Kinetics and Modeling of Perchlorate Biodegradation using Dechlorosoma Sp

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Abstract

Perchlorate is one of the highly toxic inorganic compounds present in the environment as natural deposits and as a man-made source. Significant methods of treating perchlorate compounds are by means of physical, chemical and biological processes. This work focuses on identification of Perchlorate degrading microorganisms from contaminated sources and to study its degrading ability of perchlorate along with its influencing factors. One of such microorganism was isolated from the contaminated sites from southern part of Tamilnadu and identified as Dechlorosoma Sp.The degradation process of perchlorate using Dechlorosoma Sp was influenced by various factors such as pH, initial perchlorate concentration, concentration of acetate and temperature. The microorganism degrades perchlorate faster in low concentrations and found that the optimum pH was 7. The modified Gaussian peak equation was used to incorporate the effect of pH on perchlorate degradation and the study reveals that the optimum concentration ratio of perchlorate to acetate was 1:2 and 1:3. A simple Monod model described the degradation rate and this study infers that the maximum growth rate and half saturation constant for Dechlorosoma sp were 0.309/h and 18.27mg/L.

Keywords: *Dechlorosoma Sp; Perchlorate, Monod Model, Modified Gaussian Model*

1. Introduction

Perchlorate (ClO_4^-) is one of the largest manufactured chemical substances which are widely used for industrial as well as other wide range of applications [11], [27]. The perchlorate anion (ClO_4^-) consists of a tetrahedral array of oxygen atoms around a central chlorine atom [2]. As the oxidation state of the chlorine is +7, the species is a strong oxidizing agent.

$$ClO_4^- + 8H^+ + 8e^- \rightarrow Cl^- + 4H_2O \qquad E_0 = 1.287\ V \qquad (1)$$

In this respect, perchlorate is slightly weaker than dichromate or permanganate, however on the other hand it reduces only in the presence of strong concentrated acids. Perchlorate are relatively low charge density, extremely soluble substances, it does not form any complexes with metal ions however any improper discharge of these oxyanions leads to contaminations of surface, ground water supplies, and soil [4], [21]. Because of its extreme solubility, perchlorates are not significantly broken down into its mineral compounds in the environment and make perchlorate a persistent derivate [17]. There are four mainly used perchlorate compounds namely ammonium perchlorate, sodium perchlorate, potassium perchlorate, and perchloric acid. Among this ammonium perchlorate was widely used throughout the globe for explosives, rocket fuels, air bags and paints [15].

Due to severe health hazards possessed by this compound, the United States Environmental Protection Agency (US EPA) added perchlorate as a hazardous substance in drinking water contamination list and recently issued a Reference dosage (RfD) of 0.7 µg /kg body weight /day which is equal to a drinking water equivalent level (DWEL) of 25 µg/L [3]. Due to various exposure, sources of perchlorate in drinking and food products and unavailability of long term health effects data for low concentration of perchlorates exposures, perchlorate is considered as a growing contaminant concern. Due to uncertainty in the toxicology and health hazards caused by perchlorate attracted attention of perchlorate treatment technologies by various means. Further, compared to the abiotic techniques used for removal of perchlorate like ion exchange [9], reverse osmosis [10] and electro dialysis, the biological reduction of perchlorate converts perchlorate into nontoxic chloride without the generation of high concentrated perchlorate and brains, for the purpose of regenerating ion exchange resins. In addition to that the biological reduction of perchlorate possess an additional advantage like the simultaneous reduction of nitrate, bromide and other contaminants in the same system. Therefore this research works turns out to attempt for isolating an effective microorganism from perchlorate contaminated environment, and to explore the environmental factors that affects the perchlorate biodegradation Further this research work extends to evaluate the kinetic parameters of perchlorate biodegradation.

