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Kinetics of anaerobic digestion of labaneh whey in a batch reactor

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In this work, anaerobic digestion of labanah whey was carried out in a 100 L batch reactor (RE-BIOMAS) at temperature of 30-40°C and pH 6 - 7. During the experiments, the biogas production and chemical oxygen demand (COD) concentration were recorded with time. During fermentation of labaneh whey, the pH drops dramatically due to the accumulation of volatile fatty acids that inhibits the activity of methanogens, resulting in a low gas yield and low methane content of the biogas. In a 28 days batch experiment at 36°C and pH 6.5, COD removal efficiency of 84% was achieved at initial COD of 18,000 mg/l. The cumulative biogas production was 20 L. Experimental data were fitted to the four kinetic models: Monod, Logistic, Contois and Tessier models. Comparison was made between model predictions and experiments for COD concentration. Tessier model gave marginally better fit than other models tested. Kinetic and stoichiometric coefficients were determined for the four kinetic models using Matlab nonlinear optimization function. Diluted labaneh whey (about 8000 mg/l COD) was treated to almost complete COD removal in a 10 days retention time and producing about 20 L of biogas. The COD removal was described well by first order kinetics with respect to substrate concentration. The modified Gompertz equation was used to describe the cumulative production of biogas with time. The equation kinetic constants were determined for labaneh whey and for diluted whey.

Key words: Labaneh whey, biogas, anaerobic digestion, methane production, dairy wastewater, modified Gompertz equation.

INTRODUCTION

"Labaneh" (also spelt labneh, labne or labni) is a popular type of fermented milk product in the eastern Mediterranean countries. Labaneh whey is the yellow-greenish aqueous portion of the milk that separates from the curds during labaneh manufacturing. Labaneh whey is a waste by-product of the dairy industry. It retains about 55% of the milk nutrients (Kumari et al., 2011). The disposal of labaneh whey is considered a major problem

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Abbreviations: BOD, Biochemical oxygen demand; **COD,** chemical oxygen demand; **CSTR,** continuous stirred tank reactor; **HRT,** hydraulic retention time; **UAPB,** up-flow anaerobic packed bed; **UASB,** up-flow anaerobic sludge blanket; **VFA,** volatile fatty acid.

because of its high biological oxygen demand. Whey discharge into sewage treatment plants can cause serious problems to the plants and to the surface water. This concern has been heightened in recent years by the increased volume of whey and the more stringent legislative requirements for effluent quality. Approximately 85 kg of cheese whey is produced from 100 kg of milk (Kosikowski, 1977). Whey is composed of 93% water and 7% solid. The solids are composed of lactose, proteins, lipids and various salts. Labaneh whey contains about 5% lactose sugar which can be utilized as a carbon source by bacteria in biological treatment (aerobic and anaerobic). It is a major dairy waste in many Middle East countries. There is little information published on labaneh whey. Most of the information available in literature is about cheese whey. The chemical and physical properties of labaneh whey collected from the Jordan Dairy Company (Zarka, Jordan) were reported by Batshon (1980). It has a typical pH of 3.5, which is even lower than acid whey or sweet whey. In labaneh whey there is less lactose, more calcium, phosphorus, magnesium and lactic acid as compared to sweet or acid whey (Batarseh, 1995).

Biological treatment reduces the pollutants to a level that meet even the most stringent requirements. Aerobic digestion is very energy intensive and leads to a large amount of sludge as compared to anaerobic digestion in which most of the energy from whey is conserved in the produced biogas (about 60% methane) and little is used in formation of biomass. Some of the advantages of anaerobic digestion are the high energy efficiency, lower cost in addition to simplicity of the process as compared to aerobic processes.

Anaerobic digestion of labaneh whey offers an excellent solution in terms of both energy conversion and pollution control. Labaneh whey can be classified as acid whey (pH = 3.4) that makes pH control a serious problem during anaerobic digestion. No previous studies reported on anaerobic digestion of labaneh whey. All previous studies were on anaerobic digestion of cheese whey that has different compo-sitions and pH values. Anaerobic digestion of cheese whey in two stage configuration is more efficient than a single stage because of the possibility of optimization of operating conditions in both acidogenesis and metha-nogenisis processes. Jasko et al. (2011) showed that two phase bioreactor was a good choice for anaerobic digestion of cheese whey. In their experiment, they determined methane yield that fluctuated in the range of 136.6 - 216.3 l/kg volatile solid. Stamatelatou et al. (2012) used two stages consisting of a CSTR and UASBR to overcome inhibition during anaerobic treatment of diluted cheese whey. This system achieved 95% COD removal and produced 9 l/l d biogas. Also, Patil et al. (2012) used two stages up-flow anaerobic packed bed reactor for the treatment of cheese whey, they obtained 94-96% COD removal depending on the HRT.

Biogas production from cheese whey was enhanced by co-digestion of whey with other substrates. Various co-substrates have been reported in the literature such as sewage sludge (Powell et al., 2013), cow manure (Comino et al., 2009) and olive mill waste (Martinez-Garcia et al., 2007). Comino et al. (2012) studied biogas production and COD, BOD removal of a mixture of cattle slurry and cheese whey in a 128 L continuous anaerobic reactor. Using a mixture 50% slurry, biogas production was 621 l/kg VS at HRT of 42 days. The methane content of the biogas was 55%. The maximum percentage removal of COD and BOD were 82 and 90%, respectively.

