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Anaerobic treatment of high sulphate containing pharmaceutical wastewater

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The effect of different organic loading rate (OLR) to an anaerobic reactor performance and sulphate reduction was investigated. Sulphate concentration in the feed varied from 100 to 3000 mg.L⁻¹ and up to 97% removal efficiency was observed at OLR 0.43 - 1.23 kg COD.m⁻³.d⁻¹. However, the removal efficiency showed some decline (to 53 – 67% removal) at OLR 1.53 – 3.73 kg COD.m⁻³.d⁻¹, probably due to high sulphate concentration in the feed during this period. At a reactor OLR of 1.86 kg COD.m⁻³.d⁻¹ (HRT 4 d), the soluble COD reduction was around 70 - 75%. Nevertheless, when the OLR was increased to 2.48 - 3.73 kg COD.m⁻³.d⁻¹, the COD removal efficiency decreased to 45%. The microbial aspects of sulphate reducing bacteria (SRB) results indicated that sulfidogenic bacteria, such as Desulfovibrio, had contributed substantially to the treatment process (around 16 – 36% when the reactor was operated at OLR 0.86 – 2.98 kg COD.m⁻³.d⁻¹).

Keywords: anaerobic process, microbial population, stage reactor, sulphate containing wastewater, sulphate reducing bacteria

Introduction

Industrial wastewater containing sulphate has contributed local imbalances in the natural sulfur cycle, leading to severe environmental problems¹, ². Certain industrial effluents may contain several thousands of milligrams per litre while domestic sewage contains typically less than 500 mg.L⁻¹. Wastewater containing sulphate is usually treated using physicochemical and biological methods³, ⁴. However, this process has some limitations such the need for separation and appropriate disposal of the solid phase and relatively high costs and energy consumption⁵. In contrast, the success of high-rate anaerobic technology has encouraged the treatment of high organic-strength wastewater. The presence of sulphate at high concentrations produces sulphide as a result of sulphate reduction and reported to severely impair methanogenesis⁶. Sulphate is converted by sulphate reducing bacteria (SRB) to sulphide and H₂S which are ranked as significant inhibitors of anaerobic digestion⁷. Sulphides in anaerobic digesters can also result from the presence of other sulphur containing compounds in the feed and will be established during anaerobic degradation of proteins⁸. SRB utilize the carbon source provided by the hydrolytic and acidogenic bacteria because they are unable to produce the hydrolytic enzymes necessary for protein, carbohydrate and lipid hydrolysis⁹. The main problems related to the presence of high sulphate concentrations in the influent of anaerobic reactors are recognised as: competition between sulphate reducing bacteria (SRB) and methane producing archaea (MPA) for the same substrates (H₂, acetate); sensitivity of MPA to sulphide, leading to methanogenesis inhibition when the sulphide concentration surpasses certain limits; and precipitation of trace metals, causing nutritional deficiencies in the reactor¹⁰-¹². Hydrogen sulphide toxicity of the hydrogen utilising methanogens is relatively weaker than for other microbial groups, explaining how methanogenesis can occur from complex substrates even at high concentration of sulphide. Wastewaters from the pharmaceutical industry usually contain a high concentration of sulphate as a result of sulphate use during the downstream processing of fermentation broth. The production of sulphide during anaerobic treatment of sulphate containing wastewaters can reduce the efficiency of anaerobic treatment. Moreover, high sulphide concentrations can inhibit methanogens and can precipitate nutrients essential to methanogens¹³. Consequently, the aim of this study was to investigate treatment of pharmaceutical wastewater that contains sulphate in an anaerobic reactor.

