

## Bovine Rectal Bacteria Can Solve COD Problems with Acetate Liquor and Restaurant Kitchen-Sink Wastewater

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**Abstract.** COD removal was investigated in batch anaerobic digesters fed with acetate/restaurant kitchen-sink wastewater, sulfate, and bovine rectal bacteria. Results of the experiments show that for a digestion period of 24h, COD removed from the wastewater was 55% for an initial COD to  $\text{SO}_4^{2-}$  ratio of 8.9 compared to higher COD removal (77%) for a lower COD to  $\text{SO}_4^{2-}$  ratio (4.8) for acetate. The wastewater mixed liquor showed negative voltage, indicating reducing conditions, that is, the energy from the donor wastewater was being transferred to  $\text{SO}_4^{2-}$ . For acetate the voltage was positive, indicating oxidizing conditions, that is, the energy was being transferred to oxygen. The experiments demonstrate COD removal mechanism for the wastewater was affiliated to sulfate reduction as opposed to the symbiosis of sulfate reduction and sulfide oxidation for acetate under the influence of the bovine rectal bacteria. In general COD removal shows dependency upon COD to sulfate ratio and ORP can be used to monitor wastewater treatment process control.

**Keywords:** Acetate, bovine rectal bacteria, ORP, sulfate reduction, sulfide oxidation, kitchen-sink wastewater.

### 1. Introduction

Much research has been focused on the persistency of *E. coli* harbouring the bovine terminal rectal mucosa [1]. However, to our knowledge no work has been reported in the literature that has employed bovine rectal bacteria for biological wastewater treatment or effluent remediation. The present work therefore examines the effectiveness of such bacteria for the treatment of restaurant kitchen-sink wastewater and acetate liquor. Our Division of Environment (DoE) Malaysia is most concerned with the restaurant wastewater because it is rampantly discharged to the surface drains, often secluding regulatory surveillance because it gets carried away during heavy rains to the receiving waters.

Biochemical reactions are used to describe the complex nature of bacterially mediated anaerobic processes in wastewater treatment. Oxidation removes/donates electrons; reduction adds/accepts electrons; and microbes drive these reactions by consuming the electron donor, acceptor and C-source. Insoluble organic compounds in anaerobic environments are degraded to  $\text{CH}_4$ ,  $\text{CO}_2$ , and  $\text{H}_2\text{S}$  via a syntrophic interaction of hydrolytic bacteria, acid-formers, and gas-formers, collectively known as fermentative or anaerobic bacteria. In sludge digestion, the sulphate reducers consume  $\text{H}_2$  (electron donor) when the gas is limiting and sulphate sufficiently available. Low and high COD to  $\text{SO}_4$  mass ratios appear to favour sulfidogenesis and methanogenesis, respectively [2]. Oxidation-reduction potential (ORP) measurements provide a means of process control for such anaerobic systems.

ORP indicates the flow of energy (reducing equivalents) from the electron donor to the electron acceptor; and it is the electron acceptor that determines the amount of energy released by the donor [2]. Positive ORP (+mV) shows the net reaction is potentially oxidative, inferring the energy flow is towards oxygen (that will trigger the reactions aerobically). Negative voltage (-mV) points to the net reaction being potentially

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reductive, that is, the energy flow is towards nitrate, sulphate, or carbon dioxide triggering anaerobic reaction. According to Rittmann et al 2001 [3], a fermentative reaction (where the microbes use organic compound as acceptor and donor) requires the starting donor to have a large negative voltage. Table 1 illustrates half reactions for oxidation, reduction, and cell synthesis [4,5]. Acetate is an electron donor and O<sub>2</sub> and SO<sub>4</sub><sup>2-</sup> are electron acceptors. Reaction (1) shows donor C is oxidized to CO<sub>2</sub>. Reaction (2) shows acceptor O<sub>2</sub> is reduced to H<sub>2</sub>O. Reaction (3) shows sulfate (SO<sub>4</sub><sup>2-</sup>) is reduced to sulfide. Reaction (4) shows inorganic C via autotrophic bacteria provides element C in the cell material (C<sub>5</sub>H<sub>7</sub>NO<sub>2</sub>).