Powell et al. (2013) studied the effect of cheese whey storage on the biogas production from co-digestion of whey and sewage sludge. Storage of whey affects its composition but has no effect on the methane production (m^3 CH_4/kgCOD_{added}). Najafpour et al. (2008) used successfully up-flow anaerobic sludge fixed film bioreactor to treat dairy wastewater and produce biogas. COD and lactose removal achieved were 97.5 and 98%, respectively, at HRT of 48 h. The highest production rate of biogas (3.75 l/day) was achieved at HRT of 36 h.

It is known that anaerobic digestion consists of three main steps: Hydrolysis of complex waste to monomers, oxidation of the products of the first step to acetic acids and CO_2 and the third step is formation of methane by methanogenic bacteria that use acetate, CO_2 and hydrogen to produce methane. Mathematical modeling of anaerobic digestion of labaneh whey is important for better understanding of the process. It is important for design, operation and prediction of the performance of the bioreactor that carry out the biological treatment process.

Yilmaz and Atalay (2003) developed mathematical model to describe substrate reduction and biogas production for batch reactor using five different organic wastes. The mathematical model predictions agree well with the experimental data. No mathematical model or kinetic study of anaerobic digestion is available in the literature for labaneh whey which has different composition and pH than cheese whey. The objective of this work was to study the kinetics of substrate utilization and biogas production during batch anaerobic digestion of labaneh whey and diluted labaneh whey in a 100 L pilot plant.

MATERIALS AND METHODS

Characterization of labaneh whey

Labaneh whey used in this work was collected from dairy factory at the College of Agriculture, University of Jordan, Amman, Jordan. Labaneh whey was obtained at a pH slightly less than 3.5 with a green yellowish color. Collected whey was stored at 4°C before anaerobic digestion. Typical characteristics of labaneh whey are shown in Table 1 (Batshon, 1980).

Table 1. Chemical and physical properties of labaneh whey from Jordan Dairy Company (Batshon, 1980).

Lactic acid (w %)	Specific gravity (-)	Total solids (w %)	Total ash (w %)	Glucose (w/v %)	Lactose (w/v %)	Total nitrogen (w %)	Protein (w %)	Fat (w/v %)	pH
1.568	1.03	6.2	0.9	1.009	1.234	0.298	1.862	0.683	3.4

Figure 1. RE-BIOMAS-pilot plant for the production of biogas from biomass.

Anaerobic digestion pilot plant

The RE-BIOMAS (DIDACTA Italia) pilot plant was used in the anaerobic digestion study of labaneh whey (Figure 1). The 100 L reactor has a pH and temperature control system. Mixing was carried out using circulating pump at the bottom of the reactor. Variable power electric heater was used to heat the reactor. Several temperature probes were placed inside the reactor. Base solution is used to control the pH.

In the degassing settling tank, the gas is separated from the sludge that is recycled to the reactor. After this point, the gas is subjected to a series of treatment steps and the carbon dioxide is removed by absorption in water and basic solution. The gas is dried by silica gel filter. Finally, the hydrogen sulfide is removed by activated carbon bed. The gas is stored at atmospheric pressure in a volumetric tank. Liquid and gas samples were taken every day for COD and methane concentration measurements. The cumulative biogas volume was also recorded. Biomass used in this study was obtained from nearby domestic wastewater treatment plant (bottom of the thickener).

Analytical methods

COD was determined according to the standard procedure (APHA, 2005). The gas samples were collected in rubber balloon for analysis. Organic acids and evolved gases were analyzed by capillary gas chromatography-mass spectrometry (GC-MS) (PerkinElmer, AutoSystem XL Gas Chromatograph) according to Thaxton et al., 2010 (BrukerDaltonics Inc., MA 0182, USA) and ASTM D 1946-90 (2000), respectively. Lactose concentration was measured using phenol-sulfuric acid method (Dubois et al., 1965).

Modeling of batch anaerobic bioreactor

The kinetics of biomass growth can be determined by measuring either substrate (COD) consumption or product (biogas) formation with time. The second method is fast as compared to the first method. In this work, using growth kinetics and substrate balance around the batch reactor, a mathematical model was developed that describes COD reduction with time. The mathematical model predictions were compared with the experimental results for COD reduction and biogas production. The substrate balance around the batch bioreactor results is given as follows:

$$\frac{dS}{dt} = -Y_{xs}\,\mu\,X \quad S(t=0) = S_o = COD(o) \tag{1}$$

Where, Y_{xs} is the yield coefficient (g/g), μ is the specific growth rate (d^{-1}), S_o is the initial substrate concentration (COD(o)), (g/l), and X is the cell (biomass) concentration (g/l). When the biomass yield coefficient is constant, the biomass concentration can be calculated using the following algebraic equation:

$$X = X_o + Y_{xs}(S_o - S) \tag{2}$$

The following four biomass growth kinetic equations were used in this study.

Table 2. Variation of substrate concentration with time using four different kinetic models.