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Materials and Methods

Anaerobic Reactor

The anaerobic reactor used in this study comprise of four identical cylindrical Plexiglas compartments (stages), 80 mm internal diameter by 640 mm height, linked in series. The active volume of the reactor system was 11 L (4 stages of 2.75 L). Each stage of the reactor had a 3-phase separator baffle, angled at 45° and placed 50 mm below the effluent ports, to prevent floating granules from washing out with the effluent. Each stage was equipped with sampling ports at 100 mm intervals (lowest being 30 mm from the base) that allowed biological solids and liquid samples to be withdrawn from the sludge bed. The influent wastewater entered through a 12mm internal diameter down comer tube in the head plate that extended to within 15mm of the reactor base and allowed feed to flow upward through the sludge bed. Effluent from each stage of the reactor flowed by gravity to the next, as each stage was placed on a stepped platform having a 150 mm step height. The walls of the reactors were wrapped with a tubular PVC water-jacket, 15mm internal diameter, to maintain the reactor temperature at 37 °C. Peristaltic pumps (Watson Marlow 100 series) were used to control the influent feed rate to the first stage of the reactor. Gas production was monitored separately for each stage using an optical gas-bubble counter having a measurement range of 0 – 1.5 L.h⁻¹ and precision within ±1%.

Wastewater

The untreated wastewater used in the current study was supplied by Eli Lilly & Company Ltd., Liverpool, UK and originated from pharmaceutical fermentation processes for the production of antibiotics and had the following characteristics (average); soluble COD, 7500 mg.L⁻¹; soluble BOD₅, 3500 mg.L⁻¹; Sulphate, 2500 mg.L⁻¹; Total Kjeldahl Nitrogen (TKN), 370 mg.L⁻¹ and pH, 5.7.

Reactor Operation

The start-up of the anaerobic reactor was carried out using brewery wastewater due to its ease of degradation and high COD value. The brewery wastewater comprised mainly waste beer, i.e. out-of-date product, returned to the brewery for biological treatment, which was then mixed with process wastewater in a balancing tank before treatment. In addition, the easiness of transportation to the laboratory and storage in its concentrated state in the laboratory made it an ideal wastewater for use. The trace elements deficiency of brewery wastewater was corrected by adding a trace elements solution, whereas the trace elements deficiency of pharmaceutical wastewater was corrected with a commercial micro-nutrient supplement, Nutromex TEA 310, supplied by OMEX Environmental Ltd, UK, with 0.01ml TEA supplement added for each 5000 mg COD. Urea (CH₂N₂O) and di-potassium hydrogen phosphate (K₂HPO₄) solution was prepared for Nitrogen and Phosphorous deficit. Alkalinity of the feed was adjusted to 1000 - 2000 mg.L⁻¹ by adding NaHCO₃ to the feed whenever it is required. In general, this study was carried out in four major steps; 1) start-up of anaerobic reactor, 2) acclimatisation to sulphate containing wastewater, 3) increase in OLR (0.43–1.86 kg COD.m⁻³.d⁻¹) by altering feed COD (1700–7450 mg.L⁻¹) at constant HRT (4 d), and increase in OLR (2.48–3.73 kg COD.m⁻³.d⁻¹) by reducing HRT (4–2 d) at constant feed COD (7450 mg.L⁻¹).

Seed Sludge

The reactor was seeded with anaerobic digested sewage sludge (Hexham Municipal sewage treatment plant). This was sieved to pass 2.0 mm mesh, giving solids content of 21,500 mg TSS.L⁻¹ (13,400 mg VSS.L⁻¹). 1.2 L of sieved sludge was added to each reactor stage, the remaining volume being filled with tap water, to give a final sludge concentration of 5850 mg VSS.L⁻¹. After seeding, the head plates were attached, and the headspace above each reactor was flushed with nitrogen gas to displace residual air from the system before introducing the feed. The reactors were allowed to stabilise at 37 °C for 24 h without further modification.

Sampling and Analysis

Supernatant liquor, gas and sludge samples were taken separately from each stage for analysis. In addition, gas production rate was determined separately for each stage. Sample analysis included chemical oxygen demand (COD), pH, alkalinity, total Kjeldahl nitrogen (TKN), ammonium nitrogen (NH₃-N), suspended solids (SS), volatile suspended solids (VSS), all according to Standard Methods. Microbial Community Analysis

