CHAPTER 7

A COMPUTATIONAL MODEL FOR SIMULATING THE GROWTH OF BIOGAS-PRODUCING BACTERIA FOR PREDICTING PRODUCTION RATE OF SUSTAINABLE AND AFFORDABLE CLEAN ENERGY

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Introduction

The anaerobic decomposition of cattle slurry manure or an aqueous solution composed of cow dung extract is a complex sequence of processes involving the activities of multiple bacteria types existing in symbiosis (Yilmaz and Demirer, 2011). The various stages of decomposition of cattle slurry manure are facilitated by different types of bacteria such as the hydrolyzing bacteria, digestive bacteria, acetogenic bacteria, homoacetogenic bacteria, sulfate-reducing bacteria and methanogenic bacteria (Ziemiński and Frąc, 2012). As shown in Figure 1, the process of decomposing organic wastes like cattle slurry manure can be segmented into three main stages which include hydrolysis, acidogenesis and methanogenesis.

Hydrolysis involves the conversion of the cattle waste to monomers, oligomers, amino acids and sugars. The acidogenesis stage involves the conversion of the end products of the hydrolysis stage to fatty acids, which include propionic, butylic, and valeric acid, and alcohols. Before the methanogenesis stage, the acetogenesis stage occurs whereby the fatty acids and alcohols are converted to acetic acid and hydrogen gas. The last stage of the methane fermentation process of the cow dung anaerobic decomposition is the methanogenesis stage which involves the activities of methanogenic bacteria in the conversion of acetic acid, hydrogen gas and CO_2 to methane (Park, 2004).In this paper we would focus on modelling the growth rate of the methanogenic bacteria whose anaerobic activities directly leads to methane production.

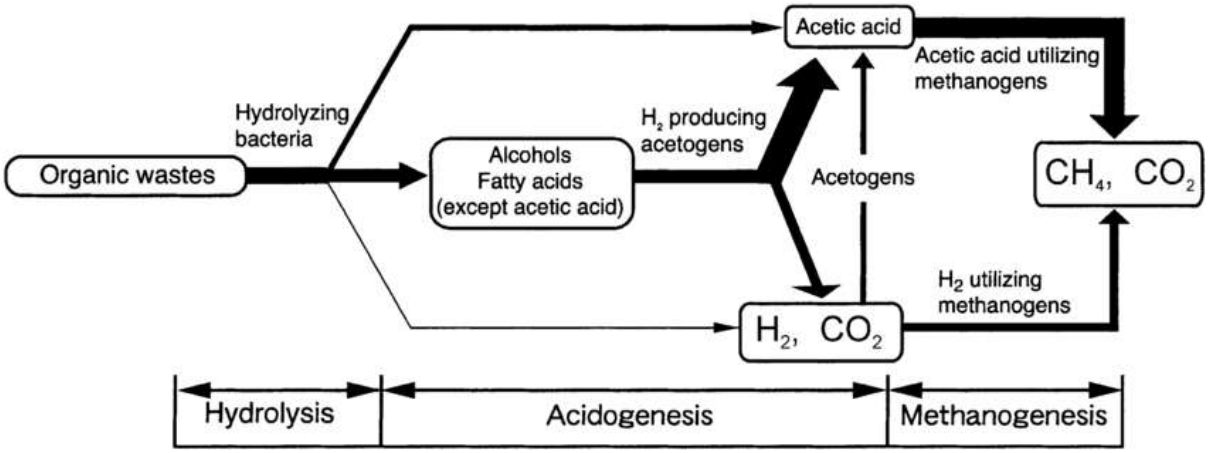


Fig. 1: Schematic Diagram of Methane Fermentation Process (Park, 2004)

The growth of methanogenic bacteria cultures refers to the increase in the population of the bacteria rather than the increase in size of each cell (Rogers &Kadner, 2019). As shown in the generalized bacteria growth curve in Figure 2, there are four phases of growth in bacteria colonies. In the lag phase, the bacteria colonies are active but do not reproduce. In the log phase, the population of bacteria (like the methanogenic bacteria) increases at a geometric or exponential rate at the rate of generation time, where the generation time is the time interval between the creation of new bacteria generations (Rogers &Kadner, 2019).

