

RESEARCH ARTICLE

Anaerobic co-digestion cow dung and corn stalk - effect of corn stalk pre-treated timing

Đồng phân hủy yếm khí phân bò và thân bắp - Ảnh hưởng của thời gian tiền xử lý thân bắp

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The study was aimed to investigate the effect of corn stalk pre-treatment duration on biogas production when cow dung and corn stalk was co-digested in an anaerobic digestion. Corn stalks were pre-treated in different durations: 2-days, 5-days, and 8-days before being added to cow dung into anaerobic co-digesters. The experiments were set up randomly by using triplicate batch anaerobic apparatus in 21 L containers that run in 60-days. The mixing ratio between a corn stalk and cow dung was 50%: 50% (based on the volatile solid value of each material), but corn stalk was cut into small pieces with around 10 cm length, while the cow dung was air dried. The results of the study indicated that all operation parameters such as temperature, pH, and alkalinity in the anaerobic batch were suitable for biogas production. The results showed that there was a significant improvement in total gas produced in the pre-treated 5-days treatment (206.4±8.4 L) compared to 2-days (153.4±9.6 L), and 8-days ones (174±11.1 L). The biogas yield of the pre-treated 2-days, 5-days, and 8-days treatments were 392.7±9.8 L/kg VS_{fermented}, 469.8±10.1 L/kg VS_{fermented} and 497.1±13.3 L/kg VS_{fermented}, respectively, that was not significantly different (5%). In all treatments, low concentration of methane in the beginning phase had been observed but increased and reached the optimum value for energy use after 10 days. The result of the study showed that it is preferable to have 5-days pre-treatment of corn stalk before the corn stalk is loaded to an anaerobic digester in combination with cow dung.

Nghiên cứu này nhằm đánh giá ảnh hưởng của thời gian xử lý thân cây bắp lên năng suất sinh khí khi ủ phối trộn phân bò và thân cây bắp trong điều kiện yếm khí. Ba mức thời gian xử lý thân cây bắp được chọn là 2 ngày, 5 ngày, và 8 ngày. Các thí nghiệm được bố trí ngẫu nhiên trong các bình ủ yếm khí theo mẻ 21 L, vận hành trong 60 ngày liên tiếp và có 3 lần lặp lại. Nguyên liệu ủ được phối trộn theo tỷ lệ 50% phân bò và 50% thân bắp, trong đó thân bắp được cắt nhỏ cỡ 10 cm. Kết quả thí nghiệm cho thấy tất cả các thông số pH, nhiệt độ, độ kiềm của mẻ ủ đều phù hợp để vận hành. Lượng khí sinh ra của các nghiệm thức xử lý ở 2 ngày, 5 ngày, 8 ngày được ghi nhận là 153,4±9,6 L, 206,4±8,4 L và 174±11,1 L; năng suất sinh khí của các nghiệm thức không khác biệt và đạt giá trị 392,7±9,8 L/kg VS_{phân hủy}, 469,8±10,1 L/kg VS_{phân hủy} và 497,1±13,3 L/kg VS_{phân hủy}. Tất cả các nghiệm thức đều sản sinh lượng CH₄ thấp ở giai đoạn đầu nhưng tăng dần theo thời gian và đạt hiệu quả sử dụng sau 10 ngày ủ. Kết quả cho thấy có thể chọn mốc thời gian 5 ngày để xử lý thân cây bắp trước khi đưa vào hầm ủ biogas.

Keywords: batch digestion, biogas, corn stalk, cow dung, pre-treatment

1. Introduction

Agriculture is a key economic sector of the Mekong Delta (MD). However, local authorities have paid a little attention to developing this sector. Livestock production has received little attention because it is mostly carried out by small farmer households and not concentrated in a big area. Therefore, livestock production is characterized by low profitability. In recent years, MD farmers have been increasingly aware of the restructuring of husbandry activities from traditional species of cattle like breeding and dairy cows. According to the General Statistics Office of Vietnam - GSO (2016), the Delta has nearly 5.5 million cows,

accounting for 12.95% of all cows in Vietnam. There are more than 32,000 milk cows in MD, representing 11.31% of the total milk cows in Vietnam. The number of cows is increasing in recent years in both sectors of livestock husbandry projects and this makes an increase in farmer households' income and profitability of raising livestock. Nevertheless, the number of livestock in each farmer household is small and there is no strategy to collect the manure from the increasing number of scattered stables. Thus, a problem arising in the MD is how to collect and use the waste of all stables in a proper way and to reduce environmental pollution caused by such waste.

