1. Introduction

An experimental operation for the treatment of fruit cordial wastewater using anaerobic continuous stirred tank reactor (CSTR) has previously been carried out [1]. That study suggested that such wastewater requires supplementary substrates to sustain its critical operational parameters such as alkalinity, pH and biomass. Observation during the operation showed that those parameters had gone below recommended levels. Subsequently, after increasing the chemical oxygen demand (COD) mass loading to the level of 1.33 kg m$^{-3}$, the whole experimental operation had failed. The failure was off-set by a sudden drop of pH and increasing concentration of volatile fatty acid (VFA). It is well known that these two factors are critical to the anaerobic digestion process, especially to the sensitive methanogen group of bacteria. However, the amount of COD loading at 1.33 kg m$^{-3}$ was found to be too low to cause failure in anaerobic CSTR [2,3]. Therefore, it was concluded that inadequate buffering control and disruption of microbial population balance between non-methanogen and methanogen to convert carbonaceous organic to CH$_4$ were the main causes of operational failure. Obstacles during operation should be rectified since this process has been shown to be an efficient alternative to treat such wastewater with similar characteristics and to produce CH$_4$ as the bioenergy [2,4].

This study proposes the use of ammonium bicarbonate (NH$_4$HCO$_3$), for its buffering requirement against acidity during the treatment operation and also for the microbial population. NH$_4$ will perform important roles in the anaerobic digester as the preferred bacterial nutrient for nitrogen and buffering capacity in an anaerobic digester [5]. However, high NH$_4$HCO$_3$ concentrations cause free ammonia toxicity especially to the methanogen [5,6]. Therefore the optimal dosage for NH$_4$HCO$_3$ used as supplement in anaerobic digestion process should be determined.

2. Materials and methods

2.1. Preparation of substrate

The fruit cordial wastewater in question is a purple soft drink that contains the juice of grapes, raspberries and blackcurrants, which has been analysed to be lacking in alkalinity and nitrogenous resources [1]. Preparation of fruit cordial wastewater was carried out according to a previous study by diluting the stock liquor [7]. The dilution of concentrated fruit cordial gave a consistent concentration of wastewater up to 2000 mg L$^{-1}$ of...
COD, which is in the range of medium strength wastewater [8]. Fibrous matter arising from the depulping process was separated at the initial stage of production either by centrifugal process or water-pressing, and managed under solid waste management. This study was carried out to represent treatment for the unmixed wastewater without the presence of fibrous components.

2.2. Toxicity test

The toxicity test of NH₄HCO₃ (BDH Ltd.) on the anaerobic digestion of soft drink wastewater was done by submerging a series of air tight containers (500 mL) in a water bath at a temperature of 35 °C. These containers were connected to biogas measurement apparatus. Prior to testing by batch operation, each container was filled with 300 mL of stabilised anaerobic sludge as the seeding sludge and 100 mL of wastewater with COD concentration of 2000 mg L⁻¹. This was to simulate the non-critical COD mass loading at 0.5 kg m⁻³, which also avoided the seed experiencing substrate shock loading. Duplicates of five containers with an incremental series of NH₄HCO₃ concentrations from 0 to 40 mg L⁻¹ were prepared by adding concentrated stock of ammonium salt. The supplementary regime from 0 to 40 mg L⁻¹ was chosen as the trial study on the optimal dosage. All containers were gently shaken every 10 min for mixing purpose and also to facilitate the release of biogas.

The optimal concentration for ammonium salt was determined according to the cumulative volumetric biogas production. It could be assumed that rapid biogas production would take place within the period of 3 h of batch operation for the same substrate [7]. Nevertheless, comparison of the maximum biogas production could indicate the toxicity of NH₄HCO₃ especially to the methanogen in the system [6]. Composition of CH₄:CO₂ in the previous study is at the ratio of 20:80. However the biogas production volume in this study was expected to be too low for composition analysis by gas analyser (GA94A, Geotechnical Instrument (UK), Ltd.). This study used the same methods of CO₂ removal by passing the biogas through a high concentration alkaline solution while measuring it by liquid displacement [1].

3. Results and discussion

The previous operational failure which was instigated by the accumulation of VFA could have occurred with other limitations such as micronutrients (Fe, Ni, Cu, Co, Mg) and phosphorus [8]. Nevertheless, hypothetically the lack of micronutrients could be dismissed by the information on mineral content given on the label of this particular softdrink cordial. Meanwhile the lack of phosphorus was also not being addressed because the inoculum was taken from freshly digested sewage sludge, in which the presence of phosphorus as the micronutrient should be adequate. Meanwhile, the presence of ammoniacal nitrogen as the macronutrient in a stabilised digested sludge is also considered to be at a residual concentration after the process of denitrification is complete [8].

The other salt that could be used to supplement the lack of nitrogenous substrate is sodium nitrate. However, as for sodium nitrate, the release of NO₃⁻ would increase the oxidation–reduction potential (ORP) of the digester. The potential ORP of the digester should be allowed to increase above −300 mV. At lower ORP methane-forming bacteria could not produce methane, for example at ORP of −300 mV [5]. While for the buffering salts, caution should be exercised in the choice of chemical used for alkalinity adjustment. Precipitation of CaCO₃ creates unwanted solids, and the large quantity of single cation, for example Ca, presents the potential for alkali metal toxicity.

3.1. Biogas production

Fig. 1 shows the cumulative production of biogas during the toxicity test of ammonium salt on the anaerobic digestion system. It shows the typical first order reaction rate on the conversion of carbonaceous organic to biogas. It decelerates with the cessation of raw materials. Total cumulative biogas production was observed to increase when the ammonium salt was added. However, the cumulative production of biogas was observed to decline when more than 20 mg L⁻¹ of ammonium salt were added into the system. The effect of C:N ratio was not investigated during this testing. The
ratio will not be consistent at all times for the batch operation due to substrate consumption and the denitrification process [8].

