate concentrations were analyzed by ion chromatography in a Model IC25 (Dionex, Sunnyvale, CA) with a 4 mm × 250 mm AS 16 column (Dionex). Biomass concentrations were determined by the dry weight (DW) method. The biomass was collected by filtering the suspension through polycarbonate membrane filters (47 mm, 0.4 μm pore diameter) and rinsed with DI water. The filters in triplicates were dried at 105 °C for 4 h and cooled in a desiccator prior to being weighed.

3. Results and discussion

3.1. The maximum observed microbial yield on perchlorate

Mixed and heterotrophic perchlorate-reducing bacteria grow on the respiration of perchlorate. Bacteria growth occurred concurrently with the perchlorate degradation. Given sufficient acetate and nutrients, the kinetics of bacterial growth and perchlorate reduction can be described by the substrate-utilizing model (Eq. (1)) and cellular maintenance model (Eq. (2)) [18,19]:

$$\frac{\mathrm{d}S}{\mathrm{d}t} = -\frac{q_{\max} X S}{S + K_s} \tag{1}$$

$$\frac{\mathrm{d}X}{\mathrm{d}t} = \mu_{\max}\left[\frac{S}{S + K_s}\right] X - bX \tag{2}$$

where S is the perchlorate concentration (mg/L), t the time (h), $\mu_{\max}$ the maximum specific growth rate (h^{-1}), $q_{\max}$ the maximum specific substrate removal rate (h^{-1}), K_s the half saturation constant (μg/L), X the microbial concentration (mg/L), and b is the endogenous decay rate (h^{-1}).

The observed yield coefficient, Y, is defined as:

$$Y = \frac{\mathrm{d}X}{-\mathrm{d}S} \tag{3}$$

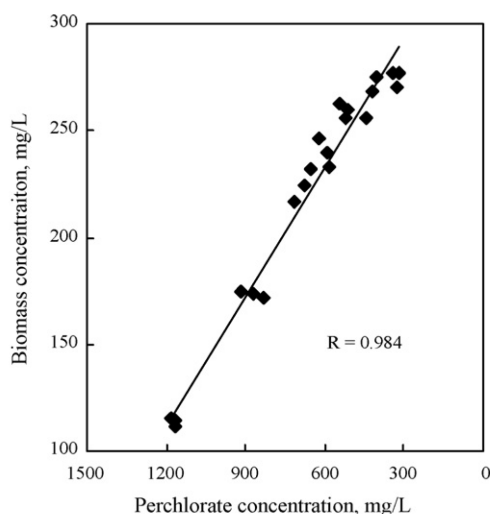

Fig. 1. The maximum observed yield on perchlorate for mixed and heterotrophic bacteria.

It can be further used to derive the maximum observed yield coefficient Y_{max} as follows:

$$Y = \frac{dX}{-dS} = \frac{dX/dt}{-(dS/dt)} = \frac{\mu_{max}[S/(S+K_s)]X - bX}{q_{max}XS/(S+K_s)} = \frac{\mu_{max}}{q_{max}} - \frac{b(S+K_s)}{q_{max}S} = \left(\frac{\mu_{max}}{q_{max}}\right)\left(1 - \frac{b(S+K_s)}{\mu_{max}S}\right) \quad (4)$$

When the perchlorate concentration is much higher than K_s ($S \gg K_s$), the Y_{max} is:

$$Y_{max} = \left(\frac{\mu_{max}}{q_{max}}\right)\left(1 - \frac{b}{\mu_{max}}\right) \quad (5)$$

Eq. (5) indicates that when $S \gg K_s$ the microbial yield on perchlorate is a constant. Thus by integrating Eq. (3) a linear relationship between X and S is derived:

$$X = X_0 + S_0Y_{max} - Y_{max}S \quad (6)$$

where X_0 is the initial biomass concentration (mg/L) and S_0 is the initial perchlorate concentration.

Y_{max} was therefore directly determined as showed in Fig. 1. The residual perchlorate concentration in Fig. 1 was controlled at a level higher than 300 mg/L, which is much larger than the known K_s values for mixed or pure perchlorate-reducing bacteria as summarized in Table 1. The results in Fig. 1 indicate that the maximum observed yield coefficient for the mixed and heterotrophic bacteria in this study is 0.20 mg-DW/mg-perchlorate.

