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Bioenergy Potential and Kinetic of Biogas Production in Anaerobic Digestion of Slaughterhouse Effluent

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Abstract: Many studies have investigated the technology of anaerobic digestion for waste treatment and its benefits. However, most of those studies have reported on solid waste. So, there are few articles on the anaerobic digestion of effluent, especially anaerobic digestion of slaughterhouse effluent and its bioenergy potential. The purpose of this study is to evaluate the bioenergy potential in slaughterhouse wastewater treatment. Then, anaerobic digestion (AD) was used in this study to assess the bio-energy potential and kinetics of biogas production during processing. The slaughterhouse wastewater collected was characterized before the experiments using french standard method "AFNOR". pH, Temperature, turbidity, dissolved oxygen (O_{2dis}.), oxidation-reduction potential (ORP), Conductivity, Chemical Oxygen Demand (COD), biochemical oxygen demand in five days (BOD_5), total Kjeldahl nitrogen (TKN) and total phosphorus (P_{tot}) were analyzed and the ratio BOD_5/COD was calculated to evaluate the biodegradability of the biomass. Laboratory-scale anaerobic batch digesters consisting of a 1 L plastic container were used in all the experiments and the biogas produced in the digesters was measured daily by the water displacement method. The wastewater produced by slaughterhouses is biodegradable with a ratio between biological oxygen demand and chemical oxygen demand (BOD_5/COD) > 0.5. An effective AD design shows that over 90% of organic matter was removed when inoculation and the carbon/nitrogen (C/N) ratio were adjusted. The cumulative volume of biogas increased from 415 mL to 2,150 mL as the substrate/inoculum (S/I) ratio has decreased from 2.028 to 0.337 and increased from 1,140 mL to 5,250 mL as the C/N ratio increased from 6 to 22. The biogas produced has a high calorific value, as the methane content is 74%. Among the three kinetic models used to describe biogas production, a modified Gompertz model was found to be the best with R^2 between 0.983 and 0.993. This study points out energy potential of slaughterhouse wastewater and its benefit as it is managed efficiently.

Keywords: Slaughterhouse Wastewater, Anaerobic Digestion, Bioenergy, Biogas Production, Kinetic of Biogas Generation, Inoculum

1. Introduction

With an increasing population, food demand has led to growing meat processing worldwide [1]. The meat processing industries are classified as industries that consume more fresh water. These industries consumed 29% of total freshwater in the agricultural sector worldwide [2]. In Togo, the slaughterhouse located in the port area consumes up to 250 m^3 of freshwater and generates about the same amount of wastewater per day. Unfortunately, the generated wastewater is directly discharged into the environment without any adequate treatment. Slaughterhouse wastewater is generally characterized by high organic content, making it one of the most harmful agricultural and industrial food wastewaters [3]. These pollutants have very long-lasting effects on the sustainability of local ecosystems and pose a serious threat to human health [4]. Due to its high organic content, slaughterhouse wastewater is usually considered as a potential energy source and the main energy contributor in a wastewater treatment plant is the biogas produced in the digester [5]. As a result, wastewater from slaughterhouses has attracted a great deal of interest for biomass energy [6]. Anaerobic digestion is an appropriate method of achieving energy.

Unfortunately, in developing countries, few are aware of the benefits of anaerobic digestion. For example, in Togo, from 1980 to 2019 only fifteen (15) plants of anaerobic digestion were installed [7]. Due to the relatively high cost of waste management, in most cases, wastewater is discharged directly into the environment, posing significant environmental challenges. Many diseases such as cholera, acute diarrhea, dysentery, typhoid fever, viral hepatitis, poliomyelitis, leptospirosis, turista or traveler's diarrhea, etc. are waterborne diseases related to water pollution [8]. According to Haras [9], in ten (10) years, more children have been killed than all armed conflicts since the end of the 2nd World War and around 6,000 deaths daily were caused by diarrheal diseases. Furthermore, wastewater is known to contain a significant amount of organic matter which can give rise to emissions of methane (CH_4) and nitrous oxide (N_2O) whose greenhouse effect is more severe than that of carbon [10].

With the development of new technologies, waste should not be considered useless, but a new biomass that can be used to generate renewable energy [11]. Anaerobic digestion (AD) is used for its effectiveness in dealing with highly polluted water. Besides its ability to produce renewable energy, anaerobic digestion is also used to reduce odours in waste management systems as well as the organic matter content of waste [12]. Anaerobic digestion technology is therefore a valuable technology for effective waste management. In addition, digestate from anaerobic digestion is a useful fertilizer for agriculture because it is rich in nitrogen (N) and has a high ammonium to total nitrogen ratio [13]. According to Baeyens et al. [14], the application of anaerobic digestion to waste water effectively reduces the operating costs of the treatment plant.

This technology essentially involves biological matter, where diverse types of bacteria work in close line for biodegradation of organic and energy production in four stages: hydrolysis, acidogenesis, acetogenesis and methanogenesis [15]. According to Appels et al. [16], regardless of the number of AD steps, the biodegradation processes of both approaches are similar. Ramatsa et al. [17] demonstrated that there are three temperature regions in which anaerobic digestion can be conducted, psychrophilic (10-20°C), mesophilic (20-45°C) and thermophilic (45-68°C), where the mesophilic conditions offer the most common temperature ranges used. The performance of this technology is influenced by operational parameters such as pH, temperature, organic load rate, substrate composition, inoculation, carbon-nitrogen balance. The pH values show the balance of the anaerobic digestion process, as well as the accumulation of volatile fatty acids (VFAs) and ammonia nitrogen (NH_3-N) [18]. Mesophilic conditions were suitable for the anaerobic digestion process with an increase of biogas production from 20°C to 35°C and a decrease after this temperature [19] while thermophilic conditions with maximum biogas production at 50°C were reported as the best condition by Deepanraj et al [20]. An inappropriate carbon/nitrogen ratio will negatively impact methane production efficiencies [21]. When using a digester, special care needs to be taken in the composition of the substrate, as the anaerobic digestion process is based on biodegradability [11]. Methane production increased with the decline in the I/S ratio and the maximum production was reached with a ratio of 1 [22]. Depending on the composition of their microbial community, different source of inoculums may influence biogas production [23]. Increased organic loading may reduce degradation of volatile solids as well as biogas production [24]. The decrease of temperature will reduce the biogas production while intermittent mixing mode will enhance its production [25].

Our aim in this study is to evaluate bioenergetic potential from slaughterhouse wastewater, the performance of certain control factors, the kinetics of organic matter removal and biogas production by anaerobic digestion process for environment preservation.

2. Materials and Methods

The site of our study is the slaughterhouse of Lomé harbor area. It is located on the coast between the Atlantic Ocean and the Ghana-Benin road. It began its activities in 1976. It was installed on an area of 4ha. On average, 100 cows, 60 small ruminants and 10 pigs are slaughtered per day and approximately 250 m^3 of water are used per day for the maintenance of slaughtered animals.