The use of rRNA-based molecular techniques such as fluorescent in situ hybridisation (FISH) has provided detail description of microbial populations present in anaerobic digestion. One advantage of
using FISH is that metabolically active cells are detected and therefore descriptions of the physiologically important population members can be obtained. Moreover, it also provides additional information for the development of new probes. Numerous rRNA-based methods have been developed to identify and quantify microorganisms in anaerobic reactors. As a result, investigation into sulphate reducing bacteria (SRB) was carried out and evaluated. Three different SRB probes; Desulfovibrio (DSV698), Desulfobacter (DSB985) and SRB385 were used in this study. Cells were visualised using a Zeiss Standard Microscope 14 (Carl Zeiss) or confocal laser scanning microscope (CLSM). The number of cells for each group specific probe was determined, and means were calculated from 5 to 10 randomly chosen FOV for each sample. Statistical analysis for valid cell counting was determined according to Davenport and Curtis.  

Results and Discussion

Reactor Performance

Figure 1 shows temporal change in the total COD removal and fractional contribution by each stage of the anaerobic reactor treating sulphate containing wastewater. At a reactor OLR of 1.86 kg COD m⁻³ d⁻¹ (HRT 4 d), the soluble COD reduction was around 70 – 75%. However, when the OLR was increased to 2.48 kg COD m⁻³ d⁻¹ (by lowering the HRT since the strength of the wastewater was limited) the COD removal efficiency decreased gradually until only 45% soluble COD removal (average removal when reactor approached steady state) was observed at an OLR of 3.73 kg COD m⁻³ d⁻¹. This high level of sulphate removal could be contributed during the pharmaceutical acclimatisation period which had lower sulphate concentration (around 100-1200 mg.L⁻¹). It was assumed that sulphate removal occurred as a result of conversion to sulphide by SRB. However, the removal efficiency showed some decline (to 53–67% removal) at OLR 1.53–3.73 kg COD.m⁻³.d⁻¹, probably due to high reactor when reactor HRT was set to 4 d (i.e. for all reactor OLR at or below 1.86 kg COD m⁻³ d⁻¹), with less contribution from Stage 2 (around 10 – 15%), and Stages 3 and 4 accounting for around 5%. This also suggests that it was the recalcitrant characteristics of the Stage 1 effluent that limited further COD degradation in subsequent stages of the reactor, rather than excessive OLR, although as the pH was reduced in all stages at the highest OLR (pH data not presented), there is a possibility that the methanogenic biomass in Stages 2–4 could also have been affected adversely by the acidic conditions generated in Stage 1 (VFA data not presented).

Sulphate Reduction

It is generally known that sulphate reduction in anaerobic digestion can cause significant effect to the methanogens. Moreover, the SRB have a higher affinity for substrates (hydrogen and acetate) and faster growth rate than methanogens. At the same time organic sulphur and sulphate could be utilized by SRB to generate sulphide. In particular, hydrogen sulphide produced during sulphate reduction can lead to poisoning of methanogens. Figure 2 shows the decrease in sulphate concentration between influent and effluent (i.e. total sulphate decrease across the reactor). Sulphate concentration in the feed varied from 100 to 3000 mg.L⁻¹ and up to 97% removal efficiency was observed at OLR 0.43-1.23 kg COD.m⁻³.d⁻¹. This high level of sulphate removal could be contributed during the pharmaceutical acclimatisation period which had lower sulphate concentration (around 100-1200 mg.L⁻¹). It was assumed that sulphate removal occurred as a result of conversion to sulphide by SRB. However, the removal efficiency showed some decline (to 53–67% removal) at OLR 1.53–3.73 kg COD.m⁻³.d⁻¹, probably due to high
sulphate concentration (around 2000–3000 mg.L\(^{-1}\)) in the feed during this period exceeding the capacity of the SRB. It is not clear in which stage the sulphate was removed largely since sulphate data was not collected for individual stages. However, a study by Freese and Stuckey\(^{22}\) on anaerobic treatment of sulphate-enriched wastewater in ABR showed the majority of the sulphate reduction occurred in the first three compartments of their reactor. These workers found that the conditions with low pH, high VFA concentrations and high hydrogen levels in the front compartment of an ABR were more favourable for sulphate reduction. Furthermore, Fox and Venkatasubbiah\(^{23}\) showed that sulphate reduction occurred in the first compartment of an ABR during the treatment of high sulphate pharmaceutical wastewater. Vossoughi et al\(^{24}\) also showed that acidogenic phase had the ability to reduce sulphate in an ABR. Consequently, similar observations were also seen in the anaerobic reactor (low pH and high VFAs in Stage 1, data not presented) and it is likely that the majority of the sulphate reduction occurred most probably in Stage 1 which is the acidogenic stage in the reactor system. Previous studies have found that there is no clear dominance between methane producing bacteria (MPB) and sulphate reducing bacteria (SRB) competition\(^{25-26}\). These researchers found that the available substrate plays an important part in the competition of SRB in anaerobic system and COD: sulphate ratios can be used as an approximate measure of competition in the system. According to Speece\(^{27}\), the theoretical COD: sulphate ratio for total sulphate reduction is 0.67. If values are less than 1, then SRB predominates; if greater then 2, then the MPB are dominant. Consequently, the COD: sulphate ratio in this study was approximately 3 (average COD of pharmaceutical wastewater is 7450 mg.L\(^{-1}\) and sulphate concentration being around 2500 mg.L\(^{-1}\)), which suggest predominance of MPB in the reactor system.