Kinetic equation	Differential equation	
Monod	$\frac{dS}{dt}=\left(\frac{-\mu_m S}{Y_{XS}}\right)\frac{\left[X_o+Y_{XS}\left(S_o-S\right)\right]}{K_S+S}$	(7)
Contois	$\frac{dS}{dt}=\left(\frac{-\mu_m S}{Y_{XS}}\right)\frac{\left[X_o+Y_{XS}\left(S_o-S\right)\right]}{k_{sx}\left[X_o+Y_{XS}\left(S_o-S\right)\right]+S}$	(8)
Logistic	$\frac{dS}{dt}=\left(\frac{-\mu_m}{Y_{xs}}\right)\left[X_o+Y_{XS}\left(S_o-S\right)-\frac{\left(X_o+Y_{XS}\left(S_o-S\right)\right)^2}{Y_{XS}\,S_o}\right]$	(9)
Tessier	$\frac{dS}{dt}=\left(\frac{-\mu_m}{Y_{xs}}\right)\left[X_o+Y_{XS}\left(S_o-S\right)\right]\left[1-e^{-\frac{S}{K_S}}\right]$	(10)

Monod equation $$\mu=\mu_m\frac{S}{K_s+S} \quad (3)$$

Contois equation $$\mu=\mu_m\frac{S}{k_{sx}\,X+S} \quad (4)$$

Logistic equation $$\mu=\mu_m\,(1-\frac{X}{X_m}) \quad (5)$$

Tessier equation $$\mu=\mu_m\,(1-e^{-\frac{S}{K_S}}) \quad (6)$$

Equation 1 that represents substrate concentration reduction with time is reduced to the following differential equations (Table 2) using the four kinetic models and assuming constant yield coefficients.

The above four kinetic models were fitted to the experimental data using the Matlab function (*fminsearch).*

First order kinetic model

In diluted labaneh whey experiments, the first order kinetic model was tested for COD reduction with time. Substrate balance around batch anaerobic digester assuming first order kinetics produces the following:

$$\frac{dS}{dt}=-k\,S \quad (S = S_o) \text{ at } t = 0 \quad (11)$$

Where, k (d^{-1}) is the first order kinetic constant, k represents a measure of biodegradation rate. The higher the k value, the higher the biodegradability of the digester. Equation 11 was used to describe biodegradation of solid waste by anaerobic digestion (Chen and Hashimoto, 1979).

Integration of equation 11 yields:

$$\ln\frac{S}{S_o}=-k\,t \quad (12)$$

Or in terms of COD $$\ln\frac{COD(t)}{COD(o)}=-kt \quad (13)$$

Or $$\ln COD(t)-\ln COD(o)=-kt \quad (14)$$

This is a straight line equation. When plotting ln COD(t) vs t, the slope will be - k and the intercept will be ln COD(o).

Kinetics of biogas production from labaneh whey

The modified Gompertz equation was used to describe the cumulative production of biogas. Equation (15) represents a modified Gompertz first order equation:

$$B_t=B\exp\left\{-\exp[\frac{R_b\times e}{B}\left(\lambda-t\right)+1]\right\} \quad (15)$$

Where B_t is the cumulative biogas produced at any time (l); B is the biogas production potential (l); R_b is the maximum biogas production rate (l/d); λ is the lag phase (d), that is the time needed for the bacteria to acclimatize to the environment or the time needed to produce biogas.

The modified Gompertz equation is used by researchers to describe the cumulative biogas production during anaerobic digestion (Budiyono et al., 2010; Yusuf et al., 2011). This equation is based on the assumption that methanogens production rate of biogas in batch reactor corresponds to its specific growth rate.

Table 3. Chemical oxygen demand removal in the 28 days batch experiment.

Day	0	1	3	7	9	10	12	13	14	21	22	23	25	28
COD (g/l)	17.92	17.3	16.5	15.7	15	14.08	13.5	12.9	12.5	8	7	5.96	4	2.9
COD removal (%)	0	3.46	7.93	12.4	16.3	21.4	24.66	28	30.25	55.36	60.94	66.7	77.7	84

RESULTS AND DISCUSSION

Anaerobic digestion of labaneh whey was carried out in a 100 L pilot plant operating in batch at controlled temperature and pH. Liquid samples were collected for COD and VFA analysis. Gas samples were collected for measurements of methane and carbon dioxide concentrations. The biogas production was measured by liquid displacement system. Table 3 shows the COD reduction with time during the 28 days of the experiment at controlled temperature of 36°C and pH of 6.5. Four kinetic models were fitted to the experimental data using the Matlab function (*fminsearch)* that find the minimum of a scalar function of several variables (unconstrained nonlinear optimization) as shown in Figure 2. All the four models fitted the experimental data closely; however Teisser model marginally fitted the data better than other models tested (higher correlation coefficient). Table 4 shows the kinetic constants and error obtained from non-linear curve fitting of the four kinetic models. Lactose in labaneh whey is converted easily to lactic acid and other volatile fatty acids by bacteria under anaerobic conditions.

This reduces the pH causing inhibition of the very sensitive methanogene bacteria. To keep the pH around 6, large amount of base was added to the reactor. The low pH caused reduction in the biogas production rate and low methane content of the biogas. Figure 3 shows the concentration of acetic and butyric acids in the reactor. The concentration of both organic acids increases with time and reached almost a steady state constant value. Anaerobic digestion studies of cheese whey at low pH showed lower productivity of biogas and low methane content of the produced biogas (Ghaly, 1996). In his experiments, he concluded that anaerobic digestion of cheese whey without pH control is not feasible.

Stamatelatou et al. (2012) used two stage systems to overcome this stability problem. In their work, they used a CSTR (2 l) followed BY UASB (6.3 l) reactor. This system produced 9 l/l d of biogas containing approximately 55% methane and achieved 95% COD removal.

