Velocity measurement in the steady state is useful to predict the operating conditions for an optimization of thermophilic anaerobic digestion

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**ABSTRACT**

This work concerns an experimental study of the biochemical conversion of waste activated sludge (WAS) by thermophilic anaerobic digestion for its optimization, by considering the impact of the content of dry matters (DM) contained in the activated sludge and the ratio of recycled biomass (BR), on the production of biogas by anaerobic digestion. The second aspect of this research relates to the kinetic modeling of this biochemical conversion of the organic matter to biogas with an adapted mathematical model while resting on the initial velocity measurement in the steady state. The results obtained show that biogas production potential and biogas production rate increased with an increasing DM concentration. The Modified Gompertz equation was employed to model the biogas production at different substrate concentrations. The equation gave a good approximation of initial velocity which equal to the maximum biogas production \((R_m)\) and the biogas yield potential \((P)\) with correlation coefficient \((R^2)\) over 0.983. The Lineweaver and Burk plot is used to estimate the parameters of optimisation from the initial methane production rate and initial DM concentration, when maximum rate \(R_{\text{max}}\) equal to 7,042 L d\(^{-1}\) and the affinity constant value \(K_m\) equal to 95,563 g kg\(^{-1}\) of DM at BR = 5%, the BR = 33% is the best in this study. The increased ratio of BR increased the rate of methane yields, and reduced the retention time.

**INTRODUCTION**

Biological methods such as anaerobic digestion is widely used for sludge stabilization, because it addresses most of the problems arising from the organic effluents: which not only reduces the quantity of sludge to be disposed of, but also produces valuable methane gas, enhanced dewatering properties of the digested sludge, high quality biosolids for land application, and as a carbon source for denitrification (Bolzonella et al., 2005; Chynoweth, 2004). The anaerobic digestion progression generally consists of four stages, hydrolysis, acidogenesis, acetogenesis and methanogenesis. In anaerobic digestion, the biological hydrolysis is recognized as the rate-limiting step (Guo et al., 2014). To moderate this restraining step, pre-treatment of waste activated sludge (WAS) is necessary such as thermal, alkaline, ultrasonic and mechanical disintegration (Sanders, 2001; Vlyssides and Karlis, 2004; Carrere et al., 2010; Donoso-Bravo et al., 2011). These treatment can speed up the solubility of WAS and reduce the particle size, which then improve the biochemical conversion (Lehne et al., 2001; Jeongsik et al., 2003). A new alternative to increase biogas production from a WAS digester is inoculation with...
residues which have better digestibility, enhanced biogas production/methane yield arising from availability of added nutrients (Gómez et al., 2006; Mshandete and Parawira, 2009).

The notion of initial rate at steady state has been frequently useful in enzymatic processes; it refers to the condition in which there is a stable and fixed flow of substrate through to product. ‘Initial steady state’ is the word occasionally used to describe the initial rate. More practical, though, is the use of the term steady state in a metabolic pathway, in which the net flow through an enzyme is determined by both the concentration of its substrate, who is always reconstructed by the previous enzyme and of its product, which is progressively taken away by the following enzyme, all enzymes having even flux, or steady state net ahead rate (Scopes, 2002). It is like the biochemical conversion of biodegradable organic pollutants into biogas, which they pass through a chain of enzymatic transformation through the metabolism of different bacterial class (Guo et al., 2014).

The application of this notion in anaerobic digestion is seldom carried in studies, Donoso-Bravo et al. (2011) evaluates the impact of the temperature on the main reactions of the anaerobic digestion, and they evaluates its applicability for predicting the performance of an anaerobic continuous-digester, and Sanders (2001) estimates kinetic parameters of the gelatin anaerobic degradation, both using the initial rate procedure.

The objective of this study was to enlarge a typical method which is useful for tracing the operating conditions for the best practices of anaerobic treatment of waste and to gauge the kinetic parameters of the anaerobic digestion of WAS, while resting on the initial rate measurement, and also to generalize its applicability in the case of anaerobic digestion.

**MATERIALS AND METHODS**

**Substrates and inoculums**

The sampling of WAS was done at the municipal wastewater treatment plants in Boumerdes, Algeria, which perform with a sludge age of 12 days in the extended aeration. WAS characteristics are shown in Table 1. The samples have been stored at 4°C in a refrigerator until usage.