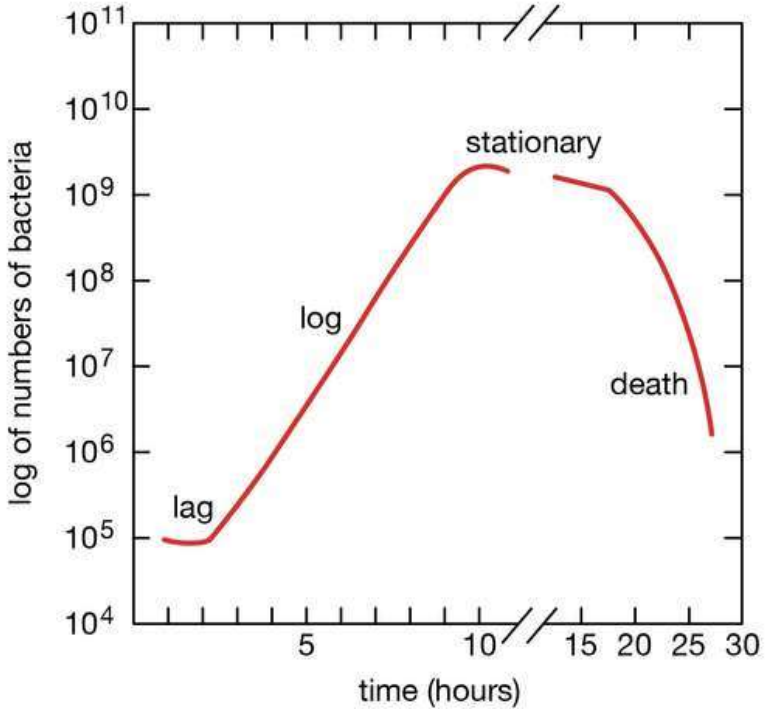


Fig. 2: Generalized bacterial growth curve showing the phases in the growth of bacterial colonies (Rogers and Kadner, 2019)

The log phase is usually followed by the stationary phase where the size of the bacteria population remains approximately constant. The stationary phase usually occurs at optimum bacteria population densityand in this phase the rate at which new cells are produced is matched by the number of bacteria cells that die. After the stationary phase is the death phase in which there are more bacteria cells dying than there are new cells being produced.This could be due to gradual depletion of substrate or excess production of toxins which could be by-products of the digestion process (Fankhauser,2004).

Most bacteria cells, including methanogenic bacteria, replicate by binary fission as shown in Figure 3. In binary fission, a bacteria cell develops till it reaches maturity before DNA replication occurs. This is then followed by the elongation of the cell after which a division septum is formed in the center of the bacteria cell. The parent bacteria cell then undergoes cell separation where two daughter cells emerge with similar form and size, each having a copy of the original parent chromosome (Margolin, 2014).

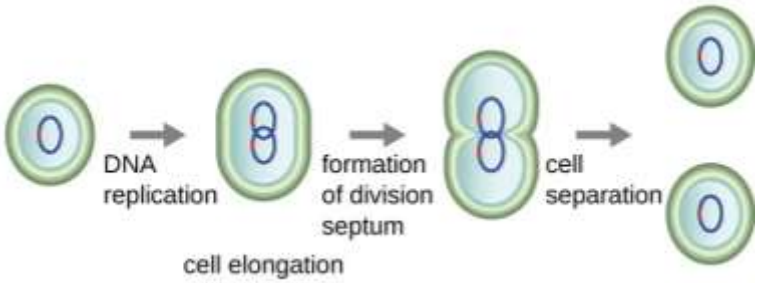


Fig. 3: Binary fission of a bacteria cell to produce two daughter cells (lumenlearning, n.d.)

It is possible to predict bacteria population size if they replicate by binary fission at a constant rate, as depicted in Figure 4. The number of cells at any generation N_n during the log growth phase can expressed as $N_n=N_0 2^n$ if the binary fission rate is constant, where N_0 is the initial number of bacteria cells and n is the number of generations (Margolin, 2014).