Biogas production was measured with time until the change in production was very small. A total of 20 L of biogas was produced with 46% methane content. Figure 4 shows a third order polynomial fitting of the cumulative biogas volume vs. time as compared to experimental data. The modified Gompertz equation was also used to fit the cumulative biogas production (liter) with time (days). Figure 5 shows the experimental results and the modified Gompertz equation predictions. The equation kinetic constants B, R_b and λ were determined using nonlinear curve fitting of the Matlab function *lsqcurvefit*. The fitting correlation coefficient is close to 1. The biogas production potential B is 19.5 L while the maximum biogas production rate R_b is 6.5 l/d. The lag time λ is low (0.3 days = 7.2 h) as compared to batch experiments using other substrates (Yusuf et al., 2011).

Another anaerobic digestion experiment (Experiment 2) was carried out using dilute labaneh whey (initial COD of 8 g/l). Figure 6 shows the decline of COD with time. It is clear from this figure that almost complete COD removal was achieved in 10 days. During this experiment, the temperature and pH were controlled at 37°C and 6.5, respectively.

Plotting ln COD(o)/COD(t) vs. t yield a straight line equation with goodness of fit (correlation coefficient 0.9924) with slope (k) equal 0.24 day^{-1}. This figure shows that first order kinetics can be used to describe the COD removal for dilute labaneh whey (Figure 7). This is expected since other kinetic models such as Monod equation reduces to first order for low substrate concentration.

Using experimental data at three different temperatures (32, 35 and 37°C), the activation energy for anaerobic digestion of labaneh whey (E) can be determined. Plotting ln K vs 1/T(K) the activation energy can be calculated, assuming K vary with temperature according to Arrhenius equation ($k = k_o\, e^{-E/RT}$). As shown in Figure 8, k_o and E can be determined from the intercept and slope (k_o= 6.146 10^{14}) and the activation energy E = 21,850 cal/mole °K.

In the second type of experiments (anaerobic digestion of diluted labaneh whey), biogas production was measured with time until the change in production is very small. A total of about 20 L of biogas was produced in 10 days retention time. Figure 9 shows the experimental results and the modified Gompertz equation predictions. The goodness of fit is high (R = 0.965). The equation kinetic constants B, R_b and λ were determined, B = 20.15 L, while the maximum biogas production rate R_b is 3.35 L/day and the lag time λ is negative (λ = - 0.2648). No lag phase was observed during anaerobic digestion of diluted labaneh whey. The concentration of VFA during the 10 days experiment is shown in Figure 10. Acetic acid has higher concentration in this dilutted whey experiment as compared to that of undiluted whey experiment.