2. Materials and methods

2.1 Chemicals and analytical methods

All the chemicals used in this study were obtained from Merck India limited, Growth media's are obtained from Hi-Media and Sodium salts of acetate and perchlorate were obtained from Sigma-Aldrich USA. In this study the biomass concentration of perchlorate reducing microorganism was determined by Speakol spectrophotometer and Dionex ion chromatography was used to analyse perchlorate concentration. For IC analysis all the solutions were prepared using Sigma- Aldrich analytical grade chemicals with deionized water. A stock solution of 1000 mg/L of perchlorate was prepared from Sodium salt of perchlorate further the working solutions are daily prepared from the stock solution. The eluent used in this study for analyzing perchlorate was Sodium hydroxide, which was prepared daily at 25, 50, 75, 100 mM by weight. The Eluent samples are continuously purged with onsite generated Nitrogen from Nitrogen gas generator. The following were used for the perchlorate analysis are Ion Pac AS 11 Suppressor column with AG 11 Guard column. The experimental conditions used for analysis of perchlorate were 100,200,500,1000 and 1500 µL sample loop,25,50,100 mM NaOH eluent and 0.5,0.75,1.0 ml/min flow rate. The obtained Chromatogram from the IC was analyzed using Peak net chromatogram software supplied by Dionex Corporation USA. The instruments were calibrated using various

perchlorate concentrations and good linearity was obtained for all concentrations with correlation coefficient of 0.991.

2.2 Isolation of perchlorate degrading microorganism

Soil containment with perchlorate from southern part of Tamilnadu was collected and enriched using autoclaved growth medium as per the isolation technique given by Song et al [25]. The medium contained 500 mg /L sodium acetate (electron donor), 100mg /L of Sodium perchlorate (electron acceptor) and NaOH which was used to adjust pH. The microbial growth was measured by optical density method and the turbid culture was transferred to a fresh medium for four times for enrichment. The enriched inoculums were streaked into a petri plate medium and it was subsequently incubated. Further selected colonies from the plate were transferred into liquid medium and consequently incubated. The successive cycle of culture transferred into plate and liquid culture, isolate perchlorate degrading microorganism and its purity was thereby confirmed using microscope.

2.3 Effect of initial perchlorate concentration

Different concentration of perchlorate, varying from 50 to 500 mg/L was added into sterilized growth medium in order to infer the ability of the perchlorate degrading microorganism to degrade perchlorate in a batch reactor. Sodium hydroxide was used to adjust the pH. The batch reactor was degassed with nitrogen gas, and kept in an incubator shaker with appropriate temperature at 150 RPM (Revolutions per minute). Samples were taken at various time interval's to quantify the microbial growth and perchlorate concentration, until steady state concentration of perchlorate was reached.

2.4 Effect of acetate addition

The ratio of electron donor to electron acceptor is very important for effective perchlorate biodegradation. The excess amount of perchlorate consumed by electron donor, may be used as a carbon source for biosynthetic reactions. The effect of acetate addition on perchlorate biodegradation was evaluated by varying the acetate concentration from 100 to 1500 mg/L, by keeping the perchlorate concentration at a fixed ratio between 100 to 500 mg/L.

2.5 Effect of pH

pH change in a growth medium affects microbial growth rate [20], accordingly the influence of pH was evaluated from 5 to 9, by adjusting the pH with help of HCl and NaOH in the batch reactor [18]. Samples were collected at different time intervals and the sample bottles were filled with Deionized (DI) water, sealed and kept in the incubator shaker at constant temperature, 150 RPM. Perchlorate concentration and the microbial growth were measured until the steady state condition in the batch was reached. The Calibrated Gaussian peak equation method [28] was adopted to derive the relationship between specific substrate removal rate and pH.

2.6 Growth kinetics of perchlorate degrading microorganism.

The kinetics of Perchlorate biodegradation was carried out in a batch reactor with various concentrations of acetate and perchlorate. The microbial cells at the late-log phase were harvested and its OD was observed. Harvested cells of perchlorate degrading bacteria's were washed at 8000 g for 10 minutes and they are re-suspended in a fresh medium with same OD value. Subsequently the suspended cultures were transferred into various bottles containing sterilized medium with different concentrations of perchlorate and acetate. Simple Monod model [15], [5] was used to calculate kinetic constants using MATLAB software.

3. Results and discussion

Three different microorganisms were isolated from a contaminated site in southern part of Tamilnadu. The microbes were enriched in a growth medium with acetate and perchlorate [25]. Among them one microorganism showed considerable amount of perchlorate degradation and it was used for this investigation. The bacterium was identified as *Dechlorosoma* sp by 16S rRNA gene sequence analysis.