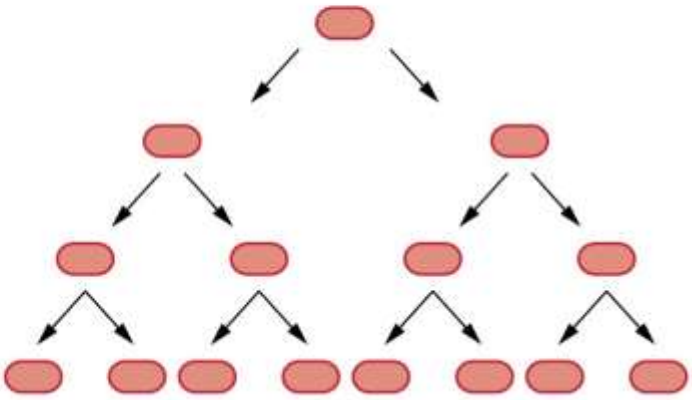
Number of generations (*n*)	Number of cells	Each division adds two new cells
0	1	
1	2	
2	4	
3	8	

Fig. 4: Depiction of bacteria cell replication during log phase at constant generation time

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Methodology

The growth of microorganisms including, methonagenic bacteria, can be modelled using the Monod equation. The Monod equation relates the microbial growth rates in an aqueous environment to the concentration of limiting nutrients. Cow dung is usually dissolved in lukewarm water to form cattle slurry manure (Rajeswari, 2016), from which biogas is generated. This means that the Monod equation can be used to model the growth rates of the various bacteria that feed on the cattle slurry manure to produce Biogas (Liu, 2006). The Monod equation possesses the same form as the Michaelis-Menten equation (Pulkkinen& Metzler 2015), as shown in equation 1, which is a model that relates the reaction rate, v, of enzymatic reactions and to the concentration, S, of a substrate like cattle slurry manure (Lin, 1995).

$$v = \frac{d[P]}{dt} = \frac{V_{max}[S]}{K_M + [S]} \qquad (1)$$

Unlike the Michaelis-Menten model, which is based on theoretical considerations, the Monod equation, as shown in equation 2, is basedon experimental observations. The Monod model, as

descried by the sample characteristic plot in Figure 5, expresses microorganism specific growth rateμ in terms of the maximum specific growth rate μ_{max}, the concentration of the substrate S, and the half-velocity constant K_s when $\mu/\mu_{max} = 0.5$. For the Monod model, the maximum growth rate and the half-velocity constant are empirical coefficients. Thus, they differ between microorganism species and ambient environmental conditions (Lin, 1995).

$$\mu = \mu_{max} \quad \frac{S}{K_S + S} \qquad (2)$$

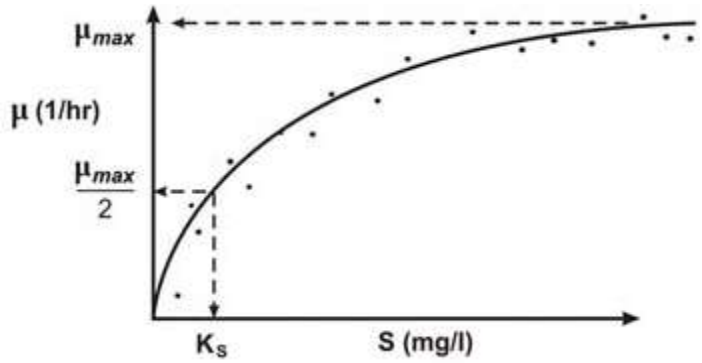

Fig. 5: Sample characteristic plot of specific growth rate against limiting substrate concentration

Many equations have been developed based on the Monod equation to model anaerobic digestion. The Contois model, as shown in equation 3, was one of such models and it described the characteristic relationship between the specific growth rate of bacteria and the microorganism population density in a continuous culture. The parameters of the Contois model include the growth rate of the microorganism per day (day^{-1}), the effluent concentration in (g/L), the microbial concentration in (g/L) and the empirical dimensionless constant (Lin, 1995).