**Digester and operation**

Microorganisms for anaerobic digestion consisted in start of those present in aerobic activated sludge (WAS) inoculated with rumen microorganisms of cattle dung (components are revealed in Table 1). The reactor for anaerobic digestion had a volume of 15 L and its working volume of 13 L (Figure 1). It was also equipped with gas and sludge sampling ports. When substrate was added, reactor was purged with helium gas to eliminate air from the reactor. The mixed sludge was stirred in the digester without oxygen contact. The reactor was incubated at 55°C and the biogas volume generated was measured by Gasholder bell (Figure 1).

The purpose of this study was to determine the effect of the DM content and the ratio of recycled biomass (BR) on the performance of methane production from WAS at thermophilic conditions, while resting on the initial velocity measurement. Thus the initial concentration of DM: 8.25, 15.5, 36.65, 41.5, 49.6, and 58 g L⁻¹ at thermophilic temperature were digested in bench scale batch with BR = 5%. The cumulative biogas maintained at room and ambient temperature along. The effect of BR ratio was tested for weight ratio: 12.5, 25, 33 and 50% in 600ml bottle serum, when the DM concentration is 36.4 g L⁻¹ for all test then the best result is confirmed in bench scale batch digester. All of the experiments were performed in duplicate. The averages of the 73 duplicates are shown in each result.

**Analytical method**

DM, volatile suspending solid (VS) were determined following the Standard Methods APHA, 1998 (Andrew, 2005). The pH of the anaerobic slurry (sludge) was measured according to NF ISO 10390 (Rodier, 2009).

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**Table 1. Composition of waste activated sludge and cattle dung.**

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Activated sludge waste</th>
<th>Cattle dung</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dry matters (g/kg)</td>
<td>35.8</td>
<td>50.7</td>
</tr>
<tr>
<td>Volatile suspending solid (g/kg)</td>
<td>27.2</td>
<td>24.6</td>
</tr>
<tr>
<td>Total chemical oxygen demand (mg/l)</td>
<td>806</td>
<td>18580</td>
</tr>
<tr>
<td>N-NH₄⁺ (mg/l)</td>
<td>32.4</td>
<td>750</td>
</tr>
<tr>
<td>P-PO₄³⁻ (mg/l)</td>
<td>18.1</td>
<td>6.9</td>
</tr>
<tr>
<td>pH</td>
<td>7.6</td>
<td>6.5</td>
</tr>
</tbody>
</table>

The different chemical elements: Chemical Oxygen Demand (COD), Total nitrogen, and total phosphorus were determined using HI 83214 COD Meter and Multiparameter Photometer. Biogas samples were collected using a gas sampling injector and a sample of 100–200 μL was used for each run. The biogas composition (CH₄+CO₂) was determined using a gas chromatograph (GC-HP 5890) equipped with a thermal conductivity detector (TCD) and stainless steel column that was 2 m long with a 5 mm OD and 2 mm ID and contained Porapak Q 100 that had a mesh range from 80–100. The carrier gas was N₂, and the analysis was carried out at a carrier gas flow rate of 30 mL.min⁻¹ with the injector, column, and detector temperatures at 120, 90 and 120°C, respectively.

Mathematic modelling

The experimental data were fitted with the modified Gompertz equation (Altas, 2009). This equation describes cumulative biogas production from batch digesters, supposing that biogas production is a function of bacterial growth. The modified Gompertz equation is given by (Equation 1), so this equation plot the cumulative methane production according to the time.

\[ M = P \cdot \exp \left( - \exp \left[ \frac{R_m}{P} (\lambda - t) + 1 \right] \right) \]  

Where; \( M \) is the cumulative methane production (L), \( P \) the methane production potential (L), \( R_m \) the maximum methane production rate (L d⁻¹), \( \lambda \) the duration of lag phase (d) and \( t \) is the duration of the assay at which cumulative methane production \( M \) is calculated (d). The parameters \( P, R_m \) and \( \lambda \) were estimated for each of the digesters using OriginPro8 software.