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Corn is a main agricultural crop in the MD with the cultivated land of 381,000 hectares (GSO, 2016). The corn is not only used as food for human and animal, but it is also used as raw material for industrial sector (Ngo Huu Tinh et al., 1997). After corn kernel is harvested, a small quantity of corn stalk could be used as feed for animals. Farmers normally burn a large quantity of corn stalk in order to save time and to be able to use the field for a next crop. This practice causes air pollution and a loss of valuable organic material. In the meantime, corn stalks could be used with high efficiency as organic material for biogas production.

As mentioned by Ofoefule et al. (2009), the anaerobic digestion of water hyacinth always needs more time than that of animal dung. Both high values of C: N ratio and lignocellulose nature of corn stalk can influence the digestion duration in an anaerobic digester (USDA, 2011). Therefore, corn stalk should be pre-treated before feeding to an anaerobic digestion process. In the previous study, Nguyen Le Phuong et al. (2015b) mentioned that co-digestion of cow dung and corn stalk together produces more biogas in comparison to a digestion of only cow dung. Yang et al. (2003) reported that the biogas production is increasing by 33.7% when biological method pre-treated corn stalk was used. This study was aimed to investigate the effect of various corn stalk pre-treating time (by effluent from an anaerobic digester) on biogas production when cow dung and corn stalk was co-digested in an anaerobic process.

2. Methodology

2.1 Input materials

Cow dung (CD) was collected from a cow farm at Long Hoa Ward, Binh Thuy district, Can Tho city. The collected dung was air dried to avoid nutrient loss, then ground (by straw cutter MB TTP 100 with a capacity of 100 kg/hour) and mixed to form a homogeneous material.

Corn stalk (CS) was collected from a corn farm, air dried and cut (by a paper-cutter) into particles with from 8 to 10 cm length. The corn stalk was pre-treated with effluent from a biogas digester; three durations of the pre-treatment of CS 2-days, 5-days, and 8-days were tested, and the CS was loosened and mixed daily.

The inoculum was collected from the biogas digester that was operating by cow dung at the same site. The inoculum was used as (i) liquid to pre-treat corn stalk, and (ii) adding only one time to each treatment at the starting date as seeding for the digestion process.

2.2 Experiment setup

Digester: an anaerobic digester designed for this experiment composing of a PVC bottle with 21 L volume was used. The bottle was connected to an aluminum bag to collect the gas (Fig. 1). The bottle was filled with 17 L input material (substrate), thereby lifting 4 L for gas production

so as to avoid the liquid coming to the gas bag. On the top of the liquid layer but inside the digester, one PVC net was placed to limit materials floating up. All bonds from the reactor body (the inlet, outlet, and gas pipe) were connected by soft joints for airtight and watertight. To avoid the development of algae, all digesters were covered with black plastic bags. There were two air valves installed in the pipe between the digester and the gas collecting aluminum bag. Thereby, the gas that flows to the bag can be regulated. Another pipe was placed in the substrate of the digester in order to monitor the digestion activity and gas development.

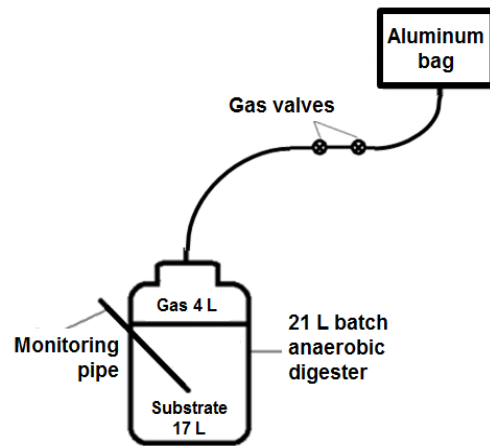


Figure 1. Scheme of experiment apparatus

After the treatments in different durations, the corn stalks were mixed with the ratio of 50%: 50% with cow dung based on their volatile solid (VS) values and then filled into the digester (Tab. 1). During the experiment, all of the four treatments run in triplicate. All digesters were manually and gently shaken every day in order to get a homogenous digestion. In this study, the temperature was not controlled so that we could test as real temperature from farmer conditions.