$$\mu = \frac{\mu_{max} S}{CX + S} \qquad (3)$$

The Contois model was altered to derive the Chen and Hashimoto model asshown in equation (4). The Chen and Hashimoto modelwas developed by incorporating the influent concentration So and the dimensionless kinetic parameter K into the Contois model, as shown in equation 4. The model was restructured to make the effluent to influent concentration ratio the subject of the equation to yield equation 5 (Lin, 1995).

$$\mu = \frac{\mu_{max} {}^{S}/_{So}}{K+(1-K)\,{}^{S}/_{So}} \qquad (4)$$

$${}^{S}/_{So} = \frac{K}{\mu_{max}\theta - 1 + K} \qquad (5)$$

Like the Contois model, the Chen and Hashimoto model could be used to describe the growth rate of bacteria during the anaerobic digestion of cattle slurry manure which leads to the production of methane. Chen and Hashimoto further developed their initial growth rate model to include methane yield B in ml methane/g VOS.Chen and Hashimoto assumed that the difference between the methane yield B and the ultimate methane yield Bo (in ml CH_4/g VOS at infinite retention time), is proportional to the quantity of unused substrate in an observed culture. The modified Chen and Hashimoto model relating microbial growth rate and methane yield is as shown in equation 6 which was then restructured to form equation 7 (Lin, 1995).

$$\frac{Bo-B}{Bo} = \frac{K}{\mu_{max}\,(\theta - 1 + K)} \qquad (6)$$

$$B = \quad Bo \quad \left[1 - \frac{K}{\mu_{max}(\theta - 1 + K)}\right] \qquad (7)$$

In this paper we would be examining the work of Karim et al., 2007, in the digestion kinetics of cattle manure slurryand apply the Chen and Hashimoto model to their results.Karim et al. performed an experiment on the anaerobic digestion of cattle manure slurry in bench-scale gas-lift digesters of 3.78liters working volume at 35^0C temperature and at eight different loading rates of volatile solids (VS) in the range of 1.11 gl^{-1}day^{-1} to 5.87 gl^{-1}day^{-1}. Methane gas at the rate of 0.44$l$day^{-1} to 1.18 $l$day^{-1}were produced by the digesters and it was observed that there was increase in methane content of the biogas output with lengthier hydraulic retention time (HRT). During their experiment, the observed ultimate methane yield was estimated to be 0.42 $l$$CH_4$ (g VS loaded)$^{-1}$ and the specific methane productivity was estimated to be 0.45 l CH_4 (g VSconsumed)$^{-1}$. The total chemical oxygen demand (COD) was estimated to be in the range of 58% to 17% and the dissolved COD was evaluated to be in the range of 78% to 43%, both at 24.4 – 4.6 days HRT.

At 4.6 days HRT, the peak concentration of volatile fatty acids in the effluent was 0.7 gl^{-1} but at HRTs lengthier than 11 days the concentration was observed to be below detection limit. Applying nonlinear regression analysis on their experimental data using a derived methane production rate equation, for a thoroughly blended anaerobic digester, including Contois kinetics with endogenous

degeneration yielded the best fit values of 0.43 day^{-1}maximum specific growth rate (μ_{max}) and 0.89 dimensionless kinetic parameter (K). The research data were evaluated to be within the 95% confidence interval of the forecast of the developed methane production rate model with the summation of the residual squared error as 0.02.

