Effect of Initial pH and Sulfide Concentrations on Sulfate Reduction Rate of *Desulfovibrio desulfuricans*

Siripha. Ratanasit, Pornpan. Panichnumsin, Penjit. Srinophakun, and Annop Nopharatana

***Abstract*—** The effect of environmental pH and initial sulfide concentrations on sulfate reduction rate of *Desulfovibrio desulfuricans* was investigated in batch experiments. The sulfate reduction rates showed dramatically decreased with decreased environmental pH ranging from 7.5 to 5.5. The rates were 0.1603, 0.1294, 0.1107, 0.0913 and 0.0857 g/L-day at pH 7.5, 7.0, 6.5, 6.0, and 5.5 respectively. For initial sulfide concentration, sulfate reduction rate decreased with the increasing of sulfide concentration ranging from 0.05-0.5 g/l and sulfate reduction rate was 0.1841, 0.1746, 0.1406, 0.1427 and 0.1458 g/L-day with in sulfide concentration of 0.05, 0.10, 0.20, 0.30 and 0.50 g/l respectively. These result indicated that the sulfate reduction rate significantly depended on environmental pH and initial sulfides concentration.

***Keywords*—**Anaerobic sulfate reduction, *Desulfovibrio desulfuricans*, Initial pH, Sulfide concentration

I. Introduction

CONCENTRATED latex is derived from natural rubber and usually contains 30-40% rubbers and 60-70% water. Centrifugation is a common process used to produce concentrated latex that contains about 60% rubber [1].The concentrated latex industries cause a big problem in water pollution because the production process generates skim latex as a byproduct. To recover latex in skim latex, large amount of sulfuric acid is used for coagulation [2]. As a result, the wastewater generated from that process is highly acidic and contains high level of sulfate. Sulfate emission is not a direct threat for environment because sulfate is inert and non-toxic. However, if sulfate-rich wastewater is discharged into surface waters, sulfate could contribute to an increase of corrosion potential of receiving waters and malodor problem due to the biological reduction of sulfate to hydrogen sulfide under anaerobic condition [4].

Generally, the wastewater treatment technologies of concentrated latex processing in Thailand are based on the conventional biological processes. For example, open systems such as anaerobic pond, these systems had high efficiency COD removal and sulfate reduction but they usually cause malodor problem from hydrogen sulfide [3]. Activated sludge is an aerobic system that has high treatment efficiency and high process stability. However, this system needs high-energy consumption and is unable to remove sulfate from wastewater. A closed system such as anaerobic digester is able to reduce sulfate along with decomposing organic matter and generates methane gas. This system is the existing treatment plant that showed low process efficiency and stability. However, in the anaerobic treatment of sulfate containing wastewater sulfate-reducing bacteria (SRB) can compete with Methanogen (MB) and acetogenic bacteria (AB) for the common substrates [5].

In order to overcome that problem and to develop treatment systems for sulfate-rich wastewater, the three-stage system of sulfate reduction, sulfide oxidation, and methane production is proposed.

The first stage is sulfate reduction of incomplete SRB (I-SRB) that decomposes organic matters with sulfate as the electron acceptor and generates acetate and sulfide as products. This stage could reduce MPB and SRB competition for common substrates. The second stage is sulfide oxidation of sulfur oxidizing bacteria (SOB) that could reduce sulfide toxicity in the system.The Final stage is methane production. This combined system is an alternative treatment system, which has generates methane gas that can be used as fuel. In addition, it would have less sulfide toxicity problem. Therefore, the system could be operated at high loading and has high stability. This study focused only on the first step, sulfate reduction of I-SRB, to increase an understanding in the role of I-SRB of anaerobic degradation.

The objective of this study aimed to develop sulfate reducing bioprocess technology for concentrated latex wastewater treatment. The specific objectives are to determine the growth kinetic parameters during exponential growth phase of SRB following Monod kinetic behavior and to develop the kinetic model for determining the sulfate reduction rate of SRB following Monod kinetic behavior. Therefore, the kinetic model is developed to study the impact

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of substrate concentration, product concentration and environmental pH on anaerobic degradation system of SRB.

II. Materials And Methods

A. Microbial Culture

Microorganism used for sulfate reduction in this research was a pure culture of the sulfate reducing bacteria species, *Desulfovibrio desulfuricans* ESSEX.