**Table 1**—Mean number of cells per ml detected in the anaerobic reactor sludge stages by group specific probes

<table>
<thead>
<tr>
<th>Probe</th>
<th>OLR (kg COD.m(^{-3}).d(^{-1}))</th>
<th>Stage 1 (Log)</th>
<th>Stage 2 (Log)</th>
<th>Stage 3 (Log)</th>
<th>Stage 4 (Log)</th>
</tr>
</thead>
<tbody>
<tr>
<td>SRB</td>
<td>2.98</td>
<td>8.2278</td>
<td>7.9049</td>
<td>7.8042</td>
<td>7.9049</td>
</tr>
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<td></td>
<td>3.73</td>
<td>7.8896</td>
<td>8.2059</td>
<td>7.8896</td>
<td>7.8404</td>
</tr>
<tr>
<td></td>
<td>0.86</td>
<td>8.0446</td>
<td>7.8575</td>
<td>7.9988</td>
<td>7.8404</td>
</tr>
<tr>
<td></td>
<td>1.86</td>
<td>7.9866</td>
<td>7.7435</td>
<td>7.7647</td>
<td>7.9049</td>
</tr>
<tr>
<td>DSV689</td>
<td>2.98</td>
<td>8.1585</td>
<td>7.8575</td>
<td>7.9049</td>
<td>7.8739</td>
</tr>
<tr>
<td></td>
<td>3.73</td>
<td>7.7435</td>
<td>8.1327</td>
<td>7.7647</td>
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<td>1.86</td>
<td>7.2206</td>
<td>7.1415</td>
<td>7.1415</td>
<td>7.1415</td>
</tr>
<tr>
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<td>7.0446</td>
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</tr>
<tr>
<td></td>
<td>3.73</td>
<td>6.9196</td>
<td>7.3456</td>
<td>7.0446</td>
<td>6.9196</td>
</tr>
</tbody>
</table>

2.98 kg COD.m\(^{-3}\).d\(^{-1}\). Based on these findings, it appears that there were favorable conditions for sulfidogenesis in reactor system, particularly the development of highly sulfidogenic populations in Stage 1 of the reactor. Furthermore, Desulfovibrio (DSV698) was the main SRB compared to Desulfobacteria (DSB985) that was present in the reactor during the investigation.

**Conclusions**

The anaerobic system is an appropriate option for pre-treatment of wastewaters with high sulphate composition. The reactor system encouraged phase separation (especially at high OLRs) by accommodating different microbial populations in different stages according to their favourable substrate conditions, thus operating as a two-stage anaerobic digestion system. However, the treatment efficiency of the anaerobic reactor was affected at high OLRs probably due the complexity of the wastewater which contained high amounts of sulphate. It was thought that as OLR was increased, the increasing sulphide production, especially in Stage 1, affected the methanogens in the subsequent stages (Stage 2, 3 and 4); therefore, the potential gains in efficiency due to phase separation were offset by these factors, contributing to the overall lower process efficiency of the system.

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References