Results

In this paper we extract the ultimate methane yield Bo, the dimensionless kinetic parameter K and the maximum specific growth rate μ_{max} values from the cattle manure slurry anaerobic digestion experiment of Karim et al., 2007.We apply the extracted experimental values to the Chen and Hashimoto model of equation 7. In the work of Karim et al., chemical oxygen demand was observed between an HRT of 4.6 days and 24.4 days. Thus, applying the extracted experimental values to the Chen and Hashimoto model for retention time values of 4.6 days to 24.4 days produces a plot of the methane yield with respect to the retention time, as shown in Figure 6. As shown in Figure 7, the Chen and Hashimoto model predicts that the methane yield to be 0.22 *l* CH_4 (g VS loaded)$^{-1}$ at a retention time of 4.6 days and 0.384*l* CH_4 (g VS loaded)$^{-1}$ at a retention time of 24.4 days. In the work of Karim et al., the concentration of volatile fatty acids in the effluent was observed to be below detection limit at HRTs lengthier than 11 days. At 11 days retention time the methane yield is predicted by the Chen and Hashimoto model to be 0.339 *l* CH_4 (g VS loaded)$^{-1}$which lies in the 'knee' region of the methane yield plot, as shown in Figure 8. The 'knee' region of the characteristic plot, spanning from a retention time of 6 days (with 0.269*l* CH_4 (g VS loaded)$^{-1}$ methane yield) to a retention time of 13 days (with 0.352 *l* CH_4 (g VS loaded)$^{-1}$ methane yield), describes a region of optimum methane yield. For instance, at a retention time of 24.4 days the methane yield is predicted to be 0.384*l* CH_4 (g VS loaded)$^{-1}$ but having two similar digesters at 11 days retention time would have a total methane yield of 0.678*l* CH_4 (g VS loaded)$^{-1}$(which is significantly greater than the yield at 24.4 HRT), where each harvester has a methane yield of 0.339 *l* CH_4 (g VS loaded)$^{-1}$.

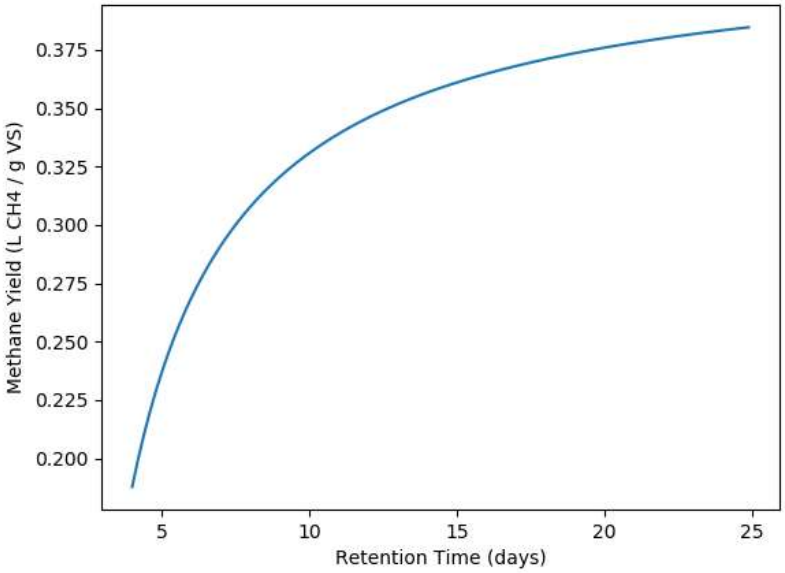

Figure 6: Result of the Chen and Hashimoto model developed with the Karim et al. parameters