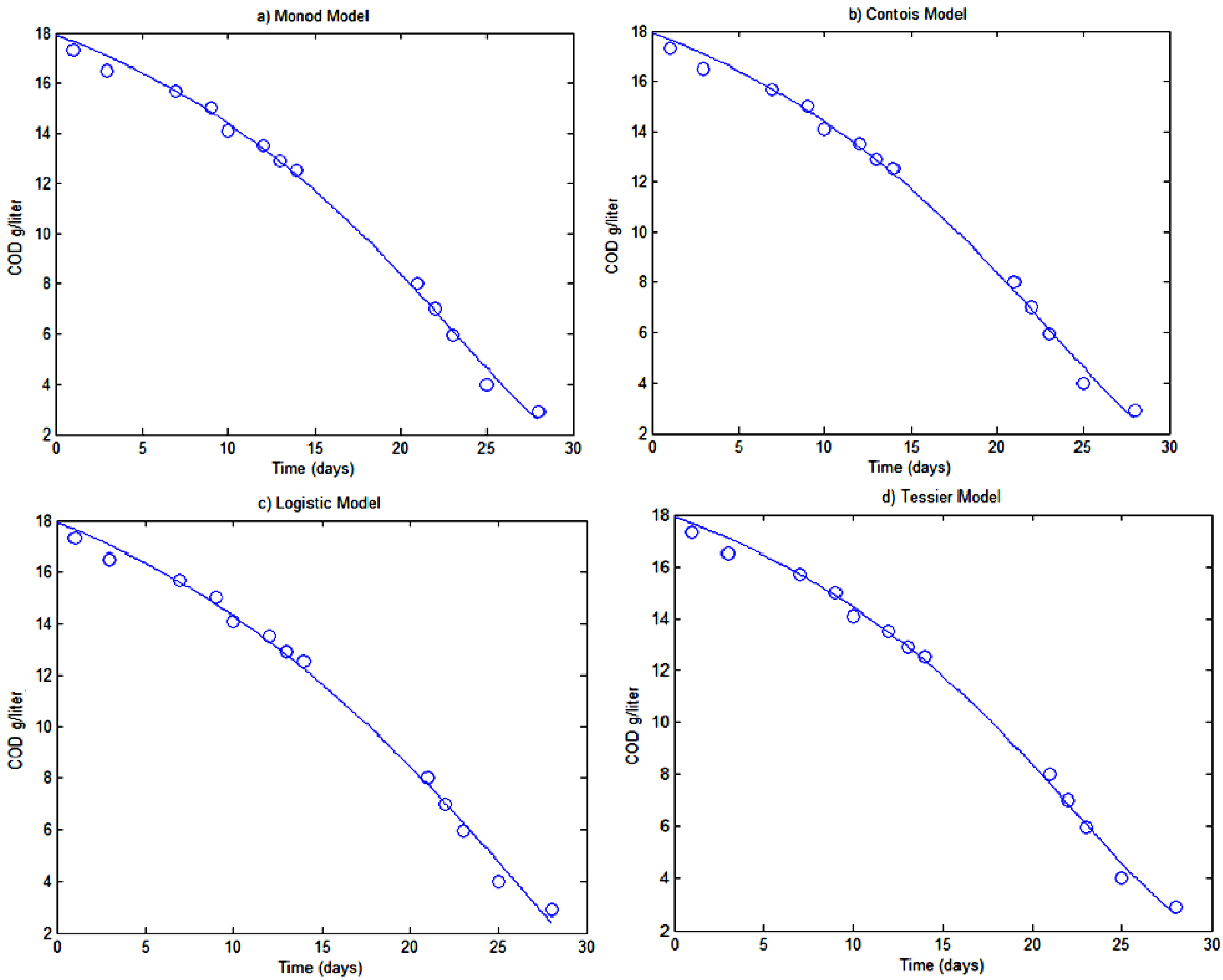

Figure 2. Biodegradation of COD with time using the 4 kinetic models (-o-o- experiment, _ model). a) Monod model; b) Contois model c) Logistic model; d) Tessier model.

Table 4. Kinetic constants and error obtained from non-linear curve fitting.

Kinetic equation	Kinetic constants	Smallest value of error
Monod	$\mu_m = 0.076\ d^{-1}$, $k_s = 3.59$ g/l	1.29
Contois	$\mu_m = 0.065\ d^{-1}$, $k_{sx} = 1.27$ g/l	1.29
Logistic	$\mu_m = 0.073\ d^{-1}$, $X_m = 4.97$ g/l	1.686
Tesseir	$\mu_m = 0.063\ d^{-1}$, $k_s = 4.17$ g/l	1.23

Conclusions

A 100 L pilot plant was used for anaerobic digestion and biogas production using labaneh whey as a substrate. Although the degradation of labaneh whey is very fast, accumulation of organic acids reduces the pH and caused instability in the system. This reduces the rate of biogas production and methane yield. The substrate

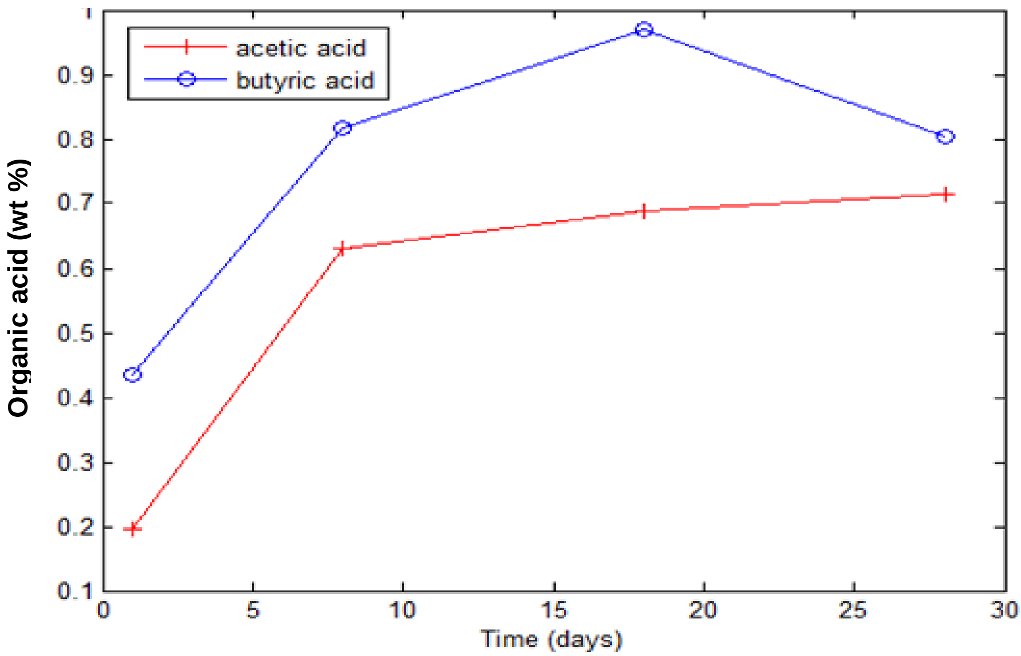

Figure 3. Concentration of acetic and butyric acid in the reactor.