3.1 Effect of initial perchlorate concentration

Batch study has been carried out to determine the degradation ability of perchlorate using *Dechlorosoma sp* with various initial concentrations of perchlorate ranging from 50 to 500 mg/L. It was observed from Figure 1 that, perchlorate concentrations ranging from 50 to 200 mg/L, the growth trend of perchlorate degrading isolated bacterial species of *Dechlorosoma* was non S Shaped with minimum lag period of 60 minutes. However lag periods of 6 hours, 9 hours, and 11 hours were observed at high initial concentrations of 300, 400 and 500 mg/L respectively. It is inferred that the lag, are delay in the growth of *Dechlorosoma sp* exposed to high concentrations of perchlorate may be due to its inability to sustain in a new environment and due to delay in re-synthetization of perchlorate reductase at elevated concentrations of perchlorate. This sort of delay in the growth of perchlorate degrading bacteria's at elevated concentrations of perchlorate were studied in [5], [7] and [8]. They have obtained that the influence of lag period was due to change in the environmental conditions and the microbial cells of perchlorate degradation bacteria's, must adopt themselves to re-synthesize of essential constitutions to initiate growth and cell division cycle, for the elevated concentrations of perchlorate.

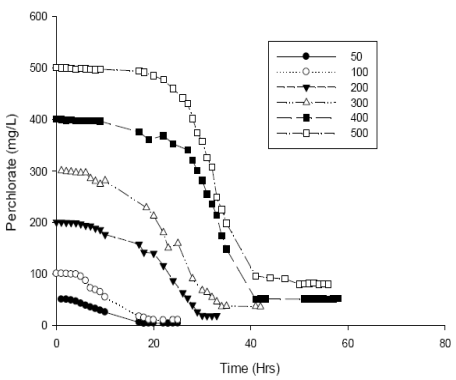

Figure 1. Effect of Initial Perchlorate Concentration

Rate constants are used to design and estimate the size of the reactor, hence the experimental data's are analyzed using Zero order and first order kinetics to find out the relations of perchlorate degradation rate with various perchlorate concentrations and the results are shown in figure 2 to 7.

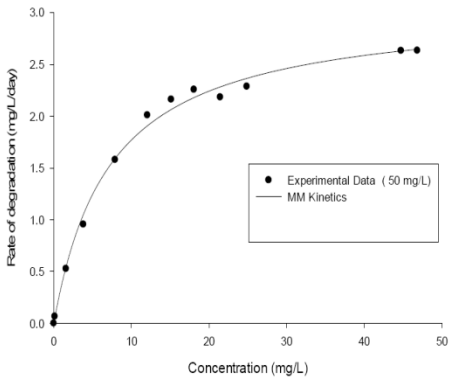

Figure 2. MM Kinetics of perchlorate degradation for 50 mg/L of initial perchlorate concentration

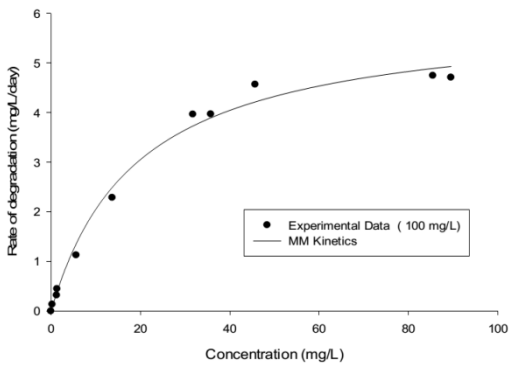

Figure 3. MM Kinetics of perchlorate degradation for 100 mg/L of initial perchlorate concentration

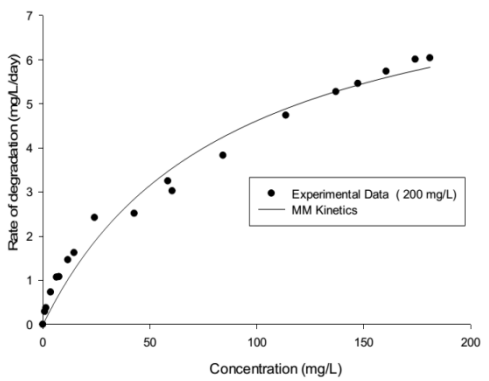

Figure 4. MM Kinetics of perchlorate degradation for 200 mg/L of initial perchlorate concentration