B. Medium

A media used in this study was reliable for cultivating and growing SRB and generally consisted of DSMZ Medium 63 Desulfovibrio medium (freshwater) which consists of the following:

Solution A: K_2HPO_4 (0.5 g/l), NH_4Cl (1.0 g/l), $CaCl_2$ x $2H_2O$ (0.1 g/l), $MgSO_4$ x $7H_2O$ (2.0 g/l), Yeast extracts (1.0 g/l), Resazurin (1.0 mg/l), and distilled water (980 ml/l). Solution B: $FeSO_4$ x $7H_2O$ (0.5 g/l), and distilled water (10 ml/l). Solution C: Na-thioglycolate (0.1 g/l), Ascorbic acid (0.1 g/l), and distilled water (10 ml/l).

Medium was prepared by bringing solution A to boil, and then cool to room temperature while gassing with oxygen-free N_2 gas. After that solutions B and C were added and pH of the medium was adjusted to 7.8 with NaOH, and dispersed under N_2 gas. During dispersion, continuously swirl the medium to keep the grey precipitate suspended and then autoclaved 15 min at 121°C.

C. Experimental procedures

Effect of initial pH

The effect of initial pH on sulfate reduction was carried out in 120-ml vial. The sulfate reducing bacteria was inoculated into each vial at 1 g/l volatile suspended solids (VSS). The cultivation temperature was 37 °C and pH was 7.8. Lactate was used as a sole carbon source at 3.3 g/l and sulfate was used as a sole electron acceptor at 2.0 g/l. Initial pH were 7.5, 7, 6.5, 6, and 5.5 consequently COD: sulfate ratios was fixed at 1.65.

Effect of initial sulfide concentration

The effect of initial sulfide concentrations on sulfate reduction was carried out in 120-ml vial. The sulfate reducing bacteria was inoculated into each vial at 1 g/l volatile suspended solids (VSS). The cultivation temperature was 37 °C and pH was 7.8. Lactate was used as a sole carbon source at 3.3 g/l and sulfate was used as a sole electron acceptor at 2.0 g/l. Initial sulfide concentration were 0.5, 0.3, 0.2, 0.1 and 0.05 g/L, consequently. COD: sulfate ratio was fixed at 1.65.

D. Analytical Method

The substrate depletion, sulfate concentration, dissolved sulfide concentration, pH and VFA were monitored over the course of the experiment. The methods were analyzed according to the standard methods for the examination of waste and water (APHA et al., 1995) Sulfate (Turbid metric), Dissolved sulfide (Iodometric).

III. Result And Discussion

Experiment 1: Effect of initial pH on sulfate reduction rate

The results in Fig.1 show the effect of initial pH on sulfate reduction and sulfate removal efficiency during 16 days of incubations. The sulfate removal efficiency and sulfate reduction decreased along with decreasing initial pH.

The high sulfate removal efficiency were 87.96 and 86.79% at pH 7.5 and 7. When pH decreased to 6.5, 6.0 and 5.5, sulfate removal efficiency dramatically decreased to 48.86, 41.02 and 38.06%. This result indicated that at low pH significantly affected SRB activity in the system even the acid formers, led to decreasing of the sulfate removal efficiency and sulfate reduction in the system. These studies agree with [6] they found that the most SRB preferable pH was in the range of 7.5 – 8.5.

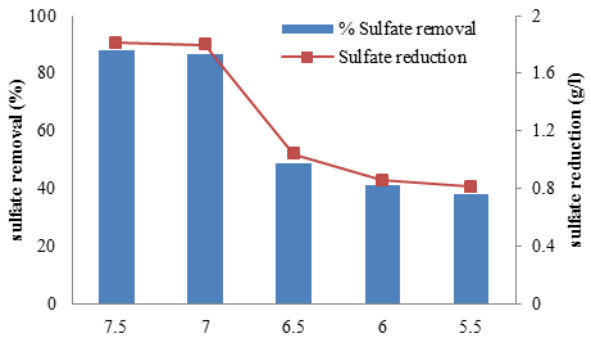

Fig. 1 Effect of initial pH on sulfate reduction and sulfate removal efficiency

The effect of initial pH on sulfate reduction rate was investigated at initial ranging pH of 7.5 to 5.5. The Fig. 2 showed sulfate reduction rate decreased with the decreasing initial pH ranging of 7.5 to 5.5 and sulfate reduction rate was in the range of 0.1603, 0.1294, 0.1107, 0.0913 and 0.0857 g/l-day at pH 7.5, 7.0, 6.5, 6.0, and 5.5 respectively. Therefore, this study indicated that the decreasing initial pH ranging from 7.5 to 5.5 were significantly effect on sulfate reduction. Similar results were found by [7].