3.2. Microbial perchlorate reduction kinetics

Microbial perchlorate reduction occurs concurrently with bacterial growth. The relationship between the time and the concentrations of biomass and perchlorate can be quantitively described by Eqs. (1) and (2). According to Eq. (5), the following equation for μ_{max} is obtained:

$$\mu_{max} = q_{max}Y_{max} + b \quad (7)$$

Substituting Eq. (7) into Eq. (2), we get:

$$\frac{dX}{dt} = (q_{max}Y_{max} + b)\left(\frac{S}{S+K_s}\right)X - bX \quad (8)$$

Thus the microbial perchlorate reduction coupled with bacteria growth can be described by Eqs. (1) and (8).

There are four parameters in these two equations. They are q_{max}, Y_{max}, b, and K_s. b should be relatively constant since it is an intrinsic property of the microorganisms [23]. The reported value of 0.05 day^{-1} for the similar type of metabolism of denitrification [17,22] was, therefore, directly applied. Y_{max} has been determined in the above section. This parameter is also relatively constant because the reaction stoichiometry involving perchlorate and acetate is expected to be at most only slightly changed in this experiment. Accordingly, the microbial yield based on thermodynamics should be stable. The other two parameters K_s and q_{max} were determined by fitting the numerically calculated data to the experimental results in Fig. 2. The reason is that the reported K_s range in Table 1 conflicts with the general conclusion that terminal electron acceptors often have K_s values below 1.0 mg/L [17,23]. Also, q_{max} proved to be the parameter most sensitive to the fitting, as discussed below.

The fitting method adopted is detailed as follows. Eqs. (1) and (8) were solved numerically by the finite-difference method with a finite-difference of $\Delta t = 0.00625$ h and with an initial guess of q_{max} and K_s. The optimal q_{max} and K_s were then obtained by changing their values in Microsoft Excel Solver to reach the minimum SSE between the model-calculated and observed data. To be noted is that the minimum available K_s in Solver and the un-detectable experimental data for the fitting were both set to 0.10 mg/L, a value that was the same as the detection limit for the perchlorate analysis in this study. The results in Fig. 2 showed that q_{max} was 0.020 (mg-perchlorate)/[(mg-DW) h] and K_s was 0.1 mg-perchlorate/L with a P value <0.01 for the fitting. How-

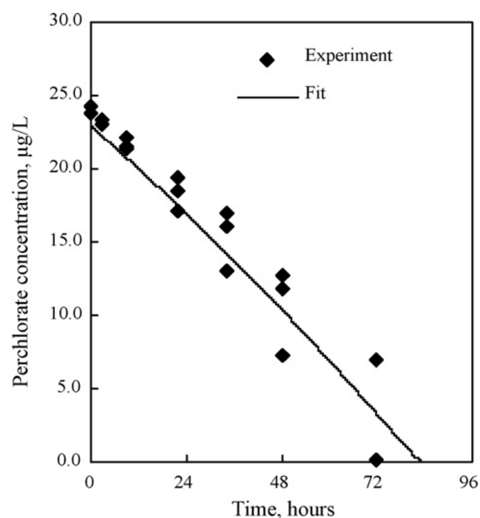

Fig. 2. Kinetics of microbial perchlorate reduction by mixed and heterotrophic bacteria, $X_0 = 11.6$ mg/L.

Table 1
Summary of kinetic parameters for pure and mixed heterotrophic perchlorate-reducing bacteria

