Anaerobic pond treatment of wastewater containing sulphate

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Abstract Anaerobic ponds are usually used for treatment of industrial and agricultural wastes which contain high organic matter and sulphate. Competition for substrate between sulphate reducing bacteria and methane producing archaea, and the inhibitory effects of sulphide produced from microbial sulphate reduction reported in the literature varied considerably. In this research, a laboratory scale column-in-series anaerobic pond reactor, consisting of five cylindrical columns of acrylic tubes, was operated to evaluate the effect of COD and sulphate ratio on pond performance treating wastewater containing high organic matter and sulphate from a tapioca starch industry. The result depicted that no adverse effect of COD:SO₄ ratios between 5 and 20 on overall COD removal performance of anaerobic pond operated with organic loading rate (OLR) of 150 to 600 g COD/m³d. Sulphate reducing bacteria could out-compete methane producing archaea for the same substrate at COD:SO₄ ratio equal to or lower than 5 and OLR greater than 300 g COD/m³d. Sulphide inhibition was not observed on overall performance of pond up to an influent sulphate concentration of 650 mg/L.

Keywords COD and sulphate ratio; methane producing archaea; organic loading rate; sulphate reducing bacteria; sulphide; volatile fatty acid

Introduction Stabilization ponds are usually classified according to the nature of the biological activity – taking place as aerobic, anaerobic and facultative. Anaerobic ponds (APs) are usually used for treatment of strong industrial and agricultural wastes (Vuillot and Boutin, 1987; Alabaster et al., 1991), or as a pretreatment step where an industry is a significant contributor to a municipal system (US EPA, 1983). Many compounds in industrial wastewater are toxic to algae, and pretreatment in anaerobic ponds ahead of facultative and maturation ponds is necessary to avoid this. The fundamental mechanisms of treatment of organic material in a fully anaerobic stabilization pond are the same as those in other anaerobic wastewater treatment systems. The basic biochemical conversions occurring in acid formation and methane fermentation (US EPA, 1983; Giraldo and Garzon, 2002). In the first step, conversion of complex organic compounds to organic acids by acid-forming bacteria will occur followed by subsequent breakdown of the acids, primarily to carbon dioxide and methane gases, by the methane producing archaea (MPA) in the second step. In anaerobic reactors treating sulphate-containing wastewaters, both sulphate reduction and methanogenesis can be the final step in the degradation process, because sulphate reducing bacteria (SRB) are capable of using many of the intermediates formed during methanogenesis (Kalyuzhnyi and Fedorovich, 1998).

Effluent from agro-based industries such as starch, pulp and paper, sugar and distillery have high sulphate content. It has been widely reported that the bioactivity of anaerobic sludge could be detrimentally affected when treating sulphate-bearing wastewater (Hilton...
The conventional hypothesis has been that bioactivity was inhibited by increased concentrations of sulphidogenous products, particularly the un-dissociated hydrogen sulphide (Speece, 1983), or even sulphate itself (Fang et al., 1997). The inhibition concentration of sulphide and sulphate reported in literature varied considerably. So, the study of pond performance under various organic matter and sulphate ratios is of prime interest. Accordingly, this research assesses the performance of laboratory scale APs treating wastewater containing sulphate from a tapioca starch industry.

**Methodology**

**Experimental setup**

A laboratory scale columns-in-series type anaerobic pond setup was constructed as presented in Figure 1. Each set up consists of five cylindrical acrylic tubes of 2.2 m depth (2.0 m working depth) and 10.0 cm diameter. Columns were connected in series such that effluent from the surface of a column flowed to 25 cm above the base of the consecutive forward column. A peristaltic pump as a feed pump was connected from a feed tank to the inlet of the first column.

**Experimental procedure**

At first, each column was seeded (about 20 cm depth) with anaerobic active sludge and filled with wastewater diluted to 500 mg/L COD. Wastewater was obtained from Sanguan wongse tapioca starch industry, and seed sludge from a full-scale anaerobic pond of the same industry, located in Nakhon Rachasima, Thailand. The seed sludge had 78.5 g/L of total solids where 58.3% was volatile. The characteristic of the starch industry wastewater is presented in Table 1. Experiments were conducted by feeding wastewater at constant hydraulic loading rates of 15 L/d, corresponding to hydraulic retention time (HRT) of 5 days, with various combinations of COD:SO₄ ratios (5 to 20) in feed and total organic loading rates (150 to 600 g COD/m³d), as presented in Table 2, at the ambient laboratory, Environmental Engineering, Asian Institute of Technology, Thailand. Change in organic loading rate (from higher to lower) was made after running the reactor at steady state conditions for about 10 to 14 days. Since organic matter content of the wastewater from the starch industry was very high, 13.5 to 26.2 g/L of COD, tap water was used to dilute the wastewater to get the required COD concentration in to the feed solution. The OLR applied into the system was calculated based on total COD. To maintain the required