During the stationary phase of reaction, the rate is constant: it is called initial rate, under these conditions, the catalytic efficiency of the enzyme is greater, and therefore the initial rate is the largest of all the rates which can be measured according to the phases of the reaction. The initial rate is the slope of initial linear part a graph of the concentration of reactants or products as a function of time (Raisonnier, 2002), so the initial rate in this study equal to \( R_m \).

Linear plots of the Michaelis–Menten equation was used to correlate \( R_m \) to DM concentration. The Lineweaver–Burk plot or double reciprocal plot is a common way of illustrating kinetic data. This is produced by taking the reciprocal of both sides of the Michaelis–Menten equation (Berg et al., 2013). This is a linear form of the Michaelis–Menten equation (Equation 2): and
produces a straight line with the equation \( y = ax + b \) with a \( y \)-intercept equivalent to \( 1/R_{\text{max}} \) and an \( x \)-intercept of the graph representing \(-1/K_m\).

\[
\frac{1}{R_m} = \frac{K_m}{R_{\text{max}}[S]} + \frac{1}{R_{\text{max}}}
\]

Where \( R_m \) equal to the initial rate (L d\(^{-1}\)), \( R_{\text{max}} \) maximum rate (L d\(^{-1}\)), \([S]\) the substrate concentration witch equal to the \([\text{DM}]\) in this study (g/kg), \( K_m \) affinity constant value (g/kg).

The modified Gompertz equation is used again to check the results of relative VS reduction and to extract kinetic parameters from the (Equation 3):

\[
R_{\text{VS}} = P'. \exp \left[ \frac{R_{\text{m}'} e^{(\lambda - t)}}{P' (\lambda - t) + 1} \right]
\]

Where \( R_{\text{VS}} \) is relative reduction VS (%), \( P' \) the VS reduction potential (%), \( R_{\text{m}'} \) the maximum VS reduction rate (% d\(^{-1}\)), \( \lambda' \) the duration of lag phase (d) and \( t \) is the duration of the assay (d). The parameters \( P', R_{\text{m}'} \) and \( \lambda \) were estimated for each of the digesters using OriginPro8 software.

RESULTS AND DISCUSSION

The data attained from the experimentation for the cumulative methane production from different DM concentration followed during 31 days as showed in Figure 2. The digestion was characterized without fluctuation of biogas production at the beginning. Degradation of substrate started almost immediately and proceeded without trouble in all digestions and methane production is significantly increased due to exponential growth of microorganisms and to their higher adaptation to the change of the concentration of substrate. After 17 - 23 days observation, biogas production for all samples at all DM content followed during 31 days as showed in Figure 2. The digestion was characterized without fluctuation of biogas production at the beginning. Degradation of substrate started almost immediately and proceeded without trouble in all digestions and methane production is significantly increased due to exponential growth of microorganisms and to their higher adaptation to the change of the concentration of substrate. After 17 - 23 days observation, biogas production for all samples tend to decrease and this is predicted tends due to stationary phase of microbial growth (Castillo et al., 1995).

The profile shape of pH variation curve during the digestion period at different DM concentration under thermophilic condition is shown in Figure 3. The results indicates that the pH values seemed to vary with operation time in a similar way in the all samples; as seen, the pH started from the same initial pH 7.49, and in the all samples it was dropped to 7.47 – 7.21 at first partly due to the heterogeneity of straw particles, subsequent hydrolysis process occurred in the reactors and the volatile fatty acids (VFA) accumulation, especially during the first three days, and then gradually increased; finally, it reached a level about 7.91 – 8.38. Indeed, the stage of hydrolysis acts on the concentration of nitrogen in the medium since it consists in converting proteins into amino acids like of polypeptides and ammoniums (Sanders, 2001).

Indeed, several studies show that a rise of pH can be responsible for an increase in the form ammonia (NH\(_3\)) in medium (Borja et al., 1996; Debattista, 2011).
same way for the temperature, its increase can also lead to the rise in the content $\text{NH}_3$ in medium (Debattista, 2011) some authors noted this effect and one explained by the rise of temperature which decreases the pKa of ammonia and thus increases the free fraction of ammonia ($\text{NH}_3$) (Rocher et al., 1999). Overall the pH varied between 7.91 and 8.38 which nearly lied in the favorable pH range of 6.6 – 7.8 for methanogenic bacteria (Jash and Ghosh, 1996).