Pure or mixed culture	Kinetic parameters							Growth model[a]	pH	T (°C)	Biomass analysis methods[b]	Electron donor	References
	μ_{max} (day^{-1})	q_{max}[c] (day^{-1})	Y_{max}	K_s (mg/L)	t_d[d] (day)	b (day^{-1})	S_{min}[e] (mg/L)						
Vibrio dechloraticans	N/A	1.67	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	DW	ROS[f]	[12]
Mixed culture	N/A	2.57	N/A	N/A	N/A	N/A	N/A	N/A	7.5	32	DW	PBCS[g]	[13]
GR-1	2.38	6.16[h]	N/A	N/A	0.29	N/A	N/A	N/A	7.0	30	P	Acetate	[14]
KJ	3.36	1.32	2.6[i]	33	0.21	N/A	N/A	Monod	N/A	N/A	OD, P	Acetate	[15]
PDX	5.04	0.41	12[i]	12	0.14	N/A	N/A	Monod	N/A	N/A	OD, P	Acetate	[15]
SN1A	1.66	4.60	0.36	2.2	0.42	N/A	N/A	Monod	N/A	N/A	CC, P	Acetate	[16]
ABL1	2.06	5.43	0.38	4.8	0.34	N/A	N/A	Monod	N/A	N/A	CC, P	Acetate	[16]
INS	1.61	4.35	0.37	18	0.43	N/A	N/A	Monod	N/A	N/A	CC, P	Acetate	[16]
RC1	2.04	6.00	0.34	12	0.34	N/A	N/A	Monod	N/A	N/A	CC, P	Acetate	[16]
Mixed culture	0.15	0.49	0.20	<0.1[k]	7.0	0.05[j]	<0.1[k]	Maintenance	∼7.0	∼27	DW	Acetate	This study

[a] If the Monod growth model was used to determine kinetic parameters in the references, b was assumed to be zero when solving for the other parameters.
[b] P, OD, and CC in the column of Biomass analysis method represent Protein, Optical Density, and Cell Count, respectively.
[c] Lower q_{max} values in the range of 0.17–0.03 day^{-1} for other bacteria isolates were also reported in the German literatures [20,21] as noted by Attaway and Smith [13].
[d] t_d, the minimum doubling time, was calculated by the equation: $t_d = \ln(2)/(\mu_{max} - b)$.
[e] S_{min} [17] was calculated by the equation: $S_{min} = K_s b/(Y_{max} q_{max} - b)$.
[f] Readily-oxidized organic substances.
[g] Rich protein-based carbon sources.
[h] It was calculated based on the assumption that one mole perchlorate yields one mole chloride [13,14]. The reported rates in the Ref. [14], 0.043 mmol chloride formed mg protein^{-1} h^{-1} possibly should be corrected as 0.043 mmol chloride formed g protein^{-1} min^{-1}.
[i] They are two high yield values. One possible reason might be that two different methods for biomass analysis were employed when determining the μ_{max} (OD methods) and q_{max} (Protein methods).
[j] This value is directly quoted from the similar type of metabolism of denitrification [17,22].
[k] They mean their values are below the detection limit of 0.1 mg/L in this study.

ever, if we set the minimum available K_s to a lower value, a better fitting with a smaller SSE could be obtained. Thus the K_s should be a value below 0.1 mg/L. The low K_s is consistent with the general conclusion that terminal electron acceptors often have K_s values below 1.0 mg/L [11]. It indicates that the perchlorate-reducing bacteria are effective at utilizing low concentrations of perchlorate. A sensitivity analysis was performed subsequently to evaluate the impact of a ±10% change of each of the q_{max}, Y_{max}, b, and K_s on the SSE [23]. This change caused less than 1.2% deviation of SSE except for q_{max}, which produced a 46.1% change of SSE. Thus the fitting is proved to be highly sensitive to q_{max}.

A summary of kinetic parameters for pure and mixed heterotrophic perchlorate-reducing bacteria, including those from previous reports, is presented in Table 1. Some factors, such as the growth model, biomass analysis methods, pH, temperature, and electron donor, affect these parameters. The weight of these factors in affecting the parameters is not explicit and sometimes is significant. Therefore, the parameters cannot be simply compared without uniform testing methods and testing conditions. The values presented in Table 1 give an alternative data source for those who work on the design of biological treatment systems, or on the prediction and evaluation of their performance.

3.3. pH effect on microbial perchlorate reduction kinetics

A kinetic study of the pH effect on perchlorate reduction by heterotrophic and mixed bacteria was examined between pH 5.0 and 9.0. Low concentrations of phosphate in the culture medium were used to buffer the pH and to minimize the possible inhibition caused by excessive buffer salt. By this method most pH values were well controlled. The final pH values deviated no more than 0.1 unit from the initial. There were two exceptions, however. For the experiment with initial pH 9.0, the final pH dropped to 8.8 after 6 days. For the experiment with the initial pH 8.5, the pH dropped to 8.3 when half of the perchlorate was reduced after 1.5 days. In this experiment the pH kept decreasing as time went by, and thus it was stopped.