![Figure 1](https://iwaponline.com/wst/article-pdf/55/11/229/439249/229.pdf)
COD:SO₄ ratio in feed, Na₂SO₄ was used as an additional sulphate source. Sodium bicarbonate (1–2 g/L) was added to the feed to maintain the pH in the range between 6.5 and 7.5 as well as to buffer the content of reactor near neutral. Influent and effluent to and from the reactor were analysed for total chemical oxygen demand (TCOD), soluble chemical oxygen demand (SCOD), total suspended solid (TSS), sulphate, sulphide, alkalinity and volatile fatty acid (VFA).

Analytical method
The physical–chemical analysis of wastewater and sludge samples was carried out according to Standard Methods for the Examination of Water and Wastewater (1998).

All the samples for soluble COD, VFA, sulphate and alkalinity analysis were filtered through a 0.45 micron GFC glass fibre filter paper. The VFA concentration was analysed by Flame Ionization Detector (FID) gas chromatograph (GC Shimadzu 14-A) with WG-100 SS column pack (1/4 O.D. x 1.8 m). The GC was set at carrier gas pressure of 3.0, 0.7 and 0.5 kg/cm² for nitrogen, hydrogen and air, respectively and column, injection and detector temperature of 180, 190 and 190°C, respectively. Oxalic acid (0.08 g/20 ml) was added and mixed thoroughly to the filtrate sample and 1 μl filtered sample injected into the GC for VFA analysis.

Results and discussion
Anaerobic ponds are one of the most widely used wastewater treatment units in developing countries. Anaerobic digestion of organic matter takes place in anaerobic ponds similar to other anaerobic digesters. In anaerobic reactors treating sulphate-containing wastewater, both sulphate reduction and methanogenesis can be the final step in the degradation process, because SRB are capable of using many of the intermediates formed during methanogenesis (Kalyuzhnyi and Fedorovich, 1998). Wastewater from a starch industry containing high organic matter and sulphate was used for this study. The process performance of the column-in-series anaerobic pond reactor is described in the following sections. Regular monitoring of pH of the columns-in-series anaerobic ponds indicated a

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Unit</th>
<th>Value</th>
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<tbody>
<tr>
<td>Total COD</td>
<td>g/L</td>
<td>13.5 – 26.2</td>
</tr>
<tr>
<td>Soluble COD</td>
<td>g/L</td>
<td>11.2 – 13.8</td>
</tr>
<tr>
<td>Suspended solid</td>
<td>g/L</td>
<td>2.3 – 13.2</td>
</tr>
<tr>
<td>Sulphate</td>
<td>mg/L</td>
<td>164 – 350</td>
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<tr>
<td>TKN</td>
<td>mg/L</td>
<td>86 – 208</td>
</tr>
<tr>
<td>PO₄</td>
<td>mg/L</td>
<td>50 – 76</td>
</tr>
<tr>
<td>pH</td>
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<td>4.5</td>
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Table 1 Starch industry wastewater characteristics

<table>
<thead>
<tr>
<th>Exp. Set</th>
<th>OLR (g COD/m²·d)</th>
<th>Influent COD (mg/L)</th>
<th>Influent sulphate (mg/L)</th>
<th>COD:SO₄</th>
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<tr>
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<td></td>
<td></td>
<td>20</td>
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<td>150</td>
<td>750</td>
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<td>50</td>
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<td>1,500</td>
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<td>100</td>
</tr>
<tr>
<td>3</td>
<td>450</td>
<td>2,250</td>
<td>112.5</td>
<td>150</td>
</tr>
<tr>
<td>4</td>
<td>600</td>
<td>3,000</td>
<td>150</td>
<td>200</td>
</tr>
</tbody>
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Table 2 COD and sulphate concentration in feed
suitable environmental condition for anaerobic digestion into the system. Since the wastewater from starch industry was acidic in nature, sodium bicarbonate (0.5 to 2.0 g/L) was added to the feed to bring the influent pH near neutral. The pH in the entire column was found almost stable at 7 to 7.5 for all the cases of organic and sulphate loading.