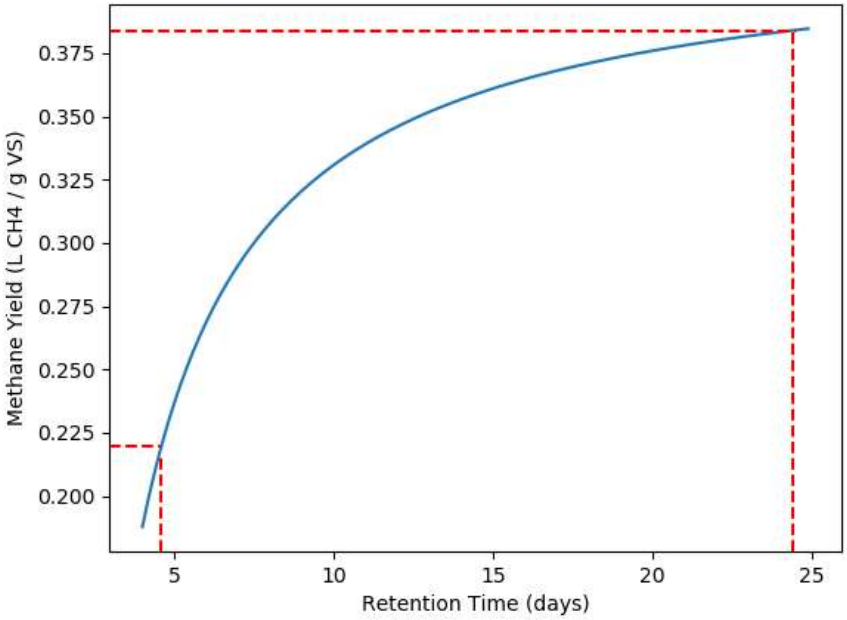

Fig. 7: Characteristic plot showing the methane yield at 4.6 days and 24.4 days

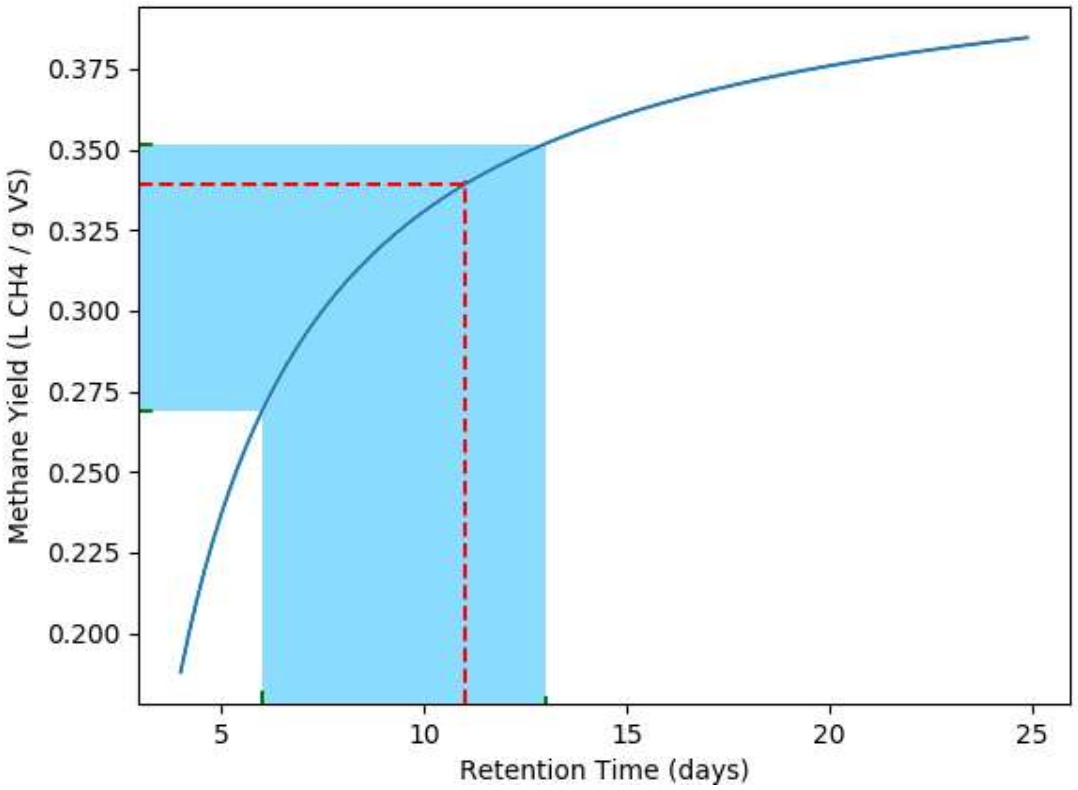

Fig. 8: Characteristic plot showing the methane yield at 11 days HRT and the 'Knee' Region