2.1. Experimental Design and Analytical Methods

Wastewater collected from a slaughterhouse was characterized according to the French standard method "AFNOR, 1986" [26]. The following parameters were analyzed: pH, Temperature, turbidity, dissolved oxygen ($O_{2dis.}$), oxidation-reduction potential (ORP), Conductivity, Chemical Oxygen Demand (COD), biochemical oxygen demand in five days (BOD_5), total Kjeldahl nitrogen (TKN), total phosphorus (P_{tot}) and the ratio BOD_5/COD was calculated to evaluate the biodegradability of the biomass. Thus, pH, temperature and ORP were measured by pHmeter acumet AP110, dissolved oxygen by oximeter WTW Multi 3630 IDS, conductivity by WTW Inolab con.730, turbidity by turbidimeter Lovibond serial n° 26018, biochemical oxygen demand by respirometry method using BODmeter VELP SCIENTIFICA serial n° 444925 and COD by titration after digestion using Lovibond RD125. A laboratory scale reactor consisting of a 1 L plastic container was constructed. The net volume was 0.9 L. The bottles were first filled with a mixture of wastewater and inoculum and sealed to exclude oxygen and preserve anaerobic condition. About 100 mL was extracted for analysis through a tap installed on the biodigester. Laboratory-scale anaerobic batch digesters were used in all the experiments and the biogas produced in the digesters was measured daily by the water displacement method [20]. Manual agitation was done once or twice a day on each bioreactor. A hydraulic retention time (HRT) of 48 days was chosen for this experiment, as biogas production became insignificant. To ensure a mesophilic condition (20 - 45°C) of operation [17] of anaerobic digestion in the bioreactor, a sample was taken and the temperature was measured during the retention time of wastewater in the biodigester. The pH of the wastewater in the bioreactor was also monitored during the hydraulic retention time. To study the organic pollutant reduction by anaerobic digestion, samples were taken daily and COD analyzed. The percentage removal of organic pollutants was calculated using the following equation:

$$\%Removal = \frac{C_0 - C_t}{C_0} * 100 \tag{1}$$

where C_0, C_t and C_f are the initial concentration, the concentration at time t and final concentration of COD in the digester, respectively, expressed in mg/L.

The anaerobic digestion process is significantly affected by parameters such as pH, temperature, organic loading rate, hydraulic retention time and carbon-to-nitrogen (C/N) ratio [17].

2.2. Effect of Initial Concentration of COD

Biogas production in the anaerobic digestion process is related to the substrate organic matter content. Therefore, biogas production with different substrate organic matter contents was studied. Three reactors R_1, R_2 and R_3 were filled with wastewater without inoculum. The initial COD concentrations of the wastewaters are 2,400; 3,100 and 4,000 mg/L for reactors R_1, R_2 and R_3, respectively. The experiments were carried out under batch condition and biogas production was measured daily by water displacement [20] for 48 days.

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2.3. Effect of Inoculum

Several major factors affect the efficiency of anaerobic digestion [27]. The inoculum was obtained from a brewery wastewater treatment plant as an anaerobic activated sludge and stored in a sealed bottle at room temperature [28]. Before anaerobic digestion, the activated sludge was characterized. pH and total volatile solid (VS) were measured and the COD/VS ratio was used to study the influence of the inoculum [6]. The substrate/Inoculum (S/I) ratio expressed as COD/VS has an important influence on biogas production [22]. Accordingly, four different biodigesters (R_1, R_2, R_3, R_4) were used with (S/I) ratios of 0.337, 0.505, 1.011 and 2.028, respectively, and a fifth one (R_5) as a control unit containing the wastewater without inoculum. In order to evaluate the net biogas production from the slaughterhouse wastewater, inoculum alone was used as control and the biogas produced from this inoculum was extracted from the sample assays [19]. The volume of biogas produced in reactors R_1, R_2, R_3, R_4 was then corrected.

2.4. Effect of COD/TKN Ratio

The COD/TKN ratio plays an important role in the performance of biogas production in the anaerobic digestion process. The performance of biogas production can be limited by an improper carbon to nitrogen (C/N) ratio [21]. Substrates with low COD/TKN ratios will have an inhibiting effect on biogas production in the anaerobic digestion process [29]. The effect of COD/TKN ratios on the performance of biogas production was studied fixing the TKN and varying the COD concentration. The variation of the COD/TKN ratio was performed by adding sodium acetate (CH_3COONa) to the effluent in different biodigesters. Five reactors with COD/TKN ratios of 6, 14, 17, 19 and 22 were studied. No nitrogen addition and operation at natural pH was conducted, i.e., no pH adjustment. The experiment was done under batch condition as described previously. The Hydraulic retention time was 48 days and manual agitation was done once or twice a day on each bioreactor. Cumulative biogas production in each bioreactor was evaluated and specific biogas production was calculated by dividing the cumulative volume of biogas at time t by the number of initial COD in the digester.

2.5. Biogas Production and Biomethane Yield Evaluation

Biogas is a mixture of methane (CH_4), carbon dioxide (CO_2) and hydrogen sulfide (H_2S) [30]. Two biodigesters R_1 and R_2 were used. The two digesters contained a mixture of 800 mL of slaughterhouse wastewater and inoculum with an S/I ratio of 1. The liquid displacement method was used. Therefore, a graduated cylinder was filled with a barrier solution and inverted in a reservoir. As biogas was produced, it was passed through the liquid vessel and displaced an equivalent liquid volume. A bottle containing a concentrated solution of NaOH (3M) [23] was inserted between the biodigester (R_2) and the graduated inverted cylinder. The two digesters were operated under the same conditions. There is no pH adjustment and the mesophilic condition was imposed. The volume of water displaced in the biodigester R_1 is considered the volume of biogas, while displacement in the biodigester R_2 is considered the volume of methane. The percentage of methane in the biogas produced is calculated as:

$$\%Methane = \frac{V_{methane}}{V_{Biogas}} * 100 \tag{2}$$

where: $V_{methane}$ is the cumulative volume or specific methane production (L or L/gCOD);

V_{Biogas} is the cumulative volume or specific biogas production (L or L/gCOD).

2.6. Evaluation of Biogas Calorific Value

The energetic potential of slaughterhouse wastewater was estimated taking into account the energy equivalent of the total biogas that can be generated per annum. Considering Vd and t respectively the average volume of slaughterhouse wastewater per day and the number of working days in the year of the slaughterhouse site, the value of COD per annum generated (χ) is calculated as:

$$\chi = A \times V_d \times t \quad (3)$$

where χ is expressed in g, A the average COD concentration of the slaughterhouse wastewater (g/L), V_d in L and t in days. The energy equivalent of 1 m^3 of biogas with 60% methane is 19.7 MJ/m^3 [31]. Taking into account this equivalence, the energy equivalent of 1 m^3 of biogas in this study was evaluated. The energy potential (Ep) of slaughterhouse wastewater was evaluated according to equation (4).

$$E_p = \varepsilon \times SB \times A \times V_d \times t \quad (4)$$

where SB is the specific biogas production (m^3/g COD), Ɛ is the energy equivalent of 1 m^3 of biogas in MJ/m^3, Ep the total energy equivalent in MJ.