Fig. 3 presents the occurrence of microbial perchlorate reduction as a function of pH. All pHs labeled in Fig. 3 were the initial values. The results in Fig. 3 indicate that microbial perchlorate reduction by the mixed and heterotrophic bacteria occurred throughout the pH range from 5.0 to 9.0. Nevertheless, the rates of perchlorate removal by a unit mass of bacteria were significantly different at various pHs. To make a better comparison, the data in Fig. 3 were then fitted by Eqs. (1) and (8). The parameters b, Y_{max}, and K_s in the equations were fixed to 0.05 day^{-1}, 0.020 (mg-perchlorate)/[(mg-DW) h], and 0.1 mg/L, respectively. q_{max} was used as the fitting parameter. This method proved to be acceptable because subsequent sensitivity analysis results showed that q_{max} was the most sensitive parameter and a ±10% change of each of Y_{max}, b, and K_s caused no more than 5% deviation of SSE. Statistical analysis also indicates that all fittings have a P value <0.01, except that at pH 5.0 there is no statistically significant relationship between perchlorate concentration and time as described by Eqs. (1) and (8). This is because at pH 5.0 the bacteria were not able to degrade perchlorate in this study. Thus one set of Y_{max}, b, and K_s together with a changing q_{max} at different pH values can well fit all experimental data in Fig. 3.

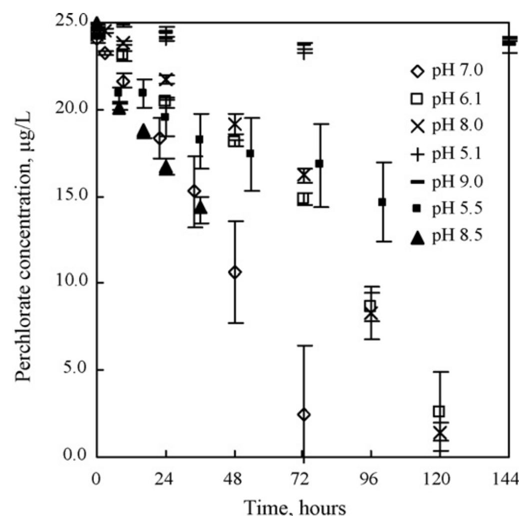

Fig. 3. pH effect on the kinetics of microbial perchlorate reduction. The experimental points were the average values of triplicate samples. The initial biomass concentration was 11.6 mg/L in all systems except for pH 5.5 and pH 8.5 systems where the concentration was 50.1 mg/L.

A plot of the q_{max} against pH is shown in Fig. 4. A transformed Gaussian peak equation, Eq. (9), was used to fit the calculated q_{max} by changing σ in Excel Solver to achieve a minimum SSE.

$$q_{max,pH} = q_{max,pH\,7.0} \exp\left[-\frac{(pH - 7.0)^2}{2\sigma^2}\right] \quad (9)$$

where $q_{max,pH}$ − q_{max} at a specific pH, $q_{max,pH\,7.0}$ − q_{max} at pH 7.0 and σ is the standard deviation. The results indicate that a σ value of 0.84 can best fit the data in Fig. 4 with a P value <0.01. Thus Eq. (9) would be expected to be useful for some practical applications by substituting σ = 0.84 into the equation when the effect of pH needs to be considered.

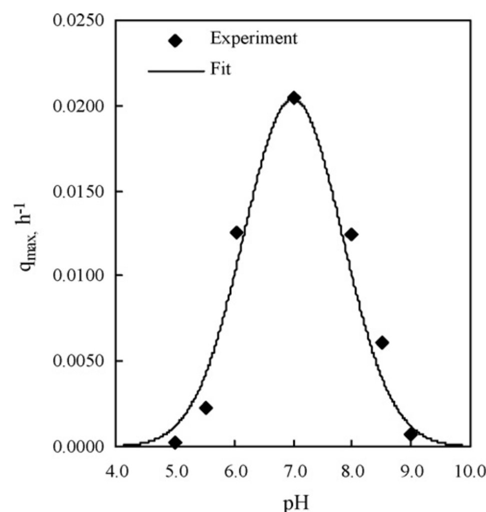

Fig. 4. Gaussian peak fit of q_{max} against pH for the microbial perchlorate reduction by mixed and heterotrophic bacteria.