One of the mainmethanogenic bacteria responsible for the production of methane from cow dung is*Methanobrevibacterthaueri* (Hook et al., 2010).It is a specie of methanogen archaeon first isolated from cow dung and named after Rolf K. Thauer(Miller, 2002).Methanogens without cytochromes, including *Methanobrevibacterthaueri*, have a generation (or doubling) time of a minimum of 1 hour (Hook et al., 2010).In the log phase of the population growth ofbacteria, like the *Methanobrevibacterthaueri*, the generation time G is expressed in terms of time t and number of generations n (Todar, 2020), as shown in equation 8. The number of bacteria b at the end of time interval t is related to the number of bacteria B at the beginning of the time interval by equation 9. Substituting n from equation 8 into equation 9 yields equation 10 (Todar, 2020).

$$G = t/n \qquad (8)$$

$$b = B \times 2^{n} \qquad (9)$$

$$b = B \times 2^{t/G} \qquad (10)$$

The duration of the lag phase of a bacteria growth curve is influenced by the number of bacteria present in a culture (Bertrand, 2019). Simulating the growth curve of the log phase of *Methanobrevibacterthaueri* with initial cell count of 1000 Cells and generation time of 1 hour produces the plot in Figure 9. The cell count of the introduced methanogenic bacteria rises exponentially form an initial count of 1000 cells to just above $2x10^6$ cells at the end of a 12-hour period, provided the substrate volume and environmental conditions in the digester are at favourable levels. From the simulated growth curve in Figure 9, it is evident that the population of methanogenic bacteria can rise to high cell count levels in less than a day. Thus, depending on the substrate and environmental conditions, the population of methanogenic bacteria in a sample of cattle manure slurry can enter the stationary growth phase within a few days. In the cattle manure slurry experiment carried out by Karim et al., the minimum retention time was 4.6 days.

If we assume that 1000 cells of *Methanobrevibacterthaueri* was present in their digested cattle manure slurry at the start of their experiment and that the generation time of the methanogenic bacteria was 1 hour, then at the end of the 4.6-day (110 hours) retention time the number of *Methanobrevibacterthaueri* cells would be in the order of 6×10^{35}, as illustrated in Figure 10. The cell count of the methanogenic bacteria would likely have peaked before reaching this value, depending on the substrate volume, which means the *Methanobrevibacterthaueri* growth curve would most likely have entered the stationary phase before the end of the 4.6-day minimum retention time. Since the methane yield in the Karim et al. experiment only starts significantly rising at a retention time of 4.6 days and beyond, as shown in Figure 8, it can be concluded that most of the significant methane yield of the Karim et al. experiment occurs when the methanogenic bacteria population of the cattle manure slurry is in the stationary phase.