2.7. Kinetics of Biogas Production

Three of the most common kinetic models were applied: First order kinetic model (5), Modified Gompertz model (6) and Logistic function model (7). The cumulative biogas production obtained from experimental data were fitted to these models [18]. According to the authors, each kinetic model has a specific benefit. The first order kinetic was used to predict biogas production, while the Modified Gompertz model was used to calculate the lag phase [32].

$$B_t = B_0 \times (1 - e^{-k.t}) \quad (5)$$

$$B_t = B_0 \exp\{-\exp[\frac{R_m \cdot e}{B_0}(\lambda - t) + 1]\} \quad (6)$$

$$B_t = \frac{B_0}{1 + \exp\ (4R_m \frac{\lambda - t}{B_0} + 2)} \quad (7)$$

where, B_t = cumulative biogas production in mL, R_m = maximum biogas production rate (mL d^{-1}), e = 2.718, λ = lag phase time (d), B_0 is the maximum biogas potential of the substrate (mL) and k is the hydrolysis rate constant (day^{-1}).

2.8. Kinetics of Biodegradation

The biodegradation of substrate is related to COD removal rate from the anaerobic digestion process. The anaerobic digestion of most substrates can be described using first-order reaction kinetics according to the following equations [31]:

$$-\frac{dY_t}{dt} = \mu Y_t \quad (8)$$

with the linear form:

$$Y_t = Y_0 e^{-\mu\ t} \quad (9)$$

where Y_t is the amount of organic matter at time t, Y_0 is the initial amount of biodegradable organic matter, and μ is the reaction rate constant. By plotting ln (Y_t) versus t, the constant μ was determined from the slope.

3. Results and Discussion

3.1. Characteristics of the Slaughterhouse Wastewater

The slaughterhouse wastewater characteristics were summarized in table 1. High content in organic matter can be observed. The values of all the parameters analyzed were in the ranges of those reported by C. F. Bustillo-Lecompte and M. Mehrvar [33] for slaughterhouse wastewater. The pH value found is similar to the pH value of drinking water, making the slaughterhouse wastewater a suitable media of microorganisms' development. In addition, the dissolved oxygen value is too weak showing the development only of anaerobic microorganisms in this kind of wastewater. As anaerobic digestion is a biologic treatment, attention was paid on the ratio BOD_5/COD. The BOD_5/COD ratio of 0.59 was found. This ratio is a good condition for the biological treatment of the target wastewater.

Table 1. *Physico-chemical Characteristics of the Slaughterhouse Wastewater.*

Parameters	Units	Value	SD
pH	-	6.48	0.3
Temperature	°C	31.2	2
EC	µS/cm	3,307	706
Turbidity	NTU	315	100
ORP	mV	8.6	23
O_{2dis}	mg O_2/L	0.037	0.01
COD	mg O_2/L	3,200	800
BOD_5	mg O_2/L	1,900	458
TKN	mg/L	504	26
P_{tot}	mg/L	140	59

3.2. Effect of Initial COD

Organic matter content plays an important part in biogas production in the anaerobic digestion process. The result of the initial COD concentration on biogas production (figure 1) revealed a higher cumulative volume of biogas (870 mL) obtained from a higher concentration (4,000 mg/L), while a lower cumulative volume of biogas (205 mL) was obtained from the lower concentration (2,400 mg/L). Filer et al. [15] reported the same findings. By varying the mass of COD from 0.1 to 3 g per gram of inoculum, the authors reported an increasing cumulative volume of biogas as COD increased. In fact, methane production is proportional to the amount of COD with a coefficient of 0.350 as 1 kg of COD is considered

[15]. According to Raposo et al. [6], biomass expressed as the content of COD in the effluent is considered as potential source for biogas production. Thus, an increase of the COD in the biodigester will increase the biogas production.

The same effect was observed on the specific biogas production. A specific biogas of 250 mL/gCOD and 100 mL/gCOD were obtained respectively for 4,000 mg/L (the highest concentration) and for 2,400 mg/L (the lowest concentration). The cumulative volume of biogas and specific biogas production were proportional to the initial COD concentration of the wastewater (figure 2). Similar results were reported by Raposo et al. [6].

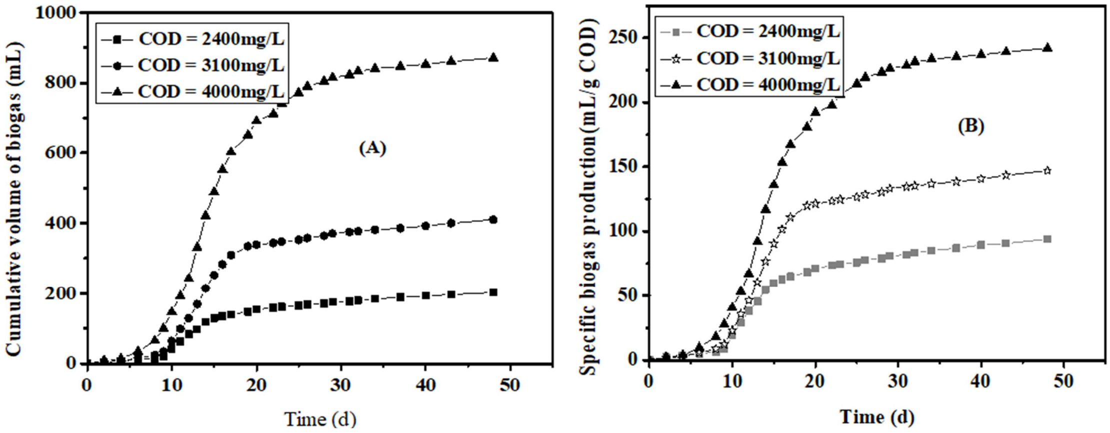

Figure 1. *Cumulative volume of biogas (A) and specific biogas production (B) as a function of time at different initial COD concentrations in wastewater.*