**COD**

The TCOD and SCOD profiles along the columns obtained from the columns-in-series reactor operated at four different organic loading rates with various COD:SO₄ ratios are presented in Figure 2 (a) and (b), respectively. Both the TCOD and SCOD profiles for each case in the figures show that most of the COD reduction took place in column C1 and C2 and very little in C3 and C4, whereas contribution of C5 on COD removal was

![Figure 2](https://iwaponline.com/wst/article-pdf/55/11/229/439249/229.pdf)
nearly zero. Most of the TCOD removal in C1 was due to settling of particulate matter in the column (Figure 2(e)). The final effluent quality in terms of COD was found not much different in all cases of OLR and COD:SO₄ ratio in feed. For 150 g COD/m³d at COD:SO₄ ratio between 5 and 20, the effluent TCOD and SCOD observed were in the range between 71 and 92 mg/L and 45 and 59 mg/L, respectively. The corresponding values for 300 g COD/m³d were 47 to 121 mg/L and 64 to 205 mg/L, for 450 g COD/m³d, 54 and 98 mg/L and 82 and 209 mg/L and for 600 g COD/m³d, 76 to 123 mg/L and 124 and 181 mg/L, respectively. However the performance of C1 was found affected due to variation in COD:SO₄ ratio at and above 300 g COD/m³d of OLR. For 300 and 450 g COD/m³d, the effluent COD concentration from C1 was found to be reduced as a result of reduction in COD:SO₄ ratio from 20 to 15 and to 10. This might be attributed to extra COD utilization for the sulphate reduction process corresponding to increase in sulphate concentration in the feed, while further reduction of COD:SO₄ ratio to 5 in both the OLR resulted in increase in effluent COD from C1. Likewise increase in effluent COD from C1 was observed at COD:SO₄ ratio of 10 and 5 compared to COD:SO₄ ratio of 20 and 15. The bulk sulphide concentrations in the above cases were above 75 mg/L, which might be inhibitory to methanogenic organisms, resulting in lower COD removal performance of C1.

**Sulphate and sulphide**

The variation of sulphate and sulphide along the column for various COD:SO₄ ratios is presented in Figure 2(c) and (d), respectively. It was observed that almost all the influent sulphate was removed in C1 except in the cases for COD:SO₄ ratio of 5 at OLR of 450 and 600 g COD/m³.d. Average sulphate concentration of 325, 467 and 647 mg/L in feed was reduced to approximately 3.5, 50 and 121 mg/L, respectively in C1 for COD:SO₄ ratio of 5 at corresponding OLR of 300, 450 and 600 g COD/m³.d. The remaining sulphate from C1 was removed in C2. Since each column had a HRT of one day, based on 5 days HRT of the overall system, two days was sufficient to remove the entire sulphate up to about 650 mg/L in the feed. Higher sulphide concentration in bulk liquid was found in C1 and reduced gradually along the columns 2 to 5. This was because of sulphide released to the atmosphere as the wastewater flowed from one column to another. The sulphide in the bulk liquid was found higher than 50 mg/L only in cases when the sulphate in the feed exceeded 300 mg/L. In this research, no adverse effect was found on pond performance for influent sulphate lower than 300 mg/L, which was found in agreement with other researchers (Van Haandel and Lettinga, 1994; Mara, 1997). Mara (1997) suggested the permissible sulphate concentration in influent of anaerobic ponds less than 300 mg/L to prevent odour problem. Likewise, Van Haandel and Lettinga (1994) argue that sulphide concentrations of up to the 50 mg/L normally expected in domestic wastewater treatment are far lower than the minimum concentration needed to cause inhibition or toxicity. The experimental data together with the studies and findings quoted above suggest that sulphate conversions do not affect process performance in the anaerobic ponds.

**TSS**

Figure 2(e) shows the variation of TSS concentration along the columns. Like COD removal, most of the TSS was removed in C1 and C2. The removal of TSS was primarily due to settling of particulate component in the feed. Finally, the solids accumulated in the sediment layer undergo various anaerobic digestion processes and are converted to methane. The overall TSS removal was found in the range between 78 and 97%.
The variation of VFA along the column for different cases of COD:SO₄ ratios obtained from the column-in-series anaerobic pond reactor are presented in Figure 3. The four different acidic fractions of the total VFA (acetic, propionic, butyric and valeric acid) were measured in influent and effluent of each of the columns and are shown in the figure. All the values in the figure are presented in terms of mg of COD per litre to compare the amount of VFA with soluble COD. The final conversion of VFA into methane was supported by the low effluent concentration (less than 45 mg COD/L) of VFA recorded throughout the experiment, irrespective of loading and feed COD:SO₄ ratio. The low effluent concentrations of VFA together with the available alkalinity and the stable pH values confirmed that there was no risk of process acidification in any experimental case. The acetic and propionic acid was the main fraction of total VFA for all the cases as butyric and valeric acid were found in trace amounts. From the figure it can be seen that the variation of SCOD and VFA in C1 effluent is small, which indicates the acidogenesis process mostly took place in C1. However, comparably higher variation in SCOD and VFA in C1 was observed in cases of COD:SO₄ ratio 5 in the feed.