Table 1. Treatments of the experiment (mixing ratio based on the VS value)

Treatment	Treatment time	Mixing ratio
NT1	-	100%CD + 0%CS
NT2	2-days	50%CD + 50%CS
NT3	5-days	50%CD + 50%CS
NT4	8-days	50%CD + 50%CS

Note: CD = Cow dung; CS = Corn stalk

According to Eder & Schulz (2007), the optimal feeding to an anaerobic digester should be $1 - 4 \text{ g VS} \times \text{day}^{-1} \times \text{L}^{-3}$. Based on this suggestion, the material discharged into the experiment was chosen to be $1 \text{ g VS} \times \text{day}^{-1} \times \text{L}^{-1}$. In this experiment, the retention time of the material had been set on 45 days. However, on the 45th day of the experiment, during collecting the samples, we found that the corn stalk did not degrade much; therefore, we left a few treatments and monitored them to continue until the 60th day to check the digestion status of the input material. The total material input was $1 \times 17 \times 45 = 765 \text{ g VS}$ per each digester (Tab. 2).

Table 2. The quantity of materials used in every digester

Ratio of CD : CS	Volatile solid - VS (g)			Total solid - TS (g)		Fresh weight - FW (g)	
	Corn stalk	Cow dung	Total	Corn stalk	Cow dung	Corn stalk	Cow dung
50% : 50%	382.5	382.5	765	407.2	500.7	475.2	695.0
100% : 0%	765.0	-	765	-	814.4	-	1389.9

2.3 Analytical methods and statistic

Before the experiment, cow dung and corn stalk were analyzed for values of VS, carbon, and total Kjeldahl nitrogen (TKN) by applying the methods described in the Standard Methods (Tab. 3). Based on these parameters, it was evaluated if the input materials suit for anaerobic digestion process or not. All analyses were conducted at the Biogas Laboratory, College of Environment and Natural Resources, Can Tho University, Vietnam.

Table 3. The physical and chemical characteristics of the input materials used in the experiments

Parameters	Unit	Cow dung	Corn stalk
VS	mg/L	76.4	93.9
Carbon	mg/L	19.6	52.2
TKN	mg/L	1.0	1.2
C : N	-	19.6	43.5

The digester’s operation parameters of pH, temperature were daily recorded directly by portable equipment (TOA 3MIP) through the monitoring pipe attached to each reactor. For alkalinity measurement, a buret method was used according to guideline by APHA, AWWA, WEF (2005).

The first gas samples were collected and recorded after 5 experimental days. Then the biogas produced was recorded by a Ritter gas meter (Germany) with the smallest scale of 20 mL; the biogas compositions were monitored at the same time by a Biogas 5000 gas analyzer (UK). After that, the same procedures were applied for gas recording every three days up to the 60th day of the experiment. The gas recording was expressed at ambient temperature and at stable atmosphere pressure (1004 - 1006 mbar) and at the same time of every testing days.

For the substrate from the experiment before and after digested, the samples were collected to analyze the values of chemical oxygen demand (COD), TKN, total phosphorus (TP), and Faecal Coliform according to the methods described in the Standard Methods (APHA et al., 2005).

For statistical evaluation, Duncan test was applied to determine significant differences among each treatment by using SPSS 16.0 software.

3. Results and discussion

3.1 Biogas production

3.1.1 Daily biogas production

In the first 5 days, the biogas produced was very low, and as result of this, no gas was recorded at this stage of the