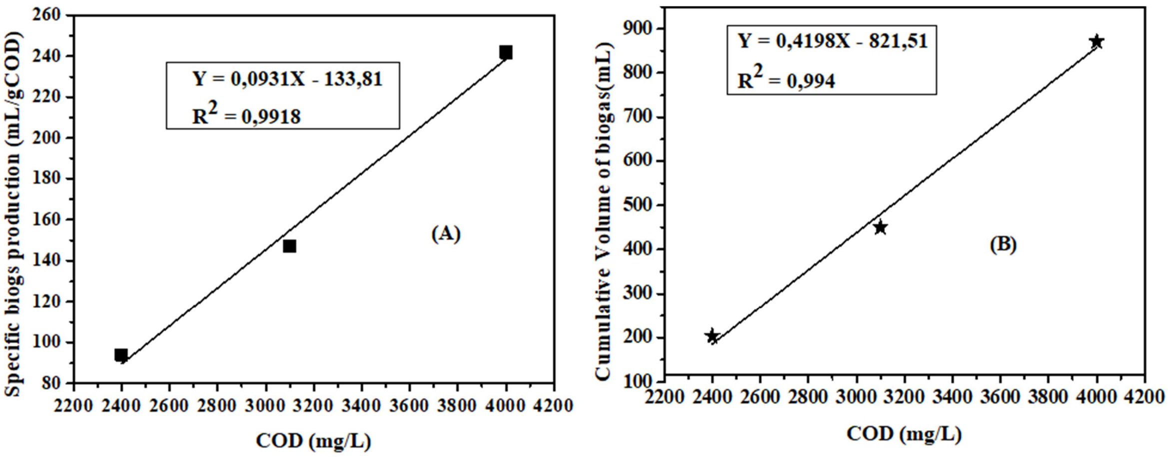

Figure 2. *Specific biogas production (A) and cumulative biogas production (B) as a function of initial COD concentration in the wastewater.*

3.3. Effect of the Inoculum

The activated sludge is characterized by a pH of 7.2 and total volatile solid (VS) of about 98,000 mg/L. Figure 3 shows the effect of the inoculum on biogas production. The cumulative volumes of biogas are 2,150 mL, 1,480 mL, 1,070 mL and 415 mL, respectively, for the ratios of R_1 = 0.337, R_2 = 0.505, R_3 = 1.011, and R_4 = 2.028 after 48 days of digestion. By dividing the cumulative volume of biogas produced after 48 days of digestion by the initial COD content, the specific biogas productions are 745 mL/gCOD, 513 mL/gCOD, 372 mL/gCOD, 142 mL/gCOD, respectively, for the ratios of R_1 = 0.337, R_2 = 0.505, R_3 = 1.011, and R_4 = 2.028.

The cumulative volume and specific biogas production increased when the S/I ratio decreased. In fact, as the ratio decreased, the quantity of microorganisms in the digester increased. Therefore, there are enough microorganisms to degrade the organic matter, enhancing its conversion to biogas. A similar finding was reported by Lawal et al. [34] on increasing biogas production with increasing inoculum dose. In addition, Figure 3 shows that the biogas production rate tends to obey a sigmoid function (S curve) as is generally observed with batch growth curves [35]. Furthermore, the results summarized in figure 3 show that, regardless of the ratio, there is no significant biogas production in the first 5 days. This duration corresponds to the lag phase, i.e., the minimum time of adaptation and acclimatization of micro-organisms in the biodigesters. During this period, there is no significant microorganism activities leading to significant cumulative volume of biogas. Widiasa et al. [35] reported a lag phase of 12 days in biogas production using anaerobic digestion in batch mode. The difference observed in the lag phase will probably be due to the nature of the substrate. In fact, Widiasa et al. [35] used cattle manure as substrate, while effluent (wastewater) was used in the present study. Using liquid effluent as substrate presents a short start-up time advantage [31].

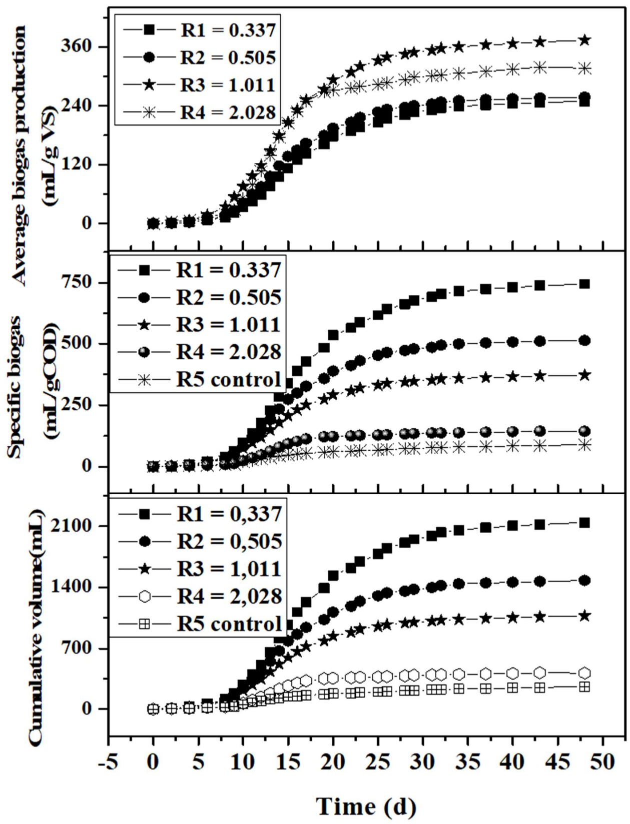

Figure 3. *Biogas production as a function of time for different substrate of inoculum ratios.*

Figure 3 also shows an average biogas production per gram of inoculum added. The ratio 1.011 presents the higher biogas production, while the ratio of 0.337 leads to the lower biogas production. In fact, the inoculum dose increases while the substrate remains constant. Therefore, there is enough inoculum for a few substrates to degrade or to convert into biogas leading to a lower biogas production per gram of inoculum as the dose of inoculum is increased.

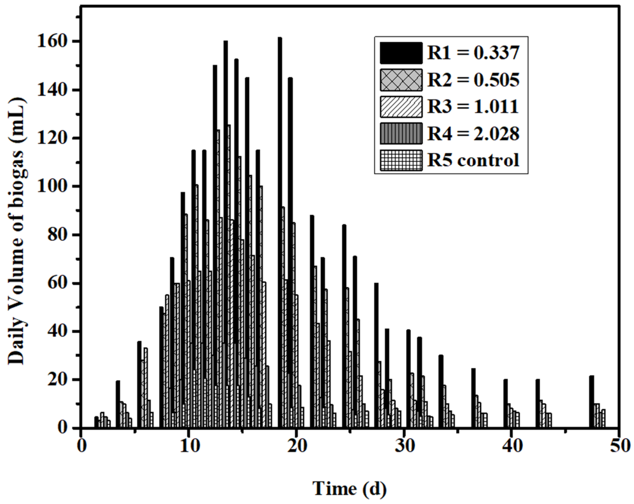

Figure 4. *Daily biogas production with different substrate to inoculum ratios as a function of time.*

The daily biogas production reveals insignificant difference between the control digester and the inoculated digesters for the first two days. But after the second day, the daily biogas production increased with the dose of inoculum while the control digester remains similar from the first to the nineth day. The daily biogas production (figure 4) shows a maximum production between the 10th and 20th days and after which daily biogas production decreased to very low values despite the ratio. In fact, the inoculation will increase the carbon availability in the effluent, helping the C/N ratio adjustment. Thus, the improvement of the C/N ratio helped to activate microbial activities early in the digesters [36].