The methane content of the biogas generated from the fermentation of studied DM concentration at $BR = 5\%$ is shown in Figure 4. We observe that there is no significant difference between them. The average methane content of the biogas generated from the fermentation of the WAS with initial concentration of DM: 8.25, 15.5, 36.65, 41.5, 49.6, and 58 g/kg at the steady state condition was
Figure 5. Relative VS reduction at different DM content under thermophilic conditions (55°C), BR = 5%.

The results of VS reduction are revealed in the Figure 5. From second day the reduction in VS is remarkable and it stabilizes towards the end of fermentation. The curves have almost the same pace, this factor depend on DM content. Kabouris et al. (2009) reports a kinetic data for COD destruction on mesophilic anaerobic digestion of primary sludge and fat, oil and grease which are comparable with ours in the profile shape but its loading rate of COD is higher comparatively to our end result. This difference in values is the result of the nature of the substrates fermented, and then primary sludge is known for its large putrescible power compared to WAS which is secondary sludge (Moletta, 2001).

The methane yield, methane produced per g VS loaded for different concentrations of DM over a 23 days digestion time at thermophilic temperature (55°C) is 166 shown in Table 2. The methane production is relatively depending to concentration of DM. Furthermore, as shown in Figures 6 and 7, so we observe a positive exponential correlation between the methane yields and DM concentration. The best performance for biogas production was the digestion with 58.5 and 49.6 g L⁻¹ of DM, give methane yield 0.335 and 0.264 L g⁻¹ VS loaded, respectively after 23 days observation. Our result appear to be within the same range as results from other studies conducted by different authors (Table 3) but in some cases our data of methane yield is lower than the information of studies listed in the table but that have reported data for DM concentration higher than ours interval. Moreover this result confirms that: the potential methane production varied noticeably according to the DM concentration. Other than it is depending to the biochemical nature according to the two last references quoted in the same table.

Kinetic parameters of anaerobic digestion process are always used to analyze the performance of digesters and design appropriate digesters, which are also helpful in understanding inhibitory mechanisms of biodegradation (Kabouris et al., 2009). With an assumption that methane produced is a function of bacterial growth in batch digesters, to quantify analytically parameters of batch growth curve, the modified Gompertz equation was selected to fit the cumulative biogas production data. Values of parameters obtained are potted in Table 4. It has been observed that the cumulative methane production was fit well with the modified Gompertz equation as is evident from the correlation coefficient R² (0.983 - 0.998) between the experimental and predicted values along with the parameter estimated. Lag phase (λ) was found between 3,263 and 5,123 day for the different DM concentrations, this lag phase might be due to low methanogenic activity and / or the number of methanogens, in the digesters (Kanwar and Kalia, 1992). The initial methane production rate (Rm) for DM concentration 8.25 is the lowest of 0.531 L d⁻¹ and the highest is shown by DM concentration 58.5 g L⁻¹ with a value of 2.750 L d⁻¹.

Therefore the amount of gas produced at the end of digestion period was highest for DM concentration 58.8 g L⁻¹ (29,152 L). This could be because WAS is rich in
Figure 6. Cumulative methane yields at different DM content under thermophilic condition (55°C), BR = 5%.

Figure 7. Positive correlation between the methane yields and DM concentration.

Table 2. Methane yields at different DM content under thermophilic condition (55 °C) BR= 5%.

<table>
<thead>
<tr>
<th>DM (g/kg)</th>
<th>Methane yield (l/ gVS)</th>
</tr>
</thead>
<tbody>
<tr>
<td>58.5</td>
<td>0.335</td>
</tr>
<tr>
<td>49.6</td>
<td>0.264</td>
</tr>
<tr>
<td>41.5</td>
<td>0.2</td>
</tr>
<tr>
<td>36.65</td>
<td>0.136</td>
</tr>
<tr>
<td>15.5</td>
<td>0.073</td>
</tr>
<tr>
<td>8.25</td>
<td>0.041</td>
</tr>
</tbody>
</table>
nutrients and contains adequate amount of carbon, oxygen, hydrogen, nitrogen, phosphorous, potassium, calcium, magnesium and a number of trace elements which are very essential for the growth of anaerobic bacterium (Chen et al., 2010). This could have optimized syntrophic interaction between acetogens and methanogens which is the most critical step in the biomethanation process (Kanwar and Kalia, 1992). However digesters for DM concentration 8.25, 15.5, 36.65, 41.5 and 49.6 g L⁻¹ produced 6.786, 9.75, 18.135, 20.114 and 22.405 L of methane respectively.