experiment. In the next 5 days, the highest biogas production was recorded in treatment NT3 (12.9 L), followed by the treatment NT2 (9.9 L) and the treatment NT4 (8.9 L). The treatment of NT1 with 100% cow dung produced the smallest gas volume with 1.9 L that is a result of low C/N ratio in cow dung itself. By the time, the gas volume of co-digestion treatments was increasing and reached peak values on the 11th day, and the peak values were 23.9 L, 22.4 L and 17.9 L for the treatment of NT2, NT3, and NT4, respectively. For the treatment of NT1, due to a technical problem (the monitoring pipe was not closed), the peak value of 8.7 L biogas was recorded on the 14th day. After these milestones, the produced gas volume decreased at all treatments but again increased on the 23rd day. On that day, the treatment NT3 produced the highest volume of gas 9.3 L, while the lowest volume recorded in the treatment NT1 (2.4 L). The increase in the gas volume at this time could be explained by the degradability of cellulose content from input material. As a major chemical component of corn stalk, lignin forms the lignin sheath and surrounds hemicellulose and cellulose, which protects the cellulose and hemicellulose from degradation by cellulase and hemicellulase (Shi et al., 2008). This means at the first 3 weeks, the lignin content from the input material was not yet degradable by the microorganism. By the time, and combination to the outdoor temperature was up to 32.4°C, the lignin content from input material could be degradable and this caused an increase in gas volume at this time. Since then, the gas produced from all treatments tended to reduce at the end of the experimental phase as the organic compound remaining in the substrate was getting lesser.

3.1.2 The accumulate biogas volume

The total gas production after 60 testing days was 69±8.65 L for the treatment NT1, 153.4±9.6 L for the treatment NT2, 206.4±8.4 L for the treatment NT3, and 174±11.1 L for the treatment NT4 (Fig. 2). The treatment NT1 (100% CD) had the lowest accumulated gas production but it had a significant difference to the other treatments ($P \leq 0.05$); this result is in line with the previous study of Yuping et al. (2012). The result of the treatment NT1 could be due to a low C/N ratio of cow dung in comparison to the other treatments with co-digestion of cow dung and corn stalk.

Within the co-digestion treatments, the treatment of NT3 produced more biogas volume compared to that of NT2 and NT4 (5%). In case of treatment NT2, pre-treatment of corn stalk in 2-days was probably too short for microorganisms’ activities. There was not enough time for hydrolysis of organic components and to convert them into a monomer form, volatile fatty acid was less produced and this was the reason for the lower amount of biogas production. For

the treatment NT4, corn stalk was treated with 8-days, it seems that part of cellulose was already degradable. The cellulose remained was not enough for microorganisms in order to convert to biogas form. Therefore, the produced gas volume was also not the highest. The produced biogas

volume was highest in case of treatment NT3; it seems a pre-treatment during 5-days is a suitable time. Hence, 5-days could be an optimal duration for pre-treating corn stalks before feeding to an anaerobic digester.

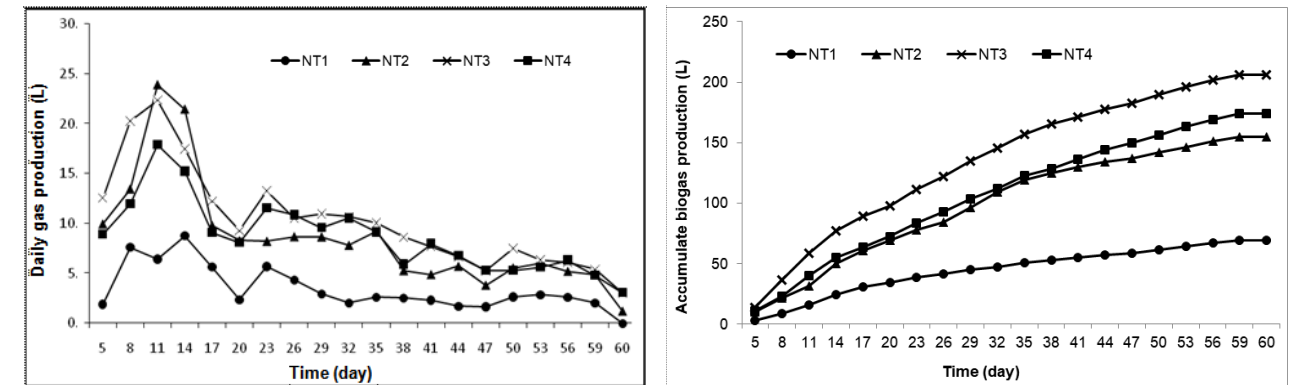


Figure 2. The daily gas production (left) and the accumulated gas production (right) of the four treatments (see Table 1)