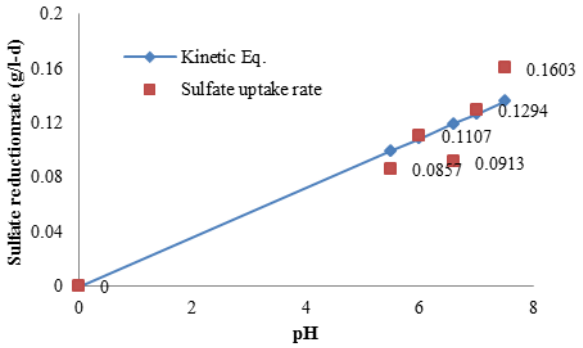

Fig. 2 Effect of initial pH on sulfate reduction rate

Experiment 2: Effect of initial sulfide concentration on sulfate reduction rate

The results in the Fig. 3 show that initial sulfide concentrations affected the sulfate reduction. The various initial sulfide concentrations were slightly influenced on sulfate reduction. The result showed that the sulfate removal efficiency in 14-day incubation were 79.59, 85.69, 80.82, 80.41, and 85.03%. The sulfate reductions were 1.6567, 1.7208, 1.6200, 1.6468, and 1.7181 g/l at initial sulfide concentration of 0.5, 0.3, 0.2, 0.1, and 0.05 respectively. The initial sulfide concentration at 0.3 g/l was the highest compared with another condition which the sulfate removal efficiency was 85.69%. While the minimum removal efficiency of sulfate was 79.59% at the initial sulfide concentration of 0.5 g/l. This result indicated that high sulfide concentration was toxic to SRB activity

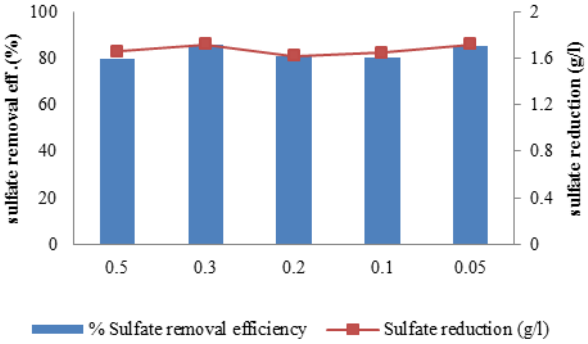

Fig.3 Effect of initial sulfide concentration on sulfate reduction and sulfate removal efficiency

Fig. 4 illustrates the effect of initial sulfide concentration on sulfate reduction rate. The figure showed sulfate reduction rate decreased with high sulfide concentration and sulfate reduction rate was in the range of 0.1841, 0.1746, 0.1406, 0.1427 and 0.1458 g/l-day with in sulfide concentration of 0.05, 0.10, 0.20, 0.30 and 0.50 g/l respectively. Resulting to the model, μ_{max} as sulfate reduction rate was 0.19 g/l-day, K_S as was 0.000045 g/l and K_I was 0.9324g/l. Therefore, the summary in this study is that the sulfate reduction rates are dependence on sulfide concentration ranging from 0.05 to 0.5 g/l.

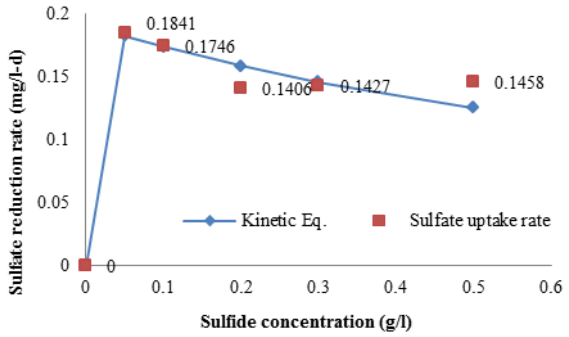

Fig.4 Effect of initial sulfide concentration on sulfate reduction rate

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