In the light of kinetic results, we have look for if there is a correlation between this parameters verves the initial DM concentration. The Figure 8 confirm the positive relation of $P$, and $R_m$ verves DM content with a good coefficient of correlation; 0.98 and 0.89 205 respectively.

Further the initial rate according to the equation of Lineweaver and Burk, allows to estimate the parameter of optimisation from the initial methane production rate and initial DM concentration (Figure 9), when maximum rate $R_{max}$ equal to 7,042 Ld⁻¹ 208 and the affinity constant value $K_m$ equal to 95,563 g/kg of DM. So we can draw a conclusion that Initial rates measurement at the steady state is key element for best practice of anaerobic digestion. So we can reach the $R_{max}$ from a [DM] = 10 Km according to the concept enzymatique (Burstein, 2000), if there is not an accumulation of inhibitors. Even if there is presence of inhibitor they have to know them, to determine the conditions of inhibitions using the same principle. Finely we can write the modified Gompertz equation as follows, when the equation plot the cumulative methane production according to the time.

### Table 3. Methane yields at different DM concentration of waste according to other research.

<table>
<thead>
<tr>
<th>Substrate type</th>
<th>Application scale</th>
<th>DM (g/kg)</th>
<th>Temperature (C)</th>
<th>HRT (day)</th>
<th>Methane yields (L gVS⁻¹)</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Waste activated sludge</td>
<td>Bench scale batch</td>
<td>150</td>
<td>55</td>
<td>10</td>
<td>0.565 Biogas (gVS)</td>
<td>(Ros and Zupancic, 2003)</td>
</tr>
<tr>
<td>Maize silage</td>
<td>Bench scale batch</td>
<td>73</td>
<td>38</td>
<td>-</td>
<td>0.299</td>
<td>(Dubrovskis et al., 2009)</td>
</tr>
<tr>
<td>Soup processing</td>
<td>Laboratory scale</td>
<td>214.8</td>
<td>50</td>
<td>28</td>
<td>0.35</td>
<td>(Chen et al., 2010)</td>
</tr>
<tr>
<td>Cafeteria</td>
<td>Laboratory scale</td>
<td>234.5</td>
<td>50</td>
<td>28</td>
<td>0.38</td>
<td>(Chen et al., 2010)</td>
</tr>
<tr>
<td>Commercial kitchen</td>
<td>Laboratory scale</td>
<td>96.9</td>
<td>50</td>
<td>28</td>
<td>0.47</td>
<td>(Chen et al., 2010)</td>
</tr>
<tr>
<td>Fish farm</td>
<td>Laboratory scale</td>
<td>558.1</td>
<td>50</td>
<td>28</td>
<td>0.86</td>
<td>(Chen et al., 2010)</td>
</tr>
<tr>
<td>Grease trap</td>
<td>Laboratory scale</td>
<td>294.0</td>
<td>50</td>
<td>28</td>
<td>0.89</td>
<td>(Chen et al., 2010)</td>
</tr>
<tr>
<td>Whey</td>
<td>Laboratory scale</td>
<td>68.6</td>
<td>40</td>
<td>40</td>
<td>0.501</td>
<td>(Dinuccio et al., 2010)</td>
</tr>
<tr>
<td>Pig slurry</td>
<td>Laboratory scale</td>
<td>69.9</td>
<td>36</td>
<td>42</td>
<td>0.317</td>
<td>(Luna-delRisco et al., 2011)</td>
</tr>
<tr>
<td>Waste activated sludge</td>
<td>Bench scale batch</td>
<td>58.5</td>
<td>55</td>
<td>23</td>
<td>0.335</td>
<td>Our study</td>
</tr>
</tbody>
</table>

### Table 4. Values of fitting functions and statistical measures for the kinetic model for Cumulative methane productions and, under thermophilic conditions (55°C), BR=5%.