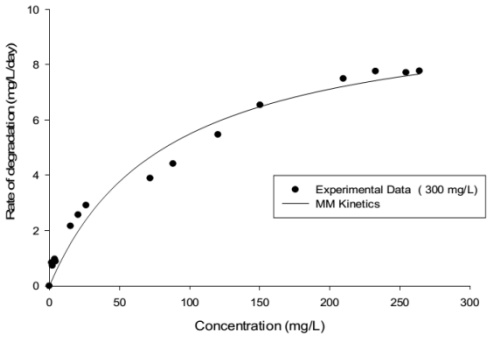

Figure 5. MM Kinetics of perchlorate degradation for 300 mg/L of initial perchlorate concentration

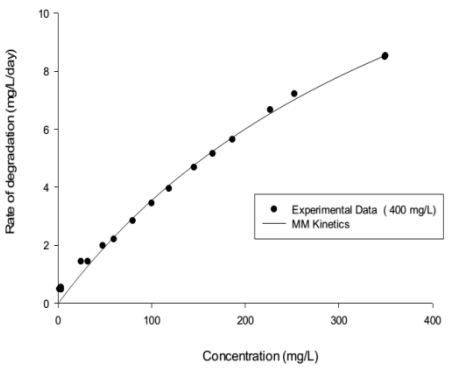

Figure 6. MM Kinetics of perchlorate degradation for 400 mg/L initial perchlorate concentration

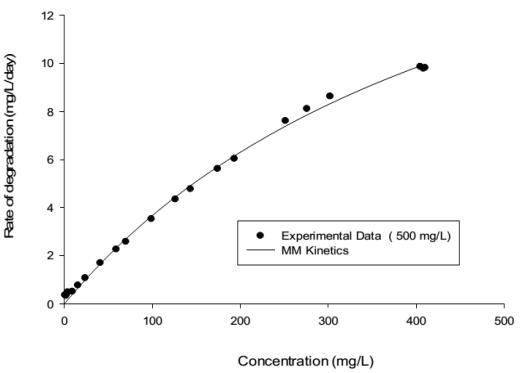

Figure 7. MM Kinetics of perchlorate degradation for 500 mg/L initial perchlorate concentration

It is inferred that based on the zero order biodegradation rate analysis the perchlorate degradation using *Dechlorosoma sp* is concentration dependent and it is found that for

concentrations ranging from 50,100,200,300,500 the rate constants observed was 2.352, 4.5190 ,6.2493, 7.9852, 9.2321 mg/L/hr respectively. The average value of first order biodegrading rate of perchlorate was found to be 0.0784/day in this study. Further Michaelis–Menton (MM) kinetics was used to infer more refined degradation rate information due to change in the value of rates constant with changing contaminant concentration The experimental data's are analyzed using MM equation in MATLAB, and found that MM kinetics fits accurately in low concentration range of 50 to 200 mg/L compared to higher concentrations range of 300 to 500 mg/L of perchlorate which is shown in Figure 8. The maximum reaction velocity was determined to be 12.050 mg/L/hr and half saturation constant was 183.480 mg/L/hr.