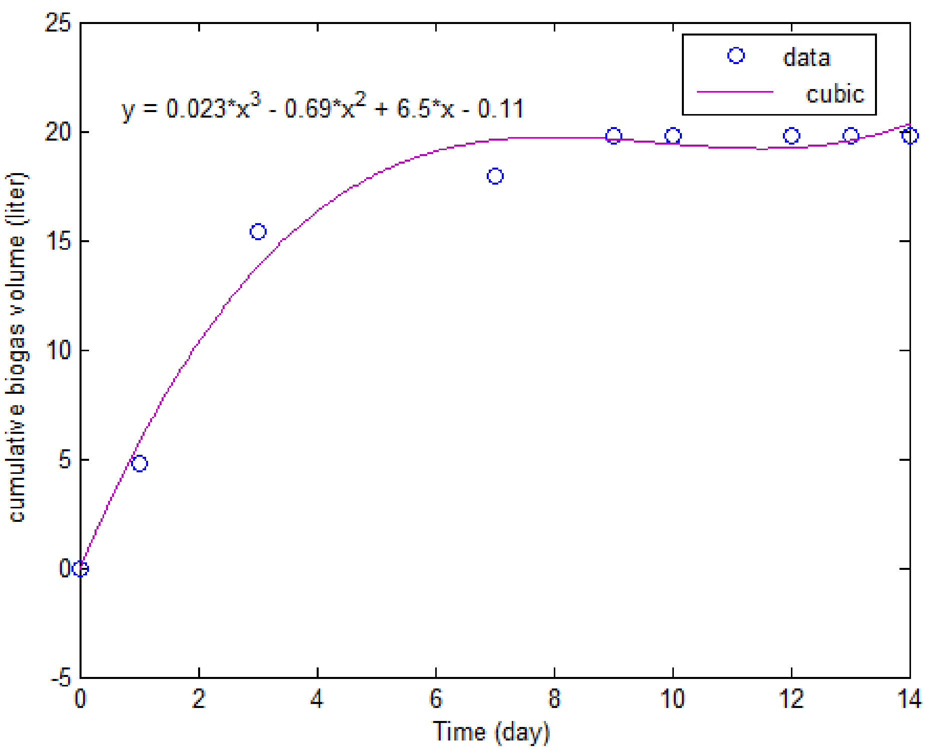

Figure 4. Cumulative biogas volume vs. time.

biodegradation was modeled and compared with the experiment. Four kinetic models were fitted closely to the experimental results of COD decline with time. Tesseir model gave marginally better fit than other models tested.

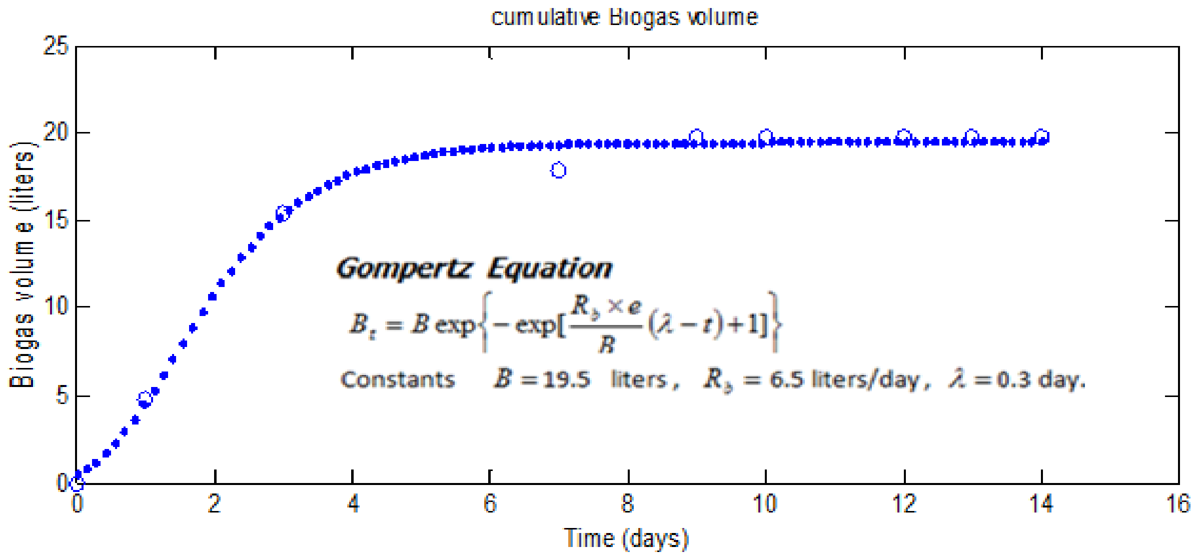

Figure 5. Kinetic constants and smallest value of error obtained from non-linear curve fitting of Gompertz equation (-o-o- experiment, ….. model).

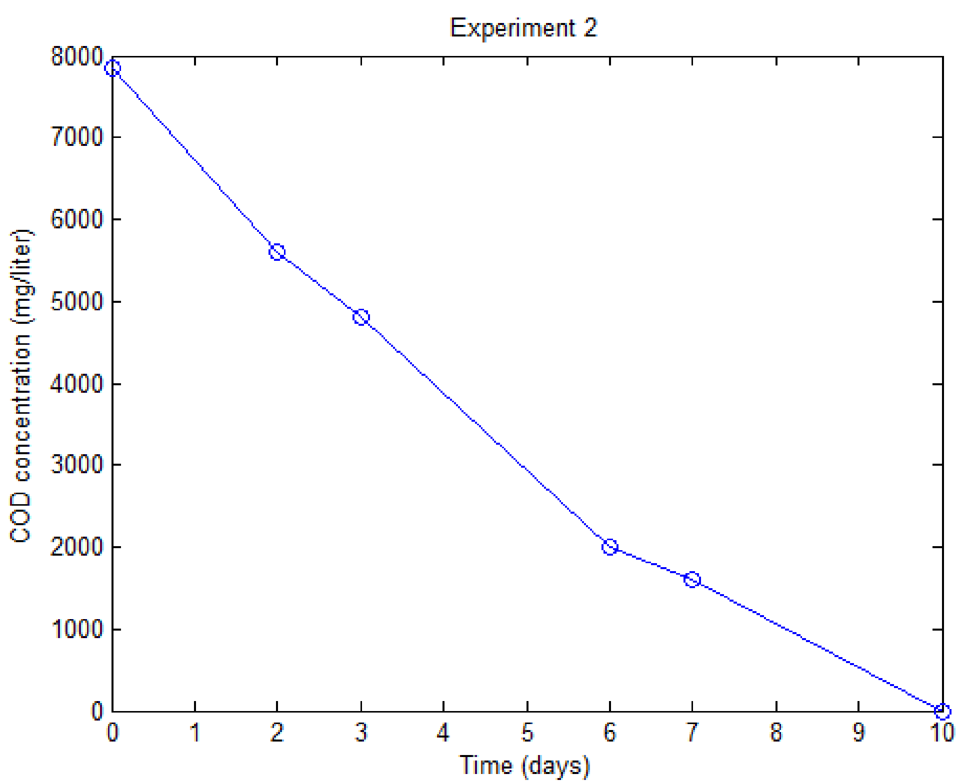

Figure 6. COD concentration vs. time during batch anaerobic digestion of diluted labaneh whey.