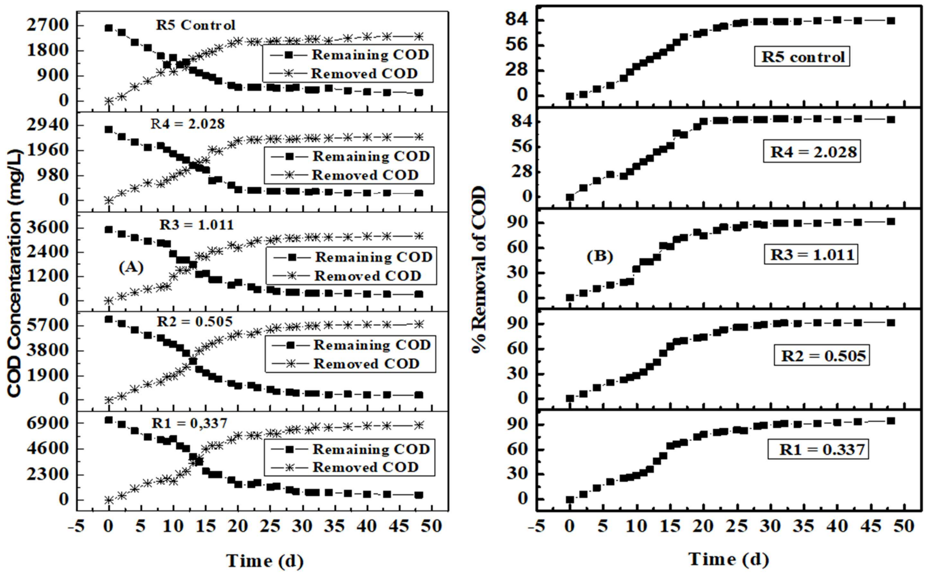

Figure 5. *Evolution of removed COD and remaining COD (A) and percentage of COD removal (B) as a function of time for different substrate/inoculum (S/I) ratios.*

COD removal in the digesters was studied. The results (figure 5-A) show an increasing COD removed with time. The degradation rate of the organic matter (COD) is higher at the beginning of the experiment (0 – 20 days) and after this period, it decreased until stationary values were reached regardless of the substrate to inoculum ratio. Marcos et al. [30] reported a similar observation in the reduction of COD in the anaerobic digestion process. As the substrate/inoculum ratio decreased, the percentage of COD removal increased (figure 5-B). A high percentage of 93% was obtained for the ratio of 0.337, while the ratio of 2.028 gave about 84% of COD removal after 48 days of digestion. The comparison of the percentage removal shows a slight increase from the ratio of 2.028 to 0.337, while the control digester (R_5) presented a lower percentage of about 83%. The inoculation or the co-digestion of slaughterhouse wastewater enhanced the efficiency of the degradation of organic matter in anaerobic digestion. In fact, the lowest ratio gave the highest anaerobic microbial populations. Therefore, as the anaerobic microbial populations consume carbon, a higher carbon consumption was observed leading to a high COD percentage reduction.

pH and temperature were monitored during the hydraulic retention time to ensure optimum operating condition in the ranges of 6 - 7 and 25 - 30°C, respectively.

3.4. Effect of C/N Ratio

The C/N ratio illustrated here by COD/TKN can significantly affect the performance of co-digestion. pH value and concentrations of total ammonium nitrogen (TAN) can be influenced by C/N ratios [29]. The evolution of pH during the digestion with different ratios is shown in figure 6-A. Despite the ratio and the time of digestion, pH values were comprised between 6.5 - 8.5, ideal condition for good anaerobic digestion [37-38]. There is an increase in the final pH in the digester comparatively to the initial pH in the digester (Figure 6-A). The same observations were reported by Dhar et al. [13] and Nurliyana et al. [17]. This situation can be explained by the fact that microorganisms produce alkalinity as they consume protein-rich organic matter [21].

An increase in pH is the result of weak volatile fatty acid (VFA) production during acidogenesis, which enhances the growth of methanogenic bacteria [27].

The evolution of temperature in the digester is plotted in figure 6-B. As temperature is comprised between 25°C and 30°C, co-digestion is then in the mesophilic condition [17]. The efficiency of anaerobic digestion is strongly dependent on temperature. According to Membere et al. [19], operation in mesophilic conditions makes the anaerobic digestion more efficient than operation in thermophilic conditions. The operation conditions (temperature comprised between 25°C - 30°C and between 6.5 - 8.5) in this study lead to a low free ammonia concentration resulting in its inhibition effect minimization [39]. According to these authors, the free ammonia concentration increases with increasing temperature and pH values which leads to the inhibition of microorganisms' activities.

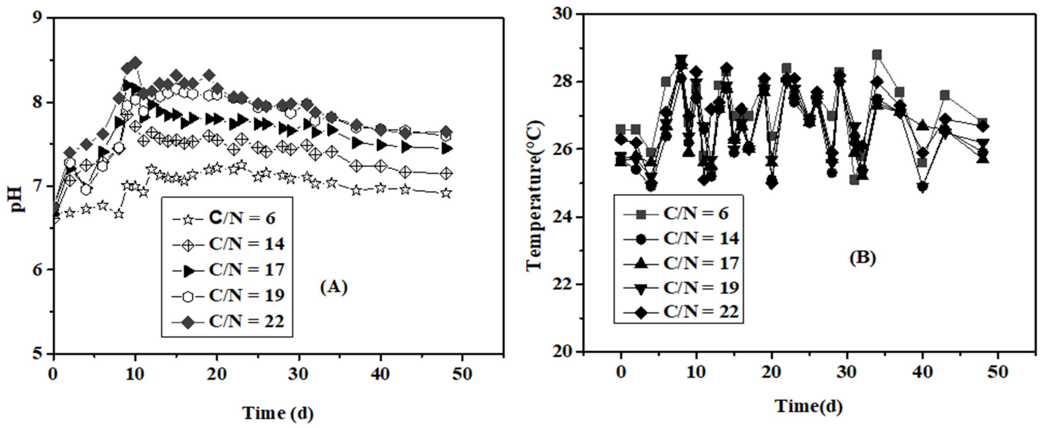

Figure 6. *pH variation (A) and temperature variation (B) during anaerobic digestion.*

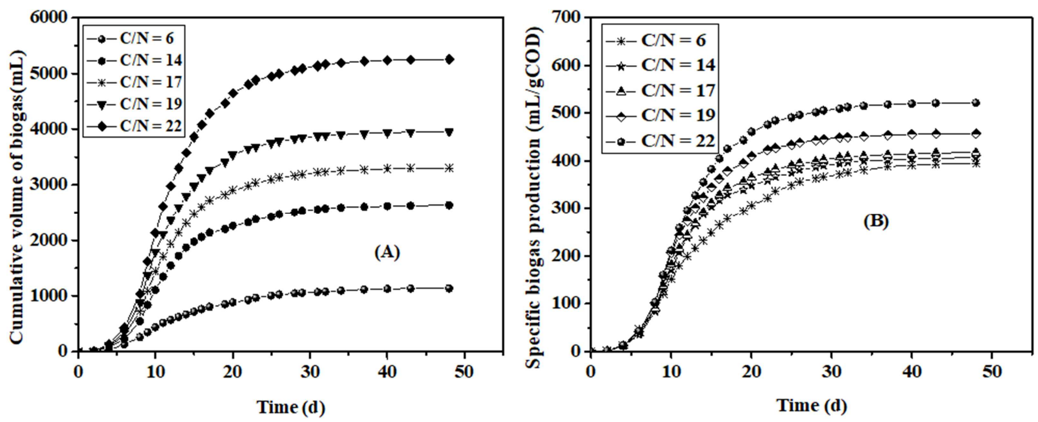

Figure 7. *Biogas production for different C/N ratios as a function of time. (A) cumulative volume of biogas (B) specific biogas production.*