<table>
<thead>
<tr>
<th>DM (g/kg)</th>
<th>$R^2$</th>
<th>$P$ (L)</th>
<th>$R_m$ (l/day)</th>
<th>$\lambda$ (day)</th>
</tr>
</thead>
<tbody>
<tr>
<td>58.5</td>
<td>0.998</td>
<td>29.308</td>
<td>2.75</td>
<td>4.119</td>
</tr>
<tr>
<td>49.6</td>
<td>0.996</td>
<td>22.635</td>
<td>1.974</td>
<td>3.961</td>
</tr>
<tr>
<td>41.5</td>
<td>0.983</td>
<td>20.679</td>
<td>1.839</td>
<td>5.123</td>
</tr>
<tr>
<td>36.65</td>
<td>0.994</td>
<td>18.309</td>
<td>2.0631</td>
<td>3.926</td>
</tr>
<tr>
<td>15.5</td>
<td>0.995</td>
<td>9.832</td>
<td>1.249</td>
<td>5.008</td>
</tr>
<tr>
<td>8.25</td>
<td>0.985</td>
<td>6.846</td>
<td>0.531</td>
<td>3.263</td>
</tr>
</tbody>
</table>
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and substrate concentration.

\[ M = P \cdot \exp - \frac{R_{\text{max}} \cdot [S]}{K_m + [S]} \cdot \exp \left[ \left( \lambda - t \right) + 1 \right] \]  \hspace{1cm} (4).

It comes to the first mention of writing the second modified Gompertz equation in function of two variables: the substrate concentration and time. Moreover the curves from the relative VS reduction data are correlated.

Figure 8. Positive correlation \( P \) or \( R_m \) and DM concentration.

Figure 9. Lineweaver and Burk plot of \( I/R_m \) verse \( I/\text{DM} \).
Figure 10. The effect of BR on methane production.

Figure 11. Cumulative methane production at different total solid content under thermophilic condition (55°C), BR = 33%.
by the DoseResp equation, one of origin basic functions with correlation coefficient $R^2$ (0.971 - 0.993), then the curves obtained are refitted by the modified Gompertz equation to improve the fit results and to extract kinetic parameters from the Equation 2, the results are summarized in the Table 5.

The experimentation at different BR ratio shows that the proportionality between BR ratio and the quantity of methane formed but beyond 33% we will have an inhibition if we compares with BR = 50% (fig.6.a), So the BR = 33% is the best at this study so we have confirmed this result at digester scale at the DM concentration 55.6 and 69.1 g L$^{-1}$. Therefore the increased ratio of BR could give the optimized syntrophic interaction between acetogens and methanogens which is the most critical step in the biomethanation process (Schink and Stams, 2005) which translates to an increase in the rate of methane yields, and reduce the retention time from digesters for DM concentration 55.6 and 69.1 g L$^{-1}$ with BR = 33% produce 33.274 and 42.237 l of methane respectively these results are revealed in the Figures 10 and 11. The best retention time is 8 d for the DM concentration (69.1 g/l).

The kinetic modelling of the last experimentations in Table 6, show the advantage of this initials conditions: For DM concentration of 69.1 g.L$^{-1}$ The lag phase is optimized, the short time to begin was 0.129 d, the best maximum methane production rate equal to 13.386 L d$^{-1}$ and the amount of methane production at the end is better.

**Conclusion**

The Modified Gompertz equation serves a starting point for the application of enzymology concept, in first time it
limit the steps transformation: (lag Phase and stationary phase), in second time for estimate the initial rate value. Finally we have confirm that we can use the Lineweaver and Burk linear equation of Michaelis and Menten model to predict the maximal operational conditions using Initials rates of methane production values, the advantage of the exploit of the concept of initial rate is the duration of the assay is limited to the steady state.

Via two mathematical models applied to different experimental variable, we showed the applicability of both methods in a realistic setting. Applying these methods in other conditions in the presence of other co-substrate or in the presence of inhibitors will be an interesting topic for further research.

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**REFERENCES**