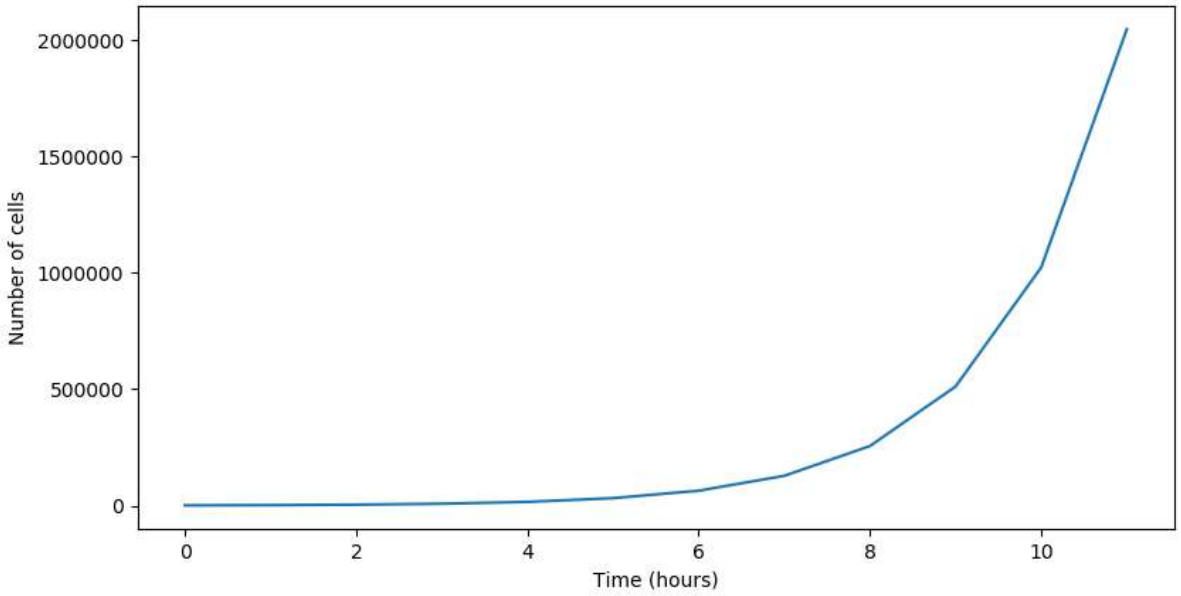

Figure 9: Simulated 12-Hour Growth Curve of the log Phase of*Methanobrevibacterthaueri*with Initial Cell Count of 1000 Cells and Generation Time of 1 hour

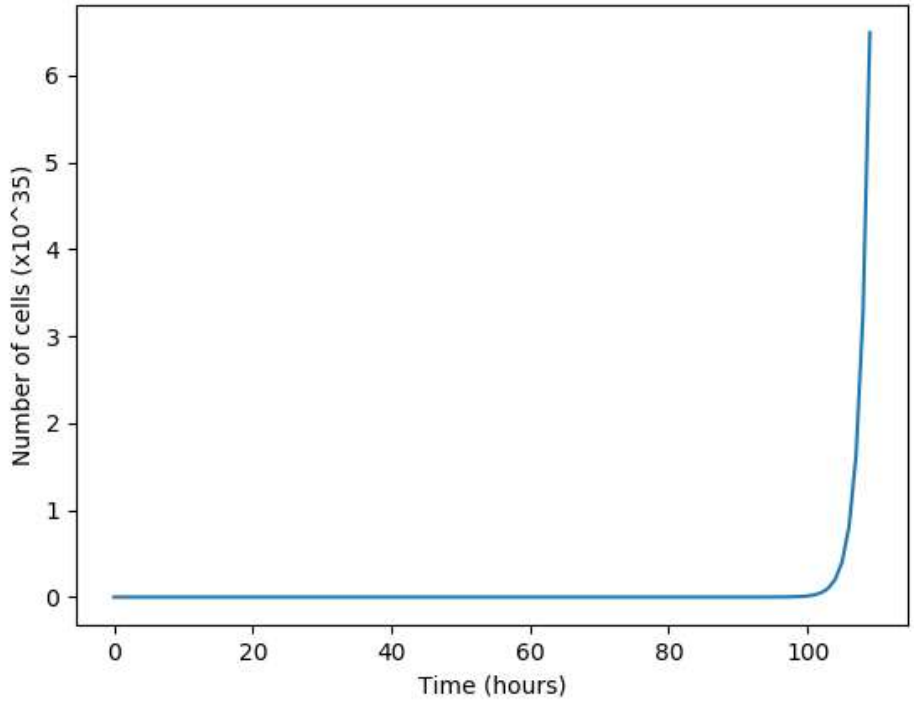

Fig. 10: Simulated 110-Hour (4.6 Days) Growth Curve of the log Phase of *Methanobrevibacterthaueri* with Initial Cell Count of 1000 Cells and Generation Time of One Hour

Conclusion

The work of Karim et al. on the digestion kinetics of cattle manure slurry was examined and the ultimate methane yield, the dimensionless kinetic parameter and the maximum specific growth rate μ_{max} parameters were extracted from their anaerobic digestion experiment. The extracted parameters were applied to the Chen and Hashimoto model to predict the methane yield of the Karim et al. anaerobic experiment with respect to the hydraulic retention time. From the characteristic plot of the applied model, the retention time range for optimal methane yield was inferred. An estimated log phase growth rate model of the cattle slurry methanogenic bacteria, most notably *Methanobrevibacterthaueri,* at a 1-hour generation time was used to imply the most probable growth phase of the methanogenic bacteria in the Karim et al. experiment that produced the most significant methane yield.

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