3.1.3 The gas yield

The biogas yield was calculated based on the difference of the total gas production and the loss of volatile solid ($VS_{fermented}$) through a digester process. Up to the end of the experimental period, the biogas yield of treatments NT1, NT2, NT3, and NT4 were respectively 196.1 ± 5.6 , 392.7 ± 9.8 , 469.8 ± 10.1 , and 497.1 ± 13.3 L/kg $VS_{fermented}$ (Fig. 3). The biogas yield of the treatment NT1 was lowest and significantly different in comparison to the other treatments ($P \leq 0.05$). There was no significant difference on biogas yields between the other treatments. These results are in line with the studies done by Pedro (2006) and Yuping et al. (2012). Co-fermented cow dung and corn stalk could improve biogas yield in an anaerobic digester and this opens a new opportunity for biogas production for livestock farmers.

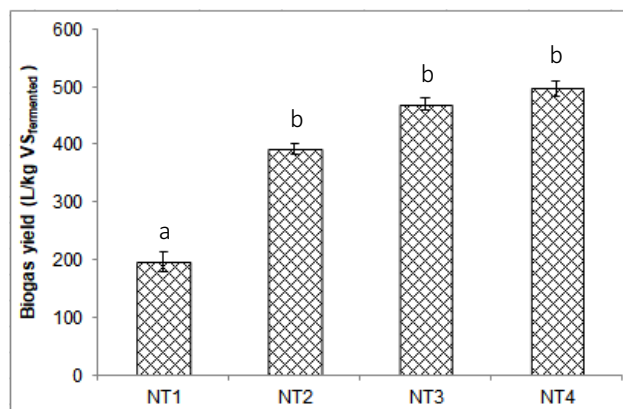


Figure 3. Biogas yield of the four treatments (see Table 1)

Error bars represent the standard deviations values of treatments; different letters indicate significant differences among all treatments ($P = 0.05$ Tukey-Test)

3.1.4 The CH_4 content during digestion

Among biogas components, CH_4 is the most important one for energy generation. In the study, the average CH_4 content in biogas was measured every 10 days until 60 days digestion in all four treatments (Fig. 4).

Based on the CH_4 content recorded, the experimental time could divide into 3 different phases. In the first phase (the first 9 days), the lowest CH_4 content was recorded with 11.8 - 37.6%, while the CO_2 content amounted from 12.1 to 51.8%. This first phase is the phase of hydrolysis and acidity in the anaerobic process, the microorganism mainly converts organic matter into volatile fatty acid, causing more CO_2 development during this time. The second phase is from day 10 to 33, the methanogenic microbes grow more and convert volatile fatty acid of the substrate to biogas; thereby, in particular, the CH_4 content increased. In this phase, CH_4 content was between 47.1 and 52.4% meanwhile the CO_2 amounted from 26 to 37.5%. At the third phase (day 34 to 60), there was a reduce trend recorded at CH_4 content with 39.9 - 46.3%, but these values were higher than the CO_2 content of 34.4 - 37.2%. At this time, the amount of the remaining volatile fatty acid was low and methanogenic microbes could not produce as much CH_4 as at the second phase.

These results showed that the hydraulic retention time (HRT) of input material should not extend more than 33 days (the preferable time is 30 days). If the HRT extends more than 33 days, the CH_4 content will probably decrease and as a result, energy generation will reduce.

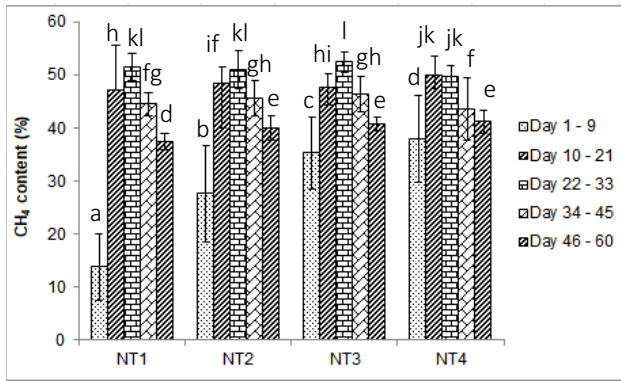


Figure 4. Percentage of CH₄ of produced biogas in the four treatments (see Table 1) measured at five dates

Error bars represent the standard deviations values of treatments, different letters indicate significant differences among all treatments ($P = 0.05$ Tukey-Test)

In general, the Fig. 4 obviously showed that CH₄ content among different treatments was not highly significantly different. One exception was in the first phase, the CH₄ content was much lower, in particular in the treatment of NT1 (100% cow dung) for the 9 first days. From day 10th to the end of the testing phase, there was no significant difference in CH₄ content among all treatments.