3.2. Use of specific mathematical function

The usual statistical analyses, i.e. ANOVA and t-test, are not suitable for a limited number of samples. The data description as shown in Fig. 1 was not able to clearly indicate the effect of ammonium salt on the production of biogas. Therefore, quantitative analysis of the volumetric production of biogas was carried out with the use of specific mathematical function as shown in Eq. (1). This specific mathematic function was introduced and later adapted for kinetic interpretation of methane gas production by anaerobic digestion [7,9]. Similarly, the equation could be used to determine the maximum volume of biogas production \( G_m \) without running the experiment to exhaustion. The linearised equation is rearranged as Eq. (2). Table 1 shows the value of biogas production \( G \) at time \( t \), which was used to plot the data points for the determination of coefficients as listed in Eq. (2). The plot of linear data points for \( \ln G \) against \( 1/t \) is shown in Fig. 2. The \( G_m \) value could be used to demonstrate the effectiveness of supplementing ammonium salts to the system.

\[
G = \frac{k}{t} \quad (1)
\]

\[
\ln G = \ln G_m + \frac{k}{t} \quad (2)
\]

where \( G \) = cumulative biogas production, \( G_m \) = maximum biogas production, \( k \) = slope coefficient in linear equation, and \( t \) = time.

All data points, which are plotted in Fig. 2, show that the \( G_m \) could be determined by the specific mathematical function because they have been plotted to be linear. Table 2 lists the estimated maximum volumetric biogas production during the toxicity test. It is determined by re-inversing the cut-off values at the y-axis in Fig. 2 as written in Eq. (2). The estimation gives the highest biogas production when 10 mg L\(^{-1}\) of ammonium salt was used in the system.

Previous study shows that the range of methane (CH\(_4\)) yield per unit of COD removed for the fruit cordial wastewater was within 0.26–0.30 [1]. In this study, the amount of CH\(_4\) yield was assumed to remain on the basis that the same substrate was being digested. The maximum CH\(_4\) yield which could be expected from this study could be equal to 60 mL, while the calculated maximum biogas production based on the specific mathematical function (Eq. (2)) ranged between 52 and 59 mL, as listed in Table 2. The actual amount of CH\(_4\) in this study was not determined due to an inadequate volume of gas for analysis. Deductively, anaerobic digestion including methanogenesis took place in this experiment without difficulty.

Table 1
Cumulative production of biogas during the toxicity test for serial concentration of NH\(_4\)HCO\(_3\) in anaerobic digestion system.

<table>
<thead>
<tr>
<th>Time (min)</th>
<th>Ammonium concentration (mg L(^{-1}))</th>
<th>Average cumulative biogas production (mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>15</td>
<td>0</td>
<td>16</td>
</tr>
<tr>
<td>30</td>
<td>1</td>
<td>20</td>
</tr>
<tr>
<td>45</td>
<td>2</td>
<td>23</td>
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<td>150</td>
<td>9</td>
<td>48</td>
</tr>
<tr>
<td>165</td>
<td>10</td>
<td>50</td>
</tr>
<tr>
<td>180</td>
<td>11</td>
<td>53</td>
</tr>
</tbody>
</table>

Table 2
Estimated maximum volumetric biogas production during the toxicity testing with serial concentration of ammonium bicarbonate.

<table>
<thead>
<tr>
<th>Ammonium concentration (mg L(^{-1}))</th>
<th>( \ln G_m )</th>
<th>( G_m ) (mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>4.04</td>
<td>57</td>
</tr>
<tr>
<td>10</td>
<td>4.08</td>
<td>59</td>
</tr>
<tr>
<td>20</td>
<td>4.01</td>
<td>55</td>
</tr>
<tr>
<td>30</td>
<td>3.95</td>
<td>52</td>
</tr>
<tr>
<td>40</td>
<td>3.98</td>
<td>53</td>
</tr>
</tbody>
</table>

Fig. 2. Determination of coefficients for \( G_m \) and \( k \) by linearising Eq. (2) for each of ammonium salt concentrations.
Sustaining methanogenesis in the anaerobic digestion process is the vital operational procedure for the removal of carbonaceous materials in wastewater. Proliferation of a specific substrate in the wastewater is equally important. It has been suggested that such wastewater with high organic content should not be mixed with other wastewater that is low in organic content to be treated with the same process even if it is generated in the same vicinity \[10\]. Therefore, individual wastewater treatment processes for specific strength and content of wastewater should be in place. Separating the wastewater treatment processes in terms of their characteristics would be more cost effective, if the treated wastewater is to be reused \[11\]. In the case of wash waters from the production operation of fruit cordial processing, i.e. pre washing of the fruit, cleaning of process vessels, pipelines and associated equipments \[4\], application of anaerobic digestion as an individual system for the treatment of its wastewater should be established, prior to the grouping of wastewaters according to their strength and composition. The establishment would then support the use of tertiary treatment for the prospect of treated wastewater being recycled and reused.

4. Conclusion

Using biogas production as the indicator, this study has shown that ammonium bicarbonate could be used as the supplement for anaerobic digestion of fruit cordial wastewater. Application of the specific mathematical function in this study suggests that 10 mg L\(^{-1}\) of ammonium salt is the optimal concentration for the substrate to be used when compared to the series of increments up to 40 mg L\(^{-1}\). This recommendation can only be applied to the current study using CSTR design. Although there are other designs of reactor that have better operational performance, CSTR should be known for the simplicity of its operational mechanism.

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Appendix A. Supplemental material

Supplementary data associated with this article can be found, in the online version, at doi: 10.1016/j.anaerobe.2009.04.008

References