Table 1. Half reactions for acetate, sulfate, and cell synthesis

$1/8 \text{CH}_3\text{COO}^- + 3/8 \text{H}_2\text{O} = 1/8 \text{CO}_2 + 1/8 \text{HCO}_3^- + \text{H}^+ + \text{e}^-$	(1)
$1/4 \text{O}_2 + \text{H}^+ + \text{e}^- = 1/2 \text{H}_2\text{O}$	(2)
$1/8 \text{SO}_4^{2-} + 19/16 \text{H}^+ + \text{e}^- = 1/16 \text{H}_2\text{S} + 1/16 \text{HS}^- + 1/2 \text{H}_2\text{O}$	(3)
$1/5 \text{CO}_2 + 1/20 \text{HCO}_3^- + 1/20 \text{NH}_4^+ + \text{H}^+ + \text{e}^- = 1/20 \text{C}_5\text{H}_7\text{NO}_2 + 9/20 \text{H}_2\text{O}$	(4)

In nature there are many donors but few acceptors. The former includes (for heterotrophic reactions) organic donors such as domestic wastewater (C<sub>10</sub>H<sub>19</sub>O<sub>3</sub>N), carbohydrate (CH<sub>2</sub>O), acetate (CH<sub>3</sub>COO<sup>-</sup>), and methanol (CH<sub>3</sub>OH); and inorganic donors (autotrophic reactions) such as Fe (II), NH<sub>4</sub><sup>+</sup>, H<sub>2</sub>S, and H<sub>2</sub> [4], [5]. As for gas production according to Clark *et al.* [6], H<sub>2</sub>S precedes CH<sub>4</sub>. Energy yield from the donor decreases according to the following donor preferential order: O<sub>2</sub>, NO<sub>3</sub><sup>-</sup>, SO<sub>4</sub><sup>2-</sup>, and CO<sub>2</sub>. Such microbial activities may employ ORP (voltage) measurements to indicate energy flow from the donor to the acceptor [2]. Carbon from acetate as in reaction (1) and nitrogen from NH<sub>4</sub><sup>+</sup> as in reaction (4), are elements required for the cell material [4]. Acetate and hydrogen are highly competitive intermediates (donors) between the methanogens and SRB which scavenge sulfate to concentrations as low as 60-150mM [7] in sediment habitats. Acetate is used in the experiment so that the SRB and SOB may remove carbon from the donor more rapidly if not substantially. The methanogens are noted for their versatility when acetate and H<sub>2</sub> (donors) and CO<sub>2</sub> (acceptor) are present for CH<sub>4</sub> production [3], [4]. Competition between these microbes, the SRB and methanogens, depends upon COD to SO<sub>4</sub><sup>2-</sup> mass ratio [4]; ratio 1.5 favours the heterotrophic SRB and ratio 6.0 the methanogens [8]. Sulfate reduction, in the presence of sodium acetate and citrate, was studied by Kleikemper *et al.* [9] using SRB obtained from contaminated aquifers. Boshoff *et al.* [10] used dried algal biomass as carbon source and found COD removal varied with influent COD to SO<sub>4</sub><sup>2-</sup> ratios (8.1, 11.2, and 15.0) which increased as SO<sub>4</sub><sup>2-</sup> decreased; and some 31% of the COD removed was affiliated to sulfate reduction. Visser *et al.* [11] investigated the SRB-SOB symbiosis using acetate in an UASB reactor and found that at pH less than 7.5, the microbes were equally affected by H<sub>2</sub>S concentrations and at higher pH, the SRB out-competed the methanogens. Work by Stams *et al.* [8] shows the SRB out-competed the methanogens for CH<sub>3</sub>COO<sup>-</sup> and H<sub>2</sub> when SO<sub>4</sub><sup>2-</sup> was sufficiently available; however, the reverse occurred under SO<sub>4</sub><sup>2-</sup> limited condition and SO<sub>4</sub><sup>2-</sup> reduction was inhibited by H<sub>2</sub>S in concentrations less than 450mg/L (as S).