Deepanraj et al. [20] reported efficiencies of anaerobic digestion in mesophilic condition. In fact, in their study they found an increase of the biogas produced as temperature was increased from 30 to 50°C and followed by a decrease in the performance of the system. On the other hand, Raposo et al. [40] reported no dependence on temperature of anaerobic digestion, but an increasing rate constant with an increasing temperature. According to Appels et al. [16], an excessive increase of temperatures (thermophilic) will increase the fraction of free ammonia, which plays an inhibiting role for the microorganisms. An increasing temperature is able to increase pKa of the VFA which will make the process more vulnerable to inhibition.

The effect of the C/N ratio on the performance of biogas production is shown in figure 7. The cumulative volume (mL) and specific biogas (mL/gCOD) production increased as the ratio increased. The cumulative volumes of 5,250 mL and 1,140 mL were obtained respectively for C/N = 22 (the highest ratio) and C/N = 6 (the lowest ratio).

The same observations were made by other authors. Panizio et al. [41] reported higher and lower values of biogas production, respectively, for C/N = 20 and C/N = 11. Nurliyana et al. [21] reported an increase in biogas production from C/N ratio of 25 to 45 of a maximum production with ratio of 45 and a decrease in biogas production for the ratios of 45 to 55. As the C/N ratio increased, carbon content increased or nitrogen content decreased. Thus, the unnecessary formation of volatile fatty acids (VFAs) and total ammonium nitrogen can be avoided by increasing the C/N ratio as the anaerobic microbial population consumes carbon 25–30 times faster than nitrogen [42].

An increase of nitrogen could have a toxic effect on methanogenic bacteria. According to Akindele and Sartaj [43], an increase of nitrogen could inhibit the utilization of COD for biogas production by the methanogenic microorganisms. These authors reported a significant effect of total ammonia nitrogen with a concentration beyond 2,500 mg/L. Therefore, a high value of C/N ratio will limit the formation of total ammonium nitrogen and its inhibitor effect, this will favor methanogenic bacterial activity for optimal biogas production. In contrast; at low ratio, anaerobic microbial population consumes nitrogen as carbon is insufficient. As consequence, there is an unnecessary formation of ammonium (NH_4^+) and ammonia (NH_3), which inhibit methanogenic activity [43].

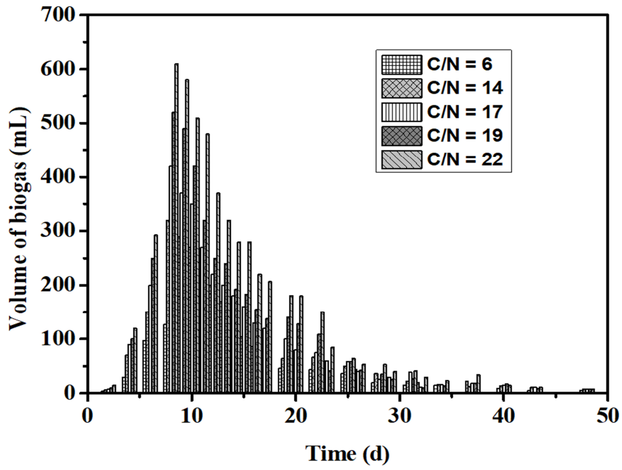

Figure 8. *Daily biogas production at different C/N ratio as a function of time.*

The daily biogas production (Figure 8) also shows a maximum between 10 and 15 days for all ratios. The C/N ratio of 22 shows a higher biogas production of 600 mL at the maximum production day (9th day). By combining inoculation and the C/N ratio adjusted, the performance of biogas production was enhanced. Its kinetic production was also increased and the maximum biogas production was obtained the day 10 for most of the ratios. Without the adjusted C/N ratio, this maximum biogas production was around the day 15 when inoculation was used at C/N ratio of 5.

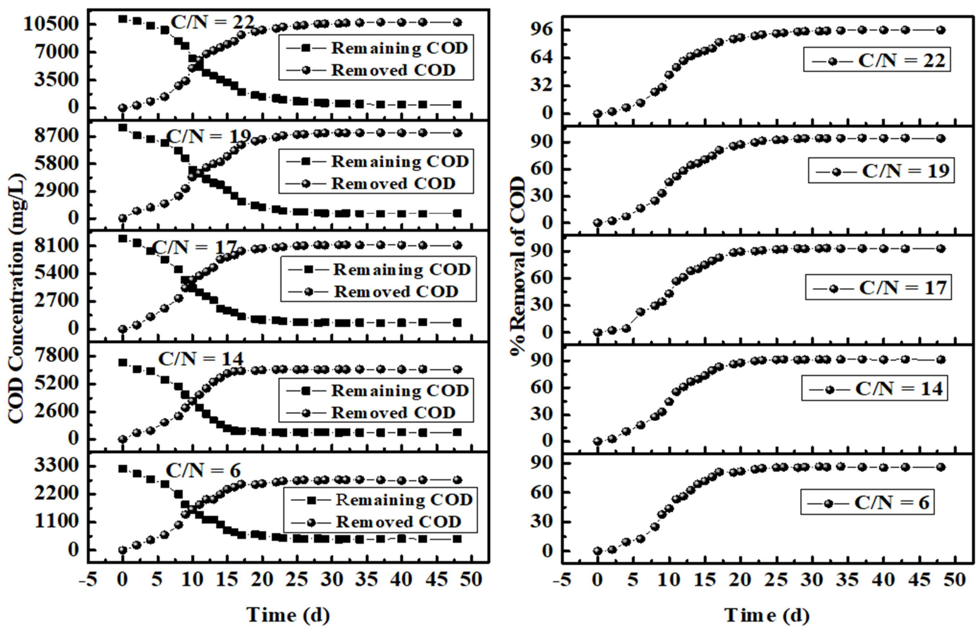

Figure 9. *Evolution of COD removed, remaining COD and percentage removal as a function of time for different C/N ratios.*

The COD removal from the digesters was evaluated for different C/N ratios. The results (Figure 9) show increasing COD removed with time. The degradation of organic matter (COD) is greater at the beginning of the experiment (day 0 – 15) and after this period, it decreased until stationary values were reached regardless of the C/N ratio. A high percentage of 96% was obtained for a ratio of 22 while the ratio of 6 gave about 86% of COD removal after 48 days of digestion.