Percentage of COD removal achieved was 84%. The digestion process was unstable due to production of organic acids and rapid drop of pH that leads to low percentage of methane in the biogas (46%). 20 L of biogas were produced in a batch during 28 days of operation. Diluted labaneh whey was treated to almost

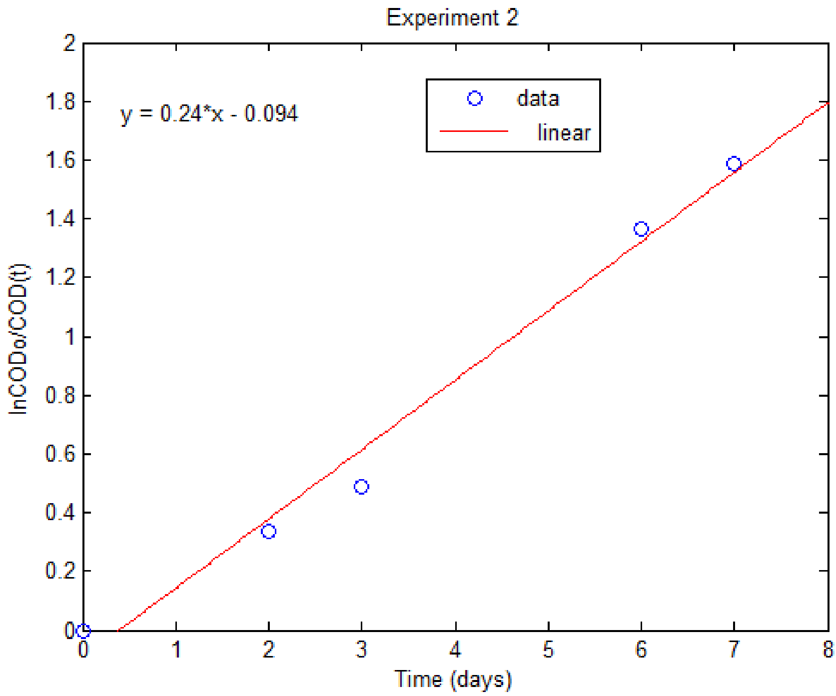

Figure 7. ln COD(t) vs. time (t) plot for anaerobic digestion of diluted labaneh whey (Experiment vs. model).

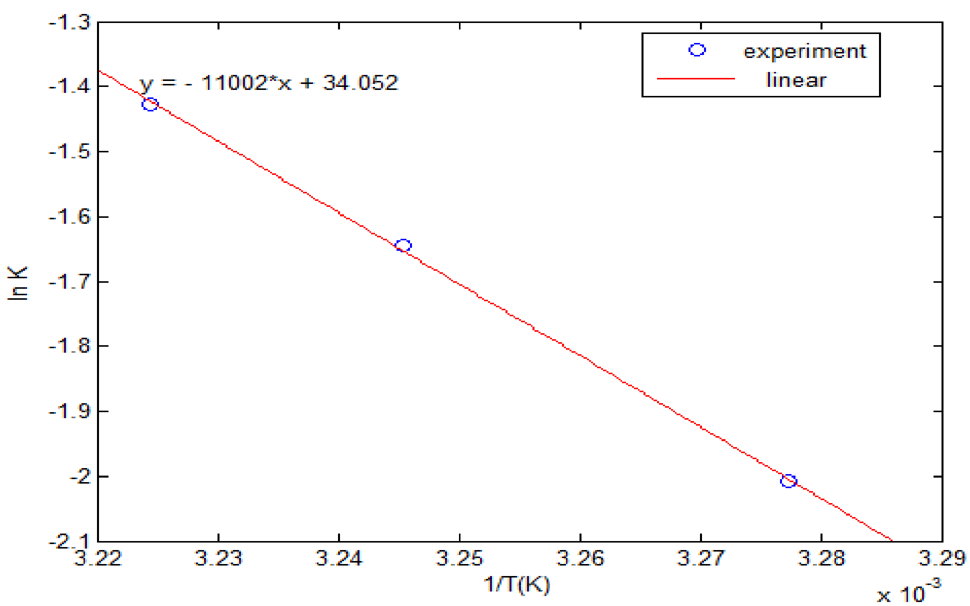

Figure 8. ln K vs. 1/T from anaerobic digestion experiments of diluted labaneh whey at three different temperatures.

complete COD removal in a shorter time and the COD removal was described well by first order kinetics. The modified Gompertz equation was used to describe the cumulative production of biogas with time. The equation kinetic constants were determined for both labaneh whey and for diluted labaneh whey.