By co-digestion of cow dung with corn stalk, the CH₄ content in this experiment was lower than that was recorded in previous studies done by Tran Trung Tinh *et al.* (2009), and Tran Sy Nam *et al.* (2015). In these studies, the authors used different input materials for co-digestion: pig dung with water hyacinth and pig dung with rice straw. However, in this study, the average value of 47.1 - 52.4% CH₄ content from day 10th to 33rd is good enough for cooking purpose.

3.2 Temperature, pH, and alkalinity during digestion in all treatments

Anaerobic digestion is strongly affected by temperature; therefore, in all four treatments, the temperature during the whole digestion period was measured (Fig.5). It can be observed that the average temperature of all treatments was 26.4 - 31.3°C, with the respective peak values in the treatments of NT1, NT2, NT3, NT4 were 30.4°C, 30.0°C, 30.3°C, and 30.1°C. These temperature ranges correspond the optimum temperature of mesophilic digester for biogas production 25 - 40°C (Monet, 2003). According to Samir *et al.* (2010), the activity and growth rate of bacteria decrease by 50% for each 10°C drop in the mesophilic range.

In the weeks 3 to 5, the recorded temperature values were rather low, in particular, at the day 28 with the treatment of NT2 was 27.4°C, NT3 - 26.5°C, NT4 - 26.3°C; at the day 35, the temperature was only 26.4°C recorded in the treatment NT1. The experiments were carried out in the rainy season; therefore, the temperature was obviously influenced by very low air temperature outdoors for several days.

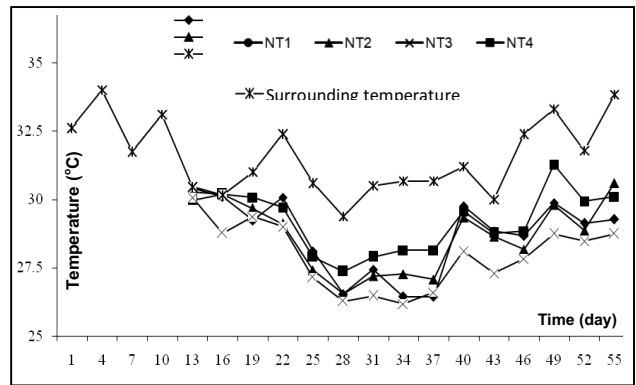


Figure 5. Temperature values during the digestion of the four treatments (see Table 1)

The pH of the anaerobic digestion process is another parameter that has a significant effect on the digestion process (Fig. 6). It can be observed that the pH in all treatments was almost in the optimum pH range in an- anaerobic digesters with 6.0 to 7.5 (Capri & Marais, 1975). For treatment of 100% cow dung, the initial pH value was 7.4 and got a peak value at day 10 that was higher than for the co-digestion treatments a general tendency of stable values of 7.1 - 7.6 after 5 weeks is confirmed. In line with NT1, the pH peak values of co-digestion treatments (NT2 - 7.2, NT3 - 6.5, and NT4 - 7.0) were lower with a peak value on day 10, then stabilizing in range of 7.0 - 7.4 from the day 35 to the end of the testing time. This indicated a better pH profile of the co-digestion during anaerobic digestion and more optimum range of pH values compared to solely digestion.

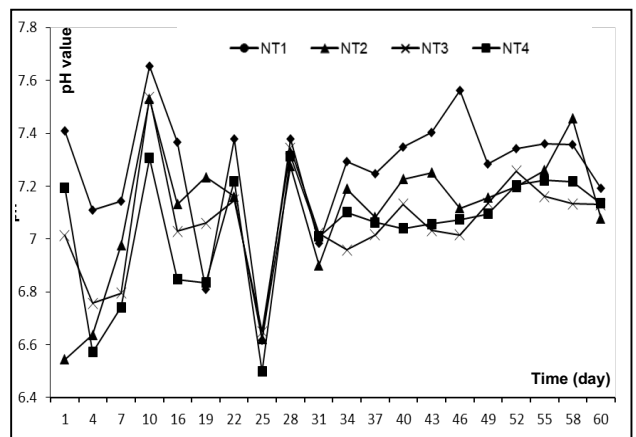


Figure 6. pH values during the digestion of the four treatments (see Table 1)