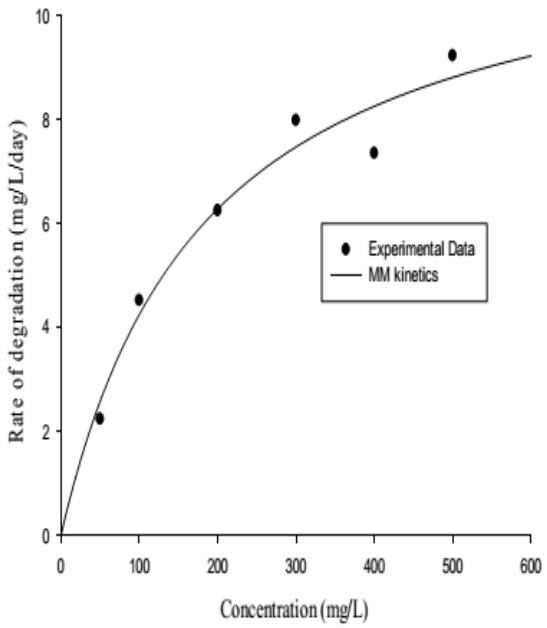

Figure 8. MM Kinetics of perchlorate degradation

3.2. Effect of pH

Experiments were conducted to test the effect of pH, varying from 5 to 9 on biodegradation of perchlorate using *Dechlorosoma* sp. During the experiments, after the initial adaptation period, perchlorate reduction was monitored at various pH levels and the results obtained are shown in Figure 9. From the figure, it was observed that when the initial pH was at 7, faster perchlorate reduction occurs. This result shows that the perchlorate reduction was optimum at pH 7 for β-Proteobacteria, and same consistent optimum level of pH at 7 was observed and reported by many researchers [29]. When the initial level of pH increased from the optimum value, reduction rate of perchlorate decreases to half. Further, the reduction of perchlorate was also observed at pH level below 7 and above 8, however its magnitude was very low. This reduction in the magnitude is may be due to generation of OH- ions, and the steady increase of pH, observed in all solution, with the amount of increase, following the order of pH 6>7>8.

However once perchlorate reduction was established, there is no decrease in the perchlorate reduction rate was observed, despite the pH increasing up to 9. In contrast at an initial pH of 6

although the pH had steadily increased to 7, the optimum pH for perchlorate reduction, little or no reduction of perchlorate can occur at pH 9 or above as observed before and slight reduction of perchlorate occurred when the initial pH was 9. Thus the initial pH appears to be important for the bacteria to initiate perchlorate reduction. Further, the enzyme responsible for perchlorate degradation into nontoxic chloride and oxygen was Chlorite dismutase which has an optimum range between 6- 7. It was reported by many authors that, if the pH was started to increase, above the optimum level, the enzyme activity of Chlorite dismutase was significantly reduced, due to the interaction of OH- ions, present in the liquid phase with enzyme molecule, and it was also found that the effect of protonation was highly stabile when the pH was in between 6 to 7. Thus the microbial community of perchlorate reducers are highly affected by the pH and it loses its buffering capacity.

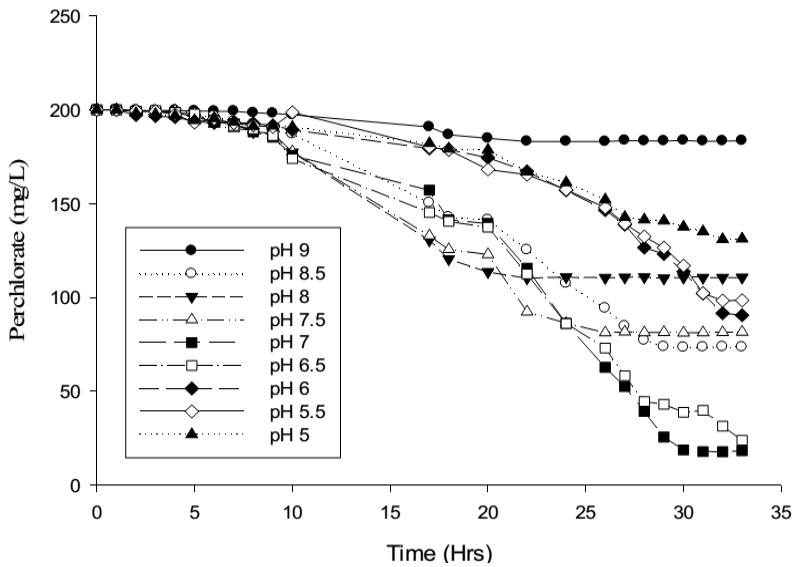

Figure 9. Effect of pH on perchlorate biodegradation

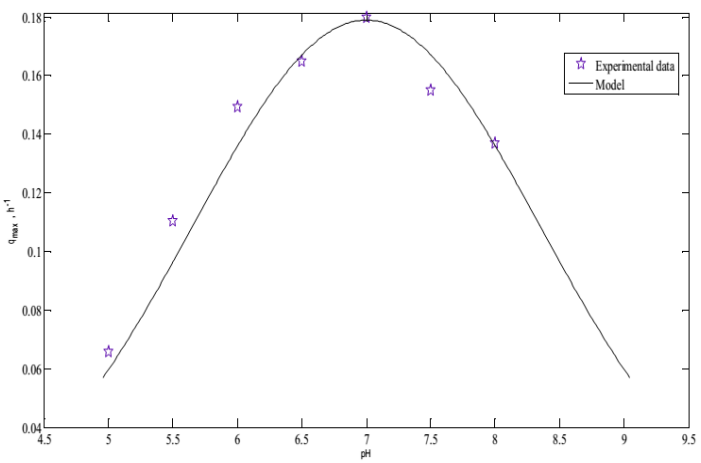

Figure 10. pH and q_{max} model

Modified Gaussian model given in the equation (2) was used to predict the relationship between pH and q_{max} in order to incorporate effect of pH in microbial degradation, if needed in real system [28].