## 2. Methodology

A cow's rectal bacteria, obtained from a local feedlot, were used to prepare bacterial slurry. After 3d of settlement (4<sup>0</sup>C) of the slurry, the slurry supernatant was used as bacteria seed for the experiments. Stock solutions of sodium acetate (C<sub>2</sub>H<sub>3</sub>NaO<sub>2</sub>·3H<sub>2</sub>O) and sodium sulfate (Na<sub>2</sub>SO<sub>4</sub>) were also prepared. Kitchen-sink wastewater, collected from a local restaurant, was filtered to remove large solid particles and oil/grease from cooking. Two shake flasks were each filled with the wastewater/sodium acetate, seed media (1mL), sodium sulfate, and de-ionized water (DW). The wastewater/acetate/sulfate volumes were adjusted to the desired COD to SO<sub>4</sub><sup>2-</sup> ratios to a total mixed liquor volume of 150mL. Prior to agitation, the mixed liquor was analyzed for soluble COD, soluble SO<sub>4</sub><sup>2-</sup>, soluble S<sup>2-</sup>, pH, dissolved oxygen, alkalinity, and temperature. The COD, SO<sub>4</sub><sup>2-</sup>, and S<sup>2-</sup> parameters were measured using the Hach DR/5000 Spectrophotometer following

the manual protocols, respectively: Method 8000 (Digestion Method); Method 8051; and Method 8131 (Methylene Blue Method). An ORP meter (YSI6600) was used to measure voltage (as mV) of the mixed liquor. The flasks were agitated for 24h under total darkness; after which the mixed liquor was settled under quiescent conditions. The mixed liquor supernatant was filtered (using 0.45 $\mu$ m filter paper) and the filtrated sample analyzed for the same parameters. Measurement of alkalinity was performed by titration to pH 4.3 according to Method 2320B [12].

### 3. Results and Discussion

The experimental results are presented and discussed as follows:

**Restaurant kitchen-sink wastewater:** The COD removed from the wastewater was 77% (from 1536 to 356mg/L soluble COD) with alkalinity of 150mg/L as CaCO<sub>3</sub> produced for an initial COD to SO<sub>4</sub><sup>2-</sup> ratio of 4.8 (1536/320). The DO concentrations decreased from 0.36mg/L (start) to 0.10mg/L (after 24h) with pH 4.7 increasing to 6.3. Soluble sulfate decreased by 41% (from 320 to 190mg/L SO<sub>4</sub><sup>2-</sup>), leading to 4-fold increase in sulfide (from 96 to 484mg/L over 12h), confirmatory of the presence of SRB. With conditions almost anoxic (0.35 decreasing to 0.1mg/L DO), it was apparent the SRB did not require such strict anaerobic conditions when present in the bulk of mixed liquor in agitation, which is in agreement with the observation of Maree *et al.* [13]. When mixed with the bacteria, the mixed liquor registered negative ORP (-280mV decreasing to -160mV over 24h), indicating reducing conditions and inferring the energy was being transferred to SO<sub>4</sub><sup>2-</sup> (320mg/l) (although NO<sub>3</sub>-N was not measured). Thus sulfate reduction occurred as SO<sub>4</sub><sup>2-</sup> was induced and under the circumstances, the reducing equivalents did not possibly flow towards O<sub>2</sub> because the dissolved oxygen concentrations were very low (0.35mg/L), following which, COD decreased (77%) and SO<sub>4</sub><sup>2-</sup> also decreased (41%). After digestion, the voltage decreased to -160mV, indicating that less reducing electrons were being transferred to SO<sub>4</sub><sup>2-</sup> as more of sulfate (together with COD) was reduced. This occurred when the COD to SO<sub>4</sub><sup>2-</sup> ratio decreased from 4.8 to 1.87 (356/190). In a real treatment situation such as an activated sludge process, ORP monitoring can help with the process control by making adjustments to pH and DO (or even SO<sub>4</sub><sup>2-</sup> for anaerobic treatment). That there was no increase in SO<sub>4</sub><sup>2-</sup> indicates the absence of SOB, which could possibly be upset or suppressed by sulfide toxicity and/or low DO concentrations in the bulk of the mixed liquor. The COD to SO<sub>4</sub><sup>2-</sup> ratio (4.8) was possibly low enough as to cause sulfide oxidation, as observed by Lens *et al.* [5].