Table 2
Summary of pH ranges for both pure and mixed heterotrophic perchlorate-reducing bacteria

Pure or mixed culture	pH range for perchlorate reducing bacteria		References
	Perchlorate reduction	Bacteria growth	
Acinetobacter	N/A	6.0–7.5, optimum 6.8–7.2	[25]
Mixed culture	6.6–7.5, optimum 7.1	Wider range	[13]
HAP-1	N/A	6.5–8.0, optimum 7.1	[26]
CKB	N/A	6.5–8.5, optimum 7.5	[27]
Perclace	6.5–8.5, optimum 7.0–7.2	N/A	[28]
Mixed culture	5.0–9.0, optimum 7.0	N/A	This study

Note: the *Acinetobacter* are 20 close chlorate-reducing strains and their capability of perchlorate reduction was not demonstrated in the reference though they are most possibly able to reduce perchlorate. The occurrences of perchlorate reduction by some strains at adverse pH values as low as 5 (heterotrophic or autotrophic is not known.) and as high as 9 (not heterotrophic but autotrophic) were also reported [29,30].

The results in Fig. 4 show that though microbial perchlorate reduction by a unit mass of mixed and heterotrophic bacteria occurred throughout the pH range from 5.0 to 9.0, the maximum reduction was obtained at pH 7.0. This optimum pH is consistent with most of the previous reports for both pure and mixed perchlorate-reducing bacteria [13,24,25,27] as summarized in Table 2. When pH shifted away from 7.0, the reduction rate accordingly decreased as described by Eq. (9). The preference for neutral pH for perchlorate-reducing bacteria indicates that they could be normally found in neutral environments [30]. This tendency along with their other preferences, such as mesophile [13,25–27] and facultative environments, and with their capability of alternative growth on nitrate and oxygen [27] as the electron acceptors and some simple organics [26,31] as the electron donors, might explain their ubiquity [10,26] in very diverse environments, since these environmental requirements for their life are commonly met in underground water, groundwater, sludge, soils, and sediments.

The mechanism of the pH effect on the perchlorate reduction by perchlorate-reducing bacteria is still not fully understood. The process of respiration of perchlorate as a terminal electron acceptor by a bacterium cell is quite complex. Some enzymatic processes might be involved in this overall biochemical reaction. Generally, it is known that pH effects on enzyme activity can be attributed to three possible mechanisms [32]: (a) variations of the pH in the environment change the ionic form of the acid and base groups on the active sites of an enzyme; (b) changes in pH alter the three-dimension shape of the enzyme; and (c) the pH in the environment affects the ionic groups on the substrates in some cases and hence varies the affinity of the substrates for the enzyme. Thus further studies on how the proton concentration outside the cell affects the key enzyme in the perchlorate reductive pathway might improve our understanding of the mechanisms.

4. Conclusions

Kinetic parameters for microbial perchlorate reduction by mixed and heterotrophic perchlorate-reducing bacteria were summarized, including those currently determined and previously reported. The half saturation constant, K_s, obtained in this study was below 0.1 mg/L, which indicated that perchlorate-reducing bacteria are effective at utilizing low concentrations of perchlorate. Microbial perchlorate reduction by mixed and heterotrophic bacteria occurred throughout the pH range from 5.0 to 9.0. However, the rates of perchlorate removal by a unit mass of bacteria were significantly different at various pH values with an optimum at pH 7.0. A Gaussian peak equation was calibrated by the experimental results and could well describe the variation of q_{max} as a function of pH. It can be used for practical applications when the pH effect needs to be taken into consideration.

Acknowledgments

This research was supported by the New Jersey Department of Environmental Protection. The authors would like to thank Dr. Chuanyong Jing and Mengqiang Zhu for their assistance. Acknowledgement should also be given to the anonymous reviewers for their helpful comments and suggestions.

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