The buffering capacity of an anaerobic digester is determined by the amount of alkalinity present in the system. Sufficient alkalinity is the main source of buffering capacity to maintain the system's pH in the optimum range. In this experiment, the initial alkalinity concentration of co-digestion treatments was from 2060 to 2580 mg CaCO₃/ L, then increasing to 2580 - 2900 mg CaCO₃/L after 60 digestion days. For treatment of 100% CD, the latter alkalinity concentration was 3400 mg CaCO₃/L, meanwhile, at the beginning of the experiment, the alkalinity was 3120 mg CaCO₃/L

(Fig. 7). The mixing of corn stalk with cow dung in the anaerobic process reduces alkalinity concentration compared to the 100% cow dung treatment that caused a negative effect on the stable operation of digesters. These results are similar to those of the study of Labatut & Gooch (2012) showing that cow manure can play an important role in co-digestion operations by increasing the pH and buffering capacity of the mixture when easily degradable industrial wastes are used as co-substrates. In the range of 2060 - 3400 mg CaCO₃/L, the alkalinity concentration of this study was in an optimum range for anaerobic digestion process of 1500 - 5000 mg CaCO₃/L (Le & Nguyen, 2013).

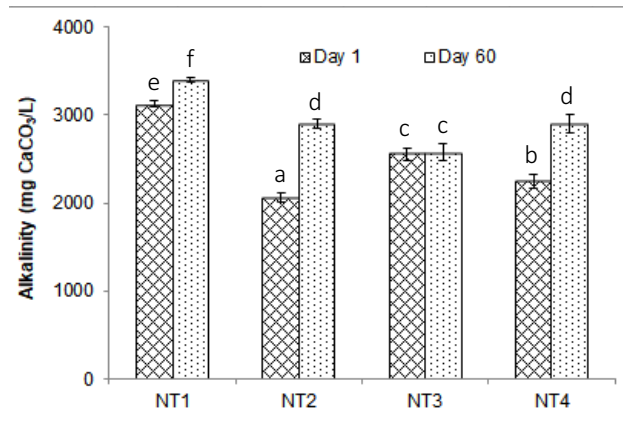


Figure 7. Alkalinity values of the four treatments (see Table 1) at the beginning and after 60 days

Error bars represent the standard deviations values of treatments, different letters indicate significant differences among all treatments (P = 0.05 Tukey-Test)

3.3 Treatment efficiency

Anaerobic digestion of organic matter to biogas has developed from a method of waste treatment. In this experiment, the waste that is needed to be treated is pig dung, and the substrates before (influent) and after (effluent) the digestion process were collected and analyzed to test treatment efficiency of cow dung. The initial substrate was rich on organic matter of COD from 23300 to 33700 mg/L, the high nutrient content of TKN from 650 to 850 mg/L, TP from 1429 to 2430 mg/L, and the Faecal Coliform from 50000 to 320000 MPN/100 mL (Fig. 8). After 60 days of digestion, the best treatment efficiency was recorded on the Faecal Coliform with up to 94%, but the worse one was from TP with less than 19%. The treatment efficiency of TKN was from 25.9 to 33.3%, and that of COD from 56.9 to 66.9%.

After digestion process, TKN that remained in the effluent - called digestate - was from 375 to 458 mg/L, TP from 1270 to 2030 mg/L; COD from 11200 to 21900 mg/L, and Faecal Coliform from 2200 to 17000 MPN/100 mL. The high organic and high nutrient remaining from the effluent causes pollution for receiving water bodies. However, as recommended by Nguyen Vo Chau Ngan (2012), Nguyen & Fricke (2014), it could be favorable to reuse the digestate as organic fertilizer for agriculture and/or aquaculture crops. However, it is a warning that bacteria could remain on a vegetable that is irrigated by digestate and such vegetable should not be eaten raw (Nguyen Vo Chau Ngan et al., 2015).