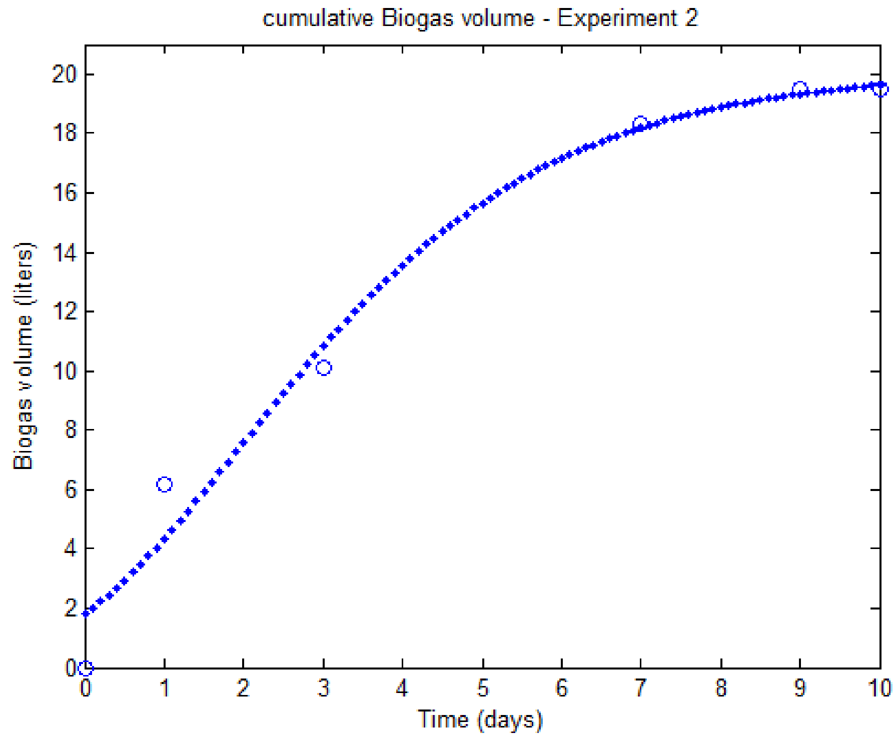

Figure 9. Kinetic constants and smallest value of error obtained from non-linear curve fitting of Gompertz equation (diluted whey) (-o-o- experiment, ….. model).

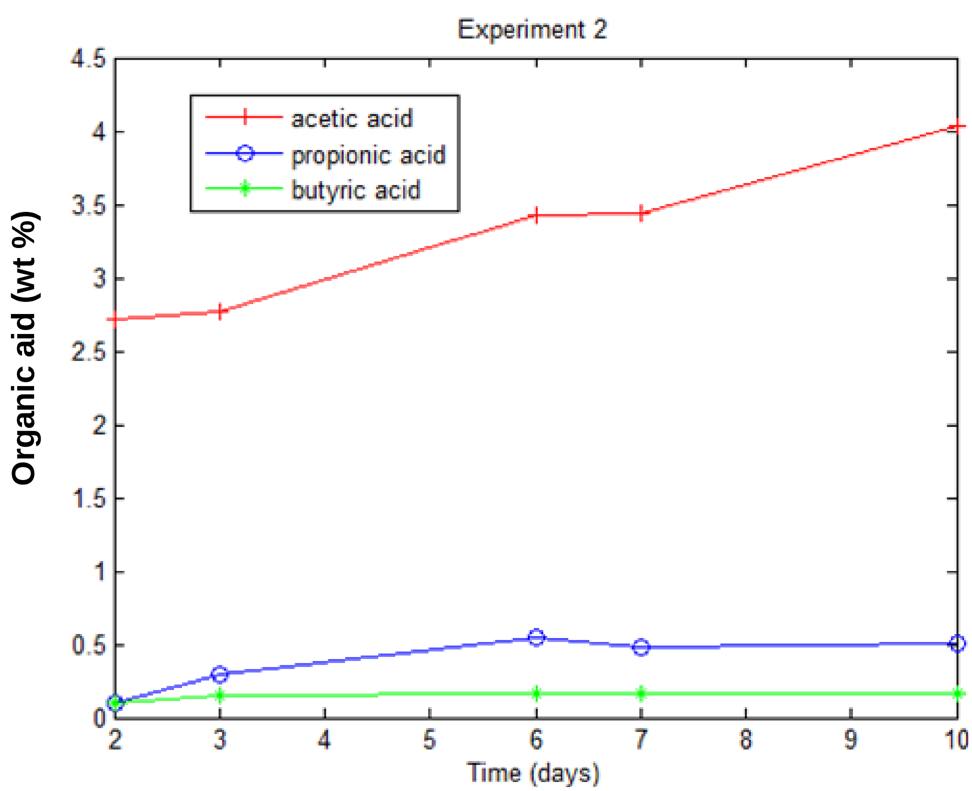

Figure 10. Concentration of volatile fatty acids in the reactor (diluted labaneh whey).

Conflict of Interests

The author(s) have not declared any conflict of interests.

Nomenclature

B, Biogas production potential (l)
B_t, Cumulative biogas production (l)
E, Activation energy in Arrhenius equation (cal/mole ^oK)
k, First order reaction rate constant (1/d)
k_o, Pre-exponential factor (1/d)
k_s, Constant in Monod, Tessier equations (g/l)
k_{sx}, Contois constant (g cell/g substrate)
R_b, Maximum biogas production rate (l/d)
S, Substrate concentration (g/l)
S_o, Initial substrate concentration (g/l)
t, Time (days)
T, Temperature (K)
X, Cell concentration (g/l)
X_o, Initial cell concentration (g/l)
X_m, Maximum cell concentration (g/l)
Y_{xs}, Cell yield coefficient (g cell/g substrate).

Greek letter

λ length of lag phase, d
μ specific growth rate, 1/d
μ_m maximum specific growth rate, 1/d

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