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

Where $q_{max\ ,pH}$ is the q_{max} at a specific pH, $q_{max\ pH,7.0}$ is the q_{max} at pH 7.0 , q_{max} is the maximum substrate utilization rate (mgS/mg-X-day) and σ is the standard deviation. In this model the σ value of 1.35 was used and the model fits well with experimental data and it's shown in Figure 10.It is inferred from our experimental data that the species, *Dechlorosoma* sp can able to utilize perchlorate at neutral pH, compared to other pH. These results matched with other microorganism isolated by various researchers from different places. Most of the perchlorate microorganism degrading microorganism can be found in neutral conditions, which aids the microorganism to grow in ground water, sludge, soils and sediments [28].

The reduction of perchlorate degradation efficiency relating to various pH can be correlated with enzymes present in the microorganisms. The enzymes degrading perchlorate ions are quite complex and its activity are depends upon (i) Change of ionic form of acid and alkaline on the active sites due to pH variation in the environment (ii) Three dimensional structural change of the enzyme due to pH change (iii) The variation of ionic groups of the substrate alternation by the variant in pH. The perchlorate degrading enzymes are sometimes inhibited and the affinity of substrate is enzyme depended and hence the degradation of perchlorate varies with pH.

3.3. Effect of Acetate on perchlorate degradation

In this study detailed analysis have been carried out to understand the importance of electron donor concentration on perchlorate degradation. Figure 11 indicates that the presence of electron donor (acetate) is very important for perchlorate degradation and it is also found that, any increase in acetate concentration will lower the lag phase of the microorganism, and further increases the rate of perchlorate biodegradation. From the Figure 12 it is observed that, the reduction rate of perchlorate reduction was linearly increases with increase in the acetate concentration up to 2 g/L (1: 2 acetate), with a reduction rate of 4.5919 $mgL^{-1}hr^{-1}$, however it remains same when it was further increased to 3g/L (1:3 acetate) . When the acetate concentration was supplied at 1g/L the reduction rate was reduced to 66.27 %. Considering 1g of acetate is equivalent to 1.085g of chemical oxygen demand, the stoichiometric equation given in equation (3) was adopted to calculate the theoretical perchlorate demand using acetate as a sole electron acceptor and assumed that all of the reducing equivalents of acetate are utilized by the microorganism for perchlorate reduction rather than carbon assimilation of the microbial biomass [24].

$$CH_3COOH + ClO_4^- \rightarrow 2CO_2 + Cl^- + 2H_2O \quad (3)$$

Based on the above equation the theoretical demand of perchlorate using acetate as carbon source was found to be 1.0 mg perchlorate per mg of acetate. However, the difference in the theoretical and observed yields was considered to be due to the fact that the utilization of acetate as a carbon source for biosynthesis reactions. The minimum ratio of complete removal of perchlorate and acetate is defined to be the optimum ratio of perchlorate removal using acetate. In this study the optimum ratio was calculate based on the model given in equation (4) [22] and found

that 1.8007 mg of acetate is required per mg of perchlorate degradation which is also equivalent to a COD value of 1.953 mg per mg of perchlorate.

$$S_{min} = K\frac{b}{Yq-b} \quad (4)$$

Where S_{min} is the minimum substrate concentration, K is the Concentration giving one half the maximum concentration is the Maximum specific rate of substrate utilization, Y is yield coefficient, b is the endogenous decay coefficient.