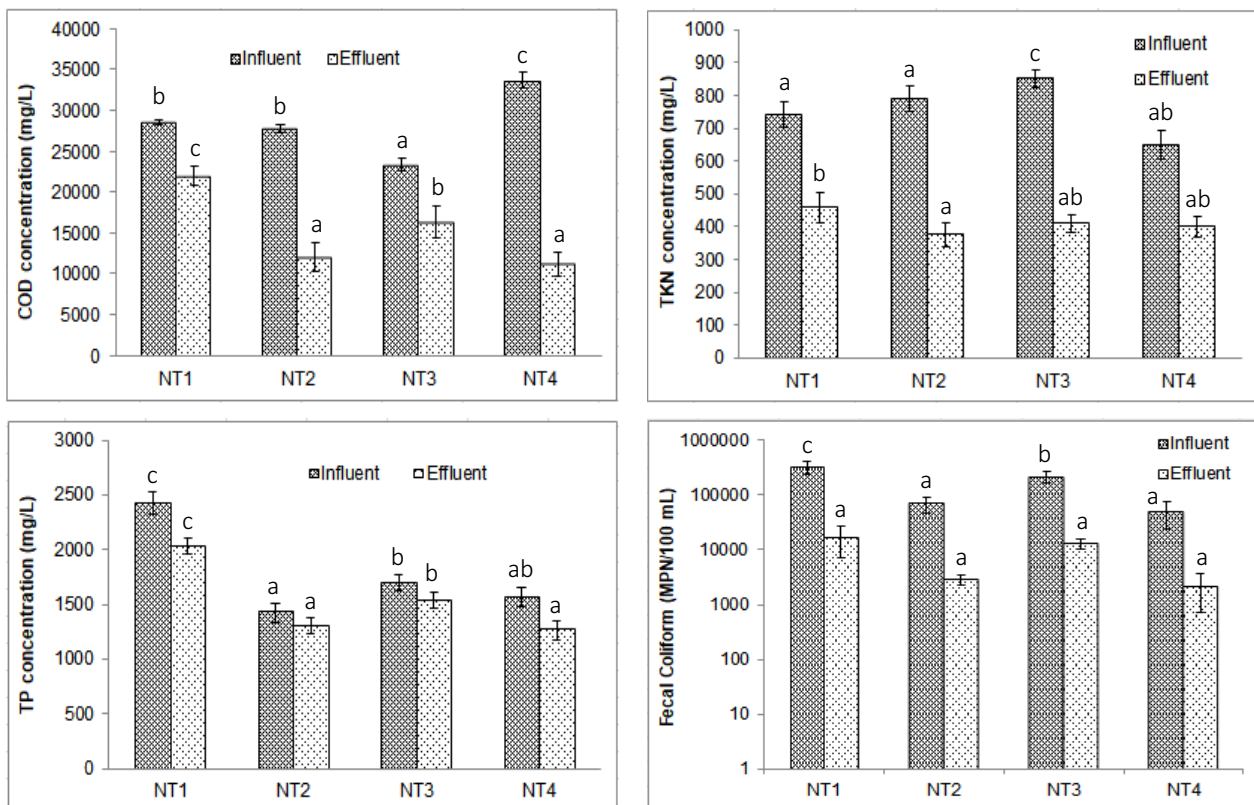


Figure 8. Comparing influent and effluent of COD, TKN, TP and Faecal Coliform of the four treatments (see Table 1)

Error bars represent the standard deviations from influent and effluent of treatments, different letters indicate significant differences among all treatments (P = 0.05 Tukey-Test)

4. Conclusions

In this study, three types of treatment duration (2-days, 5-days, and 8-days) of corn stalk were selected for the experiments of anaerobic co-digestion of cow dung and corn stalk and compared with the control (100% cow dung) as the first treatment. There was a significant difference between the 5-days treated treatment and the other treatments on the total gas produced within the testing period. However, in case of gas yield and gas composition, there was no significant difference among all treatments. In all treatments, the CH₄ concentration reached a good quality for cooking purpose after 10-days of digestion. Therefore, a 5-days treatment could be a preferable option for corn stalk to be supplementarily loaded to an anaerobic digester in combination with cow dung.

Since organic and nutrient matter remains high in digestate, it could be applied as an organic fertilizer to agriculture and/or aquaculture cultivations.

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