3.5. Biogas Production and Biomethane Yield Evaluation

Figure 10 shows the cumulative volume, specific biogas and methane yield during 48 days of co-digestion with C/N ratio of 10 and S/I ratio of 1. As biogas volume increased, methane volume also increased as well as the specific production. The maximum biogas produced was about 2,320 mL, while the maximum methane was around 1,750 mL at the end of the operating time of 48 days.

The specific biogas and methane production for the same period were about 810 and 610 mL/gCOD, respectively. The average specific productions were 560 mL/gCOD and 419 mL/gCOD for biogas and for methane, respectively. The percentage of methane in the biogas produced increased from the first day to the 25th day, whereas the highest percentage found was about 78% (15th day). After this period, the percentage of methane decreased to 75% and remained constant from the 25th day to the 48th day. The biogas produced contains 60 – 78% of methane with an average of 74%. This percentage of methane in biogas is similar to the percentage reported by Zawieja et al. [44]. In fact, these researchers reported a higher percentage of 77% of methane in the biogas produced from sonicated excess sludge.

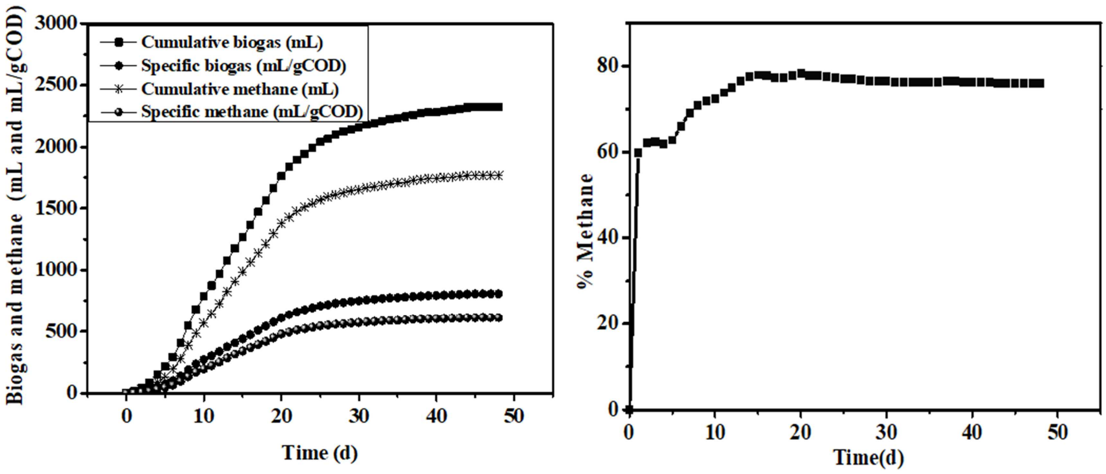

Figure 10. *Methane production as a function of time (ratio S/I = 1 and C/N = 10).*

3.6. Evaluation of Biogas Calorific Value

The average percentage of methane in the biogas produced is around 74%. The energy equivalent of 1 m^3 of this biogas with 74% methane is equal to 24.3 MJ/m^3. This energy equivalent value is within the range of those reported by Forster-Carneiro et al. [45]. According to these authors the energy equivalent of 1 m^3 of biogas is comprised between 19 and 25 MJ/m^3. The calorific value of biogas estimated from co-digestion of the slaughterhouse wastewater collected in the Lomé harbor area is summarized in table 2. Admittedly, the slaughterhouse located in the harbor area consumes up to 250 m^3 of freshwater and generates approximately the same quantity of wastewater on daily basis. An efficient management of the slaughterhouse wastewater can generate about 9.735×10^5 MJ or 270,417 KWh equivalent per annum.

Taking into account the Standard biomass-electricity-only systems efficiency of 20% reported by Liu et al. [46], the electricity transformed using the slaughterhouse wastewater energy potential Ep of 270,417 KWh was about 54,083 KWh. Therefore, the electricity produced from the slaughterhouse wastewater by anaerobic digestion can cover about 19.2% of the total electricity need per annum on the site. This percentage was obtained by dividing the electricity transformed by the average consumption of the slaughterhouse per year. In fact, the slaughterhouse site can use 22,000 – 25,000 KWh per month or 264,000 – 300,000 KWh per annum for internal consumption.

Table 2. *Annual estimated energy from slaughterhouse wastewater.*

Wastewater volume per day (L)	Days of activity per annum	COD Concentration (g/L)	Specific Biogas (m^3/g COD)	Estimated Energy Ep (MJ)/(KWh)
$250*10^3$	313	3.200	$160*10^{-6}$	$9.735*10^5$/270,417

3.7. Kinetics Study

Three kinetic models: first-order kinetics, modified Gompertz and logistic function models were used to estimate the maximum biogas production rate (mL d^{-1}), the lag phase time (d), the maximum biogas potential of the substrate (mL), and the hydrolysis rate constant (1/day). Figure 11 shows the fit between the experimental values and those obtained when Gompertz (Figure 11-A) and logistic function (Figure 11-B) models are used. The modified Gompertz model in figure 11-A seems to be closer to the experimental values than the logistic function model. The hydrolysis rate constant k (1/day) calculated from the first-order kinetic model was within the range of 0.106 and 0. 158. Apart from the hydrolysis rate constant k (1/day) obtained for the ratio of C/N = 6, all the k values fell within the range of 0.13 - 0.56, as reported by Mao et al. [47] for good degradation and biogas production. In fact, k describes the velocities of degradation and biogas production [47]. Therefore, a high value of k is synonymous with rapid degradation and biogas production. The hydrolysis rate constant obtained from first-order model was increased as the ratio C/N increased. In fact, the addition of sodium acetate (CH_3COONa) to the effluent as a high degradable matter accelerated the microbial growth and the hydrolysis rate [5]. As a consequence of this hydrolysis rate increase, acidification and methanogenesis were improved resulting in a higher biogas production.

The kinetic parameters estimated for the three models are summarized in table 3. The predicted values of biogas are closed to the experimental values for all C/N ratios using the first-order kinetic model. However, a very weak correlation with R^2 values between 0.552 and 0.704 was observed for all ratios, showing an unsuitable fit of the first-order kinetic model.