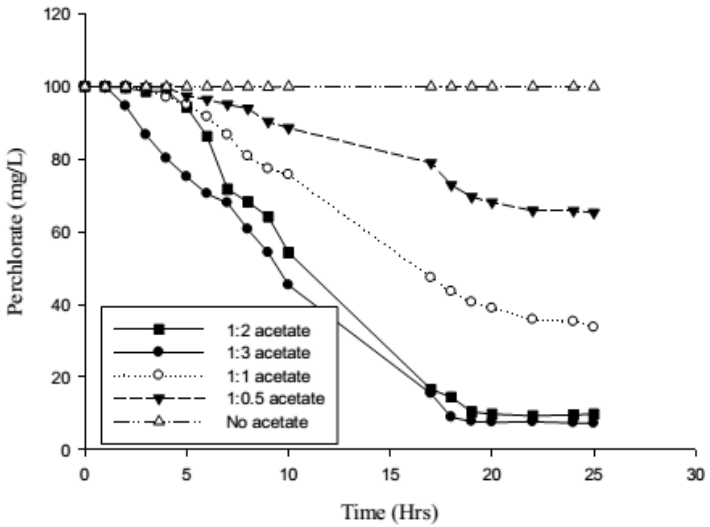

Figure 11. Effect of acetate ratio on perchlorate degradation

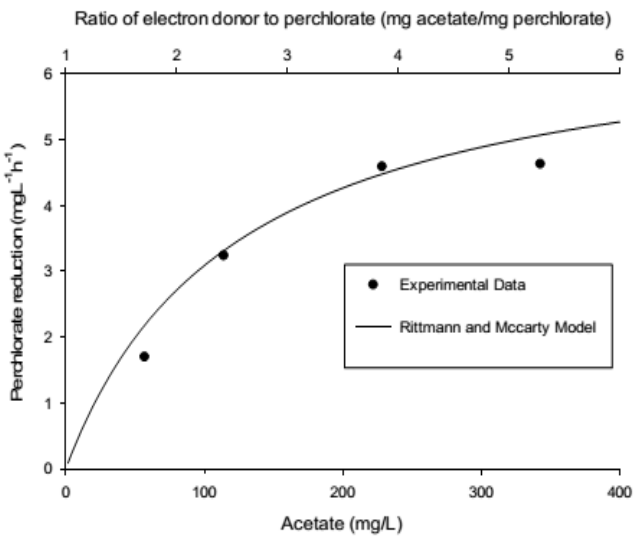

Figure 12. Effect of acetate concentration on perchlorate degradation using Rittmann and Mccarty Model

The results obtained in this study was also compared with the theoretical approach [22] using thermodynamic calculation [24] and found that 1.953 mg of COD per mg of perchlorate is very near to the theatrical values of 1.45 mg of COD per mg of Perchlorate. Further, High values of 2.1 mg COD per mg of perchlorate was also reported with acetate fed sand packed, down flow reactor [12]. From our experimental results, it was observed that when the acetate concentration was supplied in excess upto 2 g/L (1:2 acetate) linear increase in the reduction rate of perchlorate was observed, however further increase in the acetate concentration provided no contribution in the reduction rate of perchlorate which is shown in Figure 12. In order to avoid, increase in the operating cost, accumulation of excess residual electron donor and to avoid the stimulation of other sulfur reducing bacteria which provide sulfide, contributing inhibition activity of perchlorate reduction, the ratio of 1:2 acetate was adopted as a desirable concentration for perchlorate reduction for this study.

3.4. Effect of temperature on perchlorate degradation

The influence of temperature on perchlorate degradation using *Dechlorosoma* is shown in Figure 13 and it was inferred from that the bacterium was able to degrade perchlorate from 27 to 37 ^{o}C. The degradation rate of perchlorate was observed very low compared at 37 ^{o}C as compared to 32 ^{o}C in which maximum perchlorate degradation rate was observed within the temperature range. Based on the experimental data, the degradation efficiency of perchlorate for various incubation temperatures was probability due to the penetration efficiency of the perchlorate into the cells of *Dechlorosoma* sp or exclusively due to temperature effect on enzyme activities and de activation of perchlorate degrading enzymes within the cell.