Figure 3  VFA variation along the column at various feed COD:SO₄ ratios at OLR (g COD/m³d) of (a) 150 (b) 300 (c) 450 and (d) 600 (Concentrations expressed in mg COD/L)
Contribution on COD removal by each column

Figure 4 shows the contribution of COD removal by each column in the column-in-series reactor. The overall average COD removal efficiencies for each case were found above 86% and slight increase in removal rate was observed by increasing OLR. Column 1 alone removed total COD of about 60% at 150 g COD/m³d for all COD and sulphate ratio between 5 and 20. At OLR of 300 g COD/m³d the COD removal efficiency of C1 gradually increased from 54% for COD:SO₄ ratio of 20 to 64% and 72% for COD:SO₄ ratio of 15 and 10, respectively. In contrast, the COD removal of C1 reduced to 60% for COD:SO₄ ratio of 5 at the same OLR. Likewise at OLR of 450 g COD/m³d the COD removal efficiency of C1 was found to be 65, 83, 84 and 69% and 77, 81, 77 and 68% at 600 g COD/m³d for COD:SO₄ ratio of 20, 15, 10 and 5, respectively. The majority of COD removal for each of the cases, which occurred in C1 and C2, ranges from 75 to 94% of total removal.

Contribution on COD removal by MPA and SRB

If organic matter is oxidized via sulphate reduction, 0.67 g of O₂ is required per g of SO₄ (Lens et al., 1998). This means that for waste streams with a COD:SO₄ ratio of 0.67, there is theoretically enough organic matter (COD) available to completely remove the sulphate via sulphate reduction. Hence, the contribution of SRB to COD removal in this research was estimated as 0.67 times the sulphate removed, neglecting any sulphate assimilation into biomass. In this study the contribution of MPA on COD removal at the same OLR was found about constant for COD:SO₄ ratio between 10 and 20 in the feed. However, it reduced at COD:SO₄ ratio of 5 in the feed. The result indicated that SRB can out-compete MPA for the same substrate at COD:SO₄ ratio equal to or higher than 5. Lin and Yang (1991) recommend a minimum COD:SO₄ ratio of 7 to 10 in the influent, regardless of pH value. This is a safe limit to avoid problems of odour, corrosion and toxicity. Choi and Rim (1991) reported that COD:SO₄ ratios above 2.7 would ensure the predominance of acidogenic methanogens. It is obvious from Figure 2(c) that most sulphate removal was contributed by C1, nearly all the sulphate removed in the case of OLR at 150 and 300 g COD/m³d and more than 80% in the case of 450 and 600 g COD/m³d. Hence, it is more relevant to compare the contribution on COD removal by MPA and SRB in C1 alone and presented in Figure 5. The contribution of SRB to remove COD increases with decreasing COD:SO₄ ratio in the feed and found up to 25% of total COD removal at OLR of 300 g COD/m³d and COD:SO₄ of 5 in C1.
Conclusions
In this research, no adverse effect of COD:SO4 ratios between 5 and 20 were found on overall COD removal performance of an anaerobic pond operated with OLR of 150 to 600 g COD/m3d. The contribution of MPA on COD removal at the same OLR was found about constant for feed COD:SO4 ratio between 10 and 20. However, it reduced at COD:SO4 ratio of 5 in feed. From the results it can be concluded that SRB could out-compete MPA for the same substrate at COD:SO4 ratio equal to or lower than 5 for OLR greater than 300 g COD/m3d. Sulphide inhibition was not observed on overall performance of pond up to an influent sulphate concentration of 650 mg/L. A large fraction of COD and TSS removal was found in columns 1 and 2 of the column-in-series anaerobic pond reactor. Likewise almost all sulphate reduction took place in column 1. This indicated an intense microbial activity close to inlet zone, which received full strength of wastewater. Most of the fraction of effluent SCOD from each column corresponded to VFA (approximately 69 to 82% of SCOD), which can be taken as evidence of the higher activity of acid producing organisms compared to methane producing and methanogenesis as a rate-limiting step in the anaerobic digestion process in anaerobic ponds.

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References