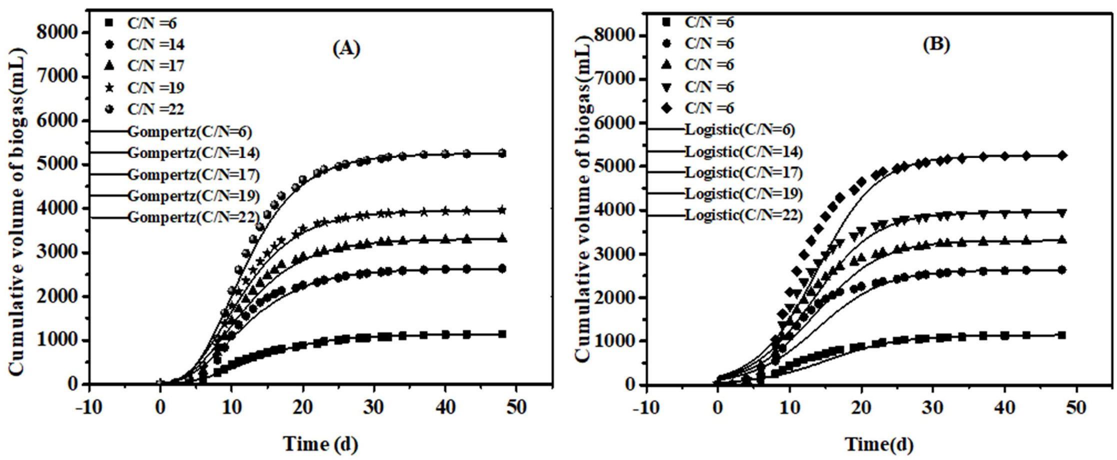

Figure 11. *Comparison between measured data and calculated data (full line) for cumulative biogas production using the modified Gompertz model (A) and the Logistic model (B) at different C/N values.*

The same findings were obtained by Pramanik et al. [18] on the fit of the first-order kinetic model. These authors reported a close predicted to experimental values with a relatively low R^2 (0.886). The predicted cumulative biogas value computed with the logistic model is closed to the experimental value, with a relatively high correlation of R^2 between 0.902 and 0.928 indicating a relatively good fit of the logistic model. The lag phase obtained from the modified Gompertz model was between 3.39 and 4 days. Similar lag phase with modified Gompertz model was reported by Ebrahimi-Nik et al. [32] in the anaerobic digestion of a mixture of drinking water sludge and food waste. The Maximum biogas rate Rm (mL/d) determined with the modified Gompertz model increased from 64.6 to 363 as the C/N ratio was increased from 6 to 22. The predicted cumulative volume of biogas production is closed to the experimental cumulative volume, with a low standard deviation (SD) and high correlation between 0.983 and 0.993. The R^2 value for the predicted volume of biogas using the modified Gompertz model was significantly higher, while the SD was lower compared to the first-order kinetic model and the logistic function model, for all C/N ratios. The Modified Gompertz model is more suitable for describing biogas production in the anaerobic digestion process followed by the logistic model. Similar findings were reported on the fit of the Modified Gompertz model for describing the kinetics of biogas production in an anaerobic digestion process [36, 48-49].

Table 3. *Estimated kinetic parameters for the three kinetic models of biogas production.*

	C/N = 6	C/N = 14	C/N = 17	C/N = 19	C/N = 22
Cumulative volume of biogas measured (mL)	1,140	2,640	3,310	3,950	5,250
Gompertz model					
Maximum biogas potential, Bo (mL)	1,136±17	2,635±45	3,305±46	3,953±47	5,256±55
Cumulative volume of biogas predicted (ml)	1,132.6±7.1	2,631±23.9	3,301±24.3	3,950±25.2	5,252.6±30.2
Maximum biogas rate Rm (mL/d)	64.6±0.13	163.4±0.6	212.8±0.5	268.1±0.5	363.1±0.59
Lag phase λ(d)	4±0.23	3.53±0.28	3.47±0.23	3.39±0.20	3.59±0.17
R^2	0.989	0.983	0.988	0.990	0.993
Logistic model					
Maximum biogas potential Bo (mL)	1,136±42	2,635±106	3,305±121	3,953±133	5,256±174
Cumulative volume of biogas predicted (mL)	1,135±32.5	2,633±89.4	3,303±100.7	3,952±110.8	5,255±145.2
Lag phase λ(d)	2.1±0.74	1.92±0.79	1.88±0.72	1.83±0.65	1.89±0.64
Maximum biogas rate Rm (mL/d)	57.5±0.28	144±0.95	185.4±1.04	230.7±1.14	312.7±1.5

	C/N = 6	C/N = 14	C/N = 17	C/N = 19	C/N = 22
R^2	0.928	0.902	0.915	0.923	0.928
First-order kinetic					
Maximum biogas potential Bo (mL)	1,143±79.8	2,637.5±178	3,308±220.5	3,955±262.4	5,259.5±369
Cumulative volume of biogas predicted (mL)	1,136±60.9	2,635±151.9	3,305±188.2	3,953±238.6	5,238±335.5
Hydrolysis rate constant k (1/day)	0.106±0.03	0.145±0.04	0.147±0.04	0.158±0.05	0.152±0.05
R^2	0.704	0.593	0.601	0.574	0.552

The biodegradability kinetic of slaughterhouse wastewater in anaerobic digestion was studied using first-order reaction kinetics according to the equation (9). The results obtained are summarized in table 4. These results show a relatively high correlation (R^2) between 0.9075 and 0.9603 as the ratio of substrate to inoculum decreased from 2.028 to 0.337, and a very weak root mean square error (RMSE) also decreasing in the range of S/I ratio. The first-order reaction kinetics can describe the biodegradability of the slaughterhouse wastewater. Furthermore, analysis of the evolution of R^2 reveals an improvement of biodegradability as inoculum dose was increased in the digester. The constant μ (d^{-1}) in the equation (9) describes the velocities of biodegradability. Therefore, an increase in this constant is synonymous with fast biodegradability and rapid biodegradable organic matter removal from the biodigester. The constant μ (d^{-1}) increased from 0.0628 to 0.0785 as the dose of inoculum was increased (decrease of S/I ratio) showing that inoculation can accelerate biodegradability giving a rapid removal of organic matter.

Table 4. *Constant of biodegradability kinetics of slaughterhouse wastewater.*

S/I Ratio	Equations	μ (d^{-1})	R^2	RMSE
R_1 = 0.337	y = - 0.0726x + 9.0297	0.0785	0.9603	0.03632
R_2 = 0.505	y = - 0.0785x + 8.8619	0.0726	0.9493	0.05483
R_3 = 1.011	y = - 0.0691x + 8.2249	0.0691	0.9312	0.05867
R_4 = 2.028	y = - 0.0628x + 7.9419	0.0628	0.9222	0.05540
R_5 Control	y = - 0.0504x + 7.6452	0.0504	0.9075	0.04307

4. Conclusion

Focusing on the bioenergy potential evaluation, this study brings out the benefit of slaughterhouse wastewater treatment by anaerobic digestion process. An efficient design of biodigesters can remove about 90% of organic matter. Slaughterhouse wastewater is a source of energy, as a sustainable approach for converting waste mass into biogas containing methane was found. Biogas produced from slaughterhouse wastewater can be considered as having a high calorific value because the methane yield can reach 74%. Therefore, effective slaughterhouse wastewater management can theoretically be a sustainable solution to the energy crisis and climate change problem facing the world.

However, the efficient slaughterhouse treatment by anaerobic digestion process is a non-negligible sludge production that have to be taken into account. This kind of sludge is hardly biodegradable with complex organic matter. Then, pretreatment processes have to be used to enhance the efficiency of the hydrolysis step in solubilization of the complex organic matter.

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