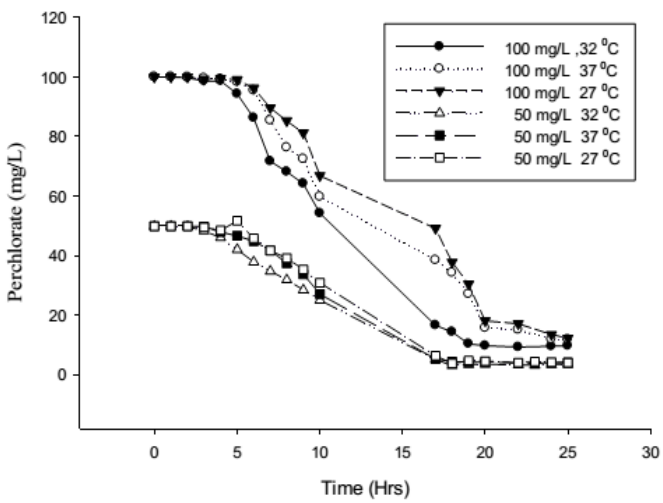

Figure 13. Effect of temperature on perchlorate degradation

3.5. Kinetics of perchlorate degradation

Batch experiments were conducted with acetate as sole electron donor in order to understand the kinetic constants and within two hours from the start of experiment the culture reaches exponential growth phase. For kinetic calculations the linear portion of the growth curve was considered and Figure 14 shows the relationship between the specific growth rate and the initial acetate concentration. The simple Monod model was used to calculate the kinetic parameters of *Dechlorosoma sp* using MATLAB. Initially the program uses, the data obtained from the batch reactor to compute the substrate concentration (S) and the specific growth rate (μ) and subsequently linear regression curve analysis were carried out to fit the experimental data using equation number 3.2 subject to the constraints of minimizing the Sum Square Error (SSE) and there by maximize the R^2 value (The values of SSE and R^2 are computed using MATLAB, wherein the function itself incorporates the effective numbers for the calculated parameter values). The computed results were found to be 0.3093±0.0084 hr^{-1} for μm, 18.2713 mg /L for Kp and 0.2474 ±0.1363 g DW/g acetate for the biomass yield in this study.

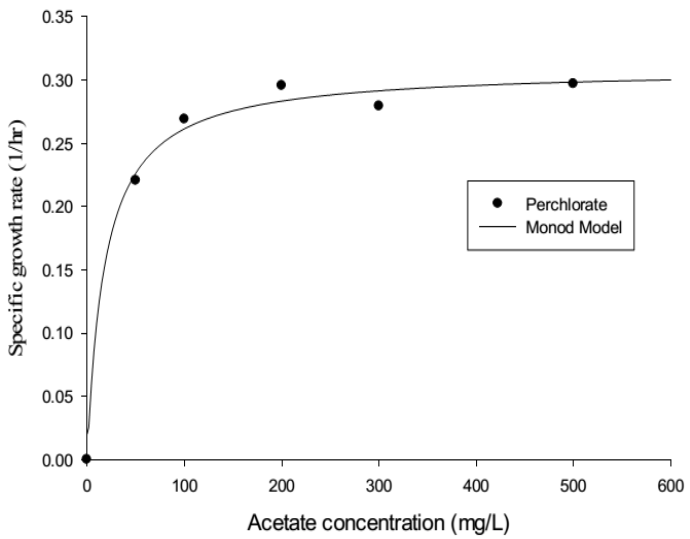

Figure 14. Microbial growth kinetics in a batch reactor

4. Conclusion

In conclusion, degradation of perchlorate into oxygen and chloride ions can be through by variety of bacterial species found in various environmental conditions. In this research work one of such microorganism was isolated from a perchlorate contaminated soil site in southern part of Tamilnadu and 16s RNA gene sequence suggest that the identified isolate belong to genera "*Dechlorosoma*". Varieties of environmental conditions affects perchlorate degradation among them are initial perchlorate concentration, pH, presence of electron donor (acetate) and temperature. Perchlorate was degraded faster at low concentration compared to high concentration and found that the rate of reduction follows first order kinetic, and its average value was 0.0784/hr. This species can have the ability to make use of perchlorate to a wider extend of pH and the rate of perchlorate degradation by unit biomass varied significantly at various pH's. The optimum pH

for this microorganism was found to be 7 and the modified Gaussian peak equation was adopted to incorporate the effect of pH on q_{max}. The addition of electron donor for perchlorate removal was found very important, without addition, perchlorate ions were not degraded and found that the excess amount of acetate addition simulates faster rate of perchlorate degradation. The optimum concentration of acetate was between 1:2 and 1:3. Temperature affects the perchlorate kinetics, low temperature resulted in low reaction growth rate and found that the optimum temperature for this culture was 32^0C. Simple Monod model provided better description of perchlorate degradation by *Dechlorosoma* species. The maximum growth rate and half saturation constant obtained by using nonlinear regression was found to be 0.3093 hr^{-1} and 18.27 mg/L and the yield of biomass was around 0.2474 gDW/g acetate. This strain shows a potential for possible perchlorate degradation in reactors and effluent wastewater treatment systems.

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