**Acetate liquor:** Soluble COD removed from acetate was 55% (from 1872 to 852mg/L) accompanied by 24% increase in soluble sulfate for an initial COD to SO<sub>4</sub><sup>2-</sup> ratio of 8.9 (1872/210). The dissolved oxygen concentrations (2.4mg/L) decreased to 1.9mg/L over 24h. Alkalinity produced was 850mg/L as CaCO<sub>3</sub> at pH 7.5, which increased from 7.22. Increase in sulfide (16% over 12h) observed indicates SO<sub>4</sub><sup>2-</sup> reduction in the presence of SRB. Increase in SO<sub>4</sub><sup>2-</sup> (24% over 24h) observed also indicates the presence of SOB which oxidized sulfide to sulfate. The rate of sulfate reduction was possibly slower than sulfide oxidation so that the increase in SO<sub>4</sub><sup>2-</sup> observed was due to SO<sub>4</sub><sup>2-</sup> replenishment (via sulfide oxidation) plus the portion of sulfate remaining (not reduced). In a real treatment situation, decrease and increase of sulfate could be monitored using short duration interval measurements, a characteristic feature of the symbiotic relationship between SRB and SOB often encountered in anaerobic environments. The mixed liquor ORP recorded positive voltage (+mV) throughout digestion, as indicated by +116mV decreasing to +100mV and to +53mV. The positive voltage indicates the reducing electrons from acetate were flowing towards oxygen (2.4mg/L initially), as evidenced by the decrease in DO by the aerobic heterotrophic bacteria plus oxygen depletion due to sulfide oxidation by the SOB. Increase in sulfide observed could be due to its higher production rate from sulfate reduction minus its lower decreasing rate used in sulfide oxidation (as indicated by 16% increase in sulfide over 12h). The net positive voltage (+mV) indicates the reducing equivalents were being transferred from acetate to oxygen as illustrated respectively in reaction (1) and reaction (2). As more carbon from acetate was removed, the reducing equivalents became less positive (as indicated by the ORP drop to +52.7mV over 24h) due to low DO availability (1.9mg/L).

**Simultaneous carbon removal:** Carbon removal possibly occurred simultaneously via the metabolic activities of the bovine rectal bacterial population that comprised the aerobic heterotrophic bacteria, SOB, SRB, and possibly methanogens (not confirmed because no CH<sub>4</sub> measurement was made) and other

facultative bacteria. The major portion of COD removed was possibly channeled for synthesis and energy production for the heterotrophic bacteria. The low DO in the wastewater reactor (0.36 mg/L diminishing to 0.1 mg/L) apparently did little or no harm to upset the SRB, indicating the SRB are not strict anaerobes and DO toxicity possibly became less effective because the DO was utilized by the SOB and the heterotrophic bacteria and also because of the agitation effect in the bulk of the mixed liquor. The DO toxicity could upset the bacteria had the habitat been sludge or sediments. The alkalinity produced could neutralize the acidic water according to the equation  $\text{HCO}_3^- + \text{H}^+ = \text{CO}_2 + \text{H}_2\text{O}$ , which possibly lessened the DO toxicity further.

#### 4. Conclusions

In conclusion, the results of the study point to the following:

For the restaurant wastewater, the COD removal mechanism was affiliated to aerobic heterotrophic oxidation and sulfate reduction combined, which proved workable at the COD to  $\text{SO}_4^{2-}$  ratio of 8.9. The COD removal for acetate was dependent on the SRB-SOB symbiosis. Reducing potential for the wastewater was observed for 55% COD removal. Oxidizing potential was observed for acetate with higher COD removal (77%) for a lower COD to  $\text{SO}_4^{2-}$  ratio (4.8). ORP proved useful for monitoring the SRB-SOB symbiotic nature of anaerobic wastewater treatment. Bovine rectum can be a sustainable source of bacteria for solving COD problems with our rampantly discharged restaurant kitchen-sink wastewater.

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