STUDY OF THE IMPACT OF THE USE OF A MIXED INOCULUM ON THE METHANE YIELD IN ANAEROBIC DIGESTION PROCESS

NGHIÊN CỨU VỀ ẢNH HƯỞNG CỦA HỖN HỢP TÁC NHÂN HỖ TRỢ ĐỐI VỚI NĂNG SUẤT SINH KHÍ MÊ TAN CỦA QUÁ TRÌNH PHÂN HỦY SINH HỌC YẾM KHÍ

ABSTRACT

Batch experiments were conducted to study the impact of the use of a mixed inoculum on the methane yield for wet, mesophilic, discontinuous anaerobic digestion of food waste. Three different inocula were used: cow rumen, AD (anerobic digestion) sludge and UASB (Upflow anaerobic sludge blanket) sludge. The results indicated that the use of a mixed inoculum does not lead to any improvements in the methane yield. On the contrary, the yield decreases and the kinetic of the reaction slows down. The best-performing inoculum is AD sludge, while cow rumen and UASB sludge appear to be inadequate choices of inoculum for food waste AD.

Keywords: Food waste, anaerobic digestion, biogas, energy, fertilizer.

TÓM TẮT

Nghiên cứu này tập trung vào đánh giá ảnh hưởng của các tác nhân khác nhau đến khả năng sinh khí mê-tan đối với quá trình phân hủy ướt theo mẻ đối với chất thải thực phẩm trong điều kiện ưa ấm. Ba tác nhân được sử dụng là hỗn dịch trong dạ dày bò, bùn từ bể AD (bể phân hủy yếm khí) và bùn từ bể UASSB (bể kỵ khí). Kết quả nghiên cứu chỉ ra rằng việc sử dụng hỗn hợp của ba tác nhân trên không cải thiện năng suất sinh khí mê-tan. Việc sử dụng tác nhân là bùn từ hệ thống AD có hiệu quả tốt hơn hỗn dịch từ dạ dày bò Trong khí đó nào về năng suất khí mêtan. Ngược lại, năng suất giảm và động học của phản ứng chậm lại. Chất cấy có hiệu suất tốt nhất là bùn AD, trong khi dạ cỏ bò và bùn UASB dường như không phù hợp với lựa chọn cấy cho chất thải thực phẩm AD.

Từ khóa: Chất thải thực phẩm, phân hủy yếm khí, khí sinh học, năng lượng, phân bón.

¹School of Environmental Science and Technology, Hanoi University of Science and Technology
²National Institute of Applied Sciences of Lyon
*Email: hung.dinhquang@hust.edu.vn
Ngày nhận bài: 25/5/2020
Ngày nhận bài sửa sau phản biện: 30/6/2020
Ngày chấp nhận đăng: 18/8/2020

1. INTRODUCTION

In our world, where the population and its needs in terms of energy and waste management are constantly increasing, anaerobic digestion (AD) can be part of a global solution. AD is a biological process, which degrades biodegradable waste in the absence of oxygen; it allows a

Dinh Quang Hung^{1,*}, Do Trong Mui¹, Damien Lagar², Mai Thị Linh¹, Hoang Thi Linh¹

waste recovery and an energy production without plundering natural resources.

According to Gustavsson et al. (2011) working with the food and agriculture organization, 1.3 billion tonnes of food produced in the world for human consumption every year gets lost or wasted, which represents roughly one third of the production [1]. Knowing that the food waste has a high biochemical methane potential (BMP), and the huge quantity of food waste available, one can appreciate the potential of AD.

The AD process needs an inoculum to provide the methanogenic bacteria needed. The quality and the quantity of the inoculum is a key-parameter of the process. The use of a mixed inoculum might provide more diverse nutrients and microorganism to achieve AD than a single inoculum.

The effectiveness of different types of food waste and different types of inoculum and their proportion have already been the subject of many publications. However, there are still many scientific and technical locks, which require more research. The objective of this study is to measure the impact of the use of a mixed inoculum on the methane yield. This study will focus on wet, mesophilic, discontinuous anaerobic digestion of food waste.

2. MATERIALS AND METHODS

2.1. Experimental set-up

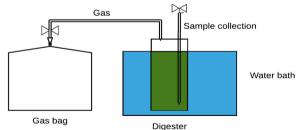


Figure 1. Set-up of a digester

The experimental set-up is made of a water bath containing 30 digesters kept at a temperature of 37°C. Each of them has a rubber stopper with 2 valves. The first valve lets the gas produced in the digester to go in a gas bag; the second one allows the specimen collection for

measurements. An illustration of the set-up is available below in Figure 1.

2.2. Gas measurements

In the absence of volumetric measurement device, the water displacement method has been used. The premise of this system is that 1mL of water occupies as much volume as 1mL of gas.

2.3. Parameters analysis

AD process is a complex operation with a large number of factors either due to environmental conditions or operational parameters; both affecting the methane production. They have to be cautiously controlled at all time. All the measured parameters and their determination methods are detailed in the Table 1.

The food waste has been collected from the canteen of one industrial company. It has been stored for 2 days at 5°C to prevent biodegradation. The food waste was mainly composed of rice, noodles, shrimp, beef, chicken, cabbage, water spinach, chayote, chilli, lemon and water. Before use, it has been crushed to get a size smaller than 1.5mm.

Table 1. Measured parameters and determination methods

Parameters	Determination method
TS	Drying sample in a proofer at 105°C during 12h [2]
VS	Ashing sample in muffle furnace at 550°C during
	2h [2]
рН	pH-paper
Temperature	Thermometer
Alkalinity	Titration using Kapp method [3]
VFA	Titration using Kapp method [3]
Biogas production	Water displacement method
Methane production	NaOH solution + water displacement method

Inocula have been collected from three different plants:

- Cow rumen from a local slaughterhouse.

- AD sludge from a homemade AD set-up. The feedstock of the AD plant is pig manure and human feces.

- Upflow anaerobic sludge blanket (UASB) sludge from a local wastewater treatment plant.

2.4. Substrate and inocula

Table 2. Characteristics of the food waste and of the inocula

Parameters $(n = 3)$	Food Waste			UASB sludge	
TS (%)	19.79	19.28	1.15	8.27	
VS (%)	18.33	16.40	0.84	5.45	
VS/TS (%)	92.06	85.04	72.61	65.92	
рН	4.47	7.49	7.56	7.34	
Alkalinity (gCaCO ₃ /L)	1.024	11.65	4.26	5.17	
VFA (mgCH ₃ COOH/L)	3741.0	6591.8	1377.4	1490.9	

The characteristics of the food waste and the *inocula* shown in Table 2 have been compared with literature data. The TS and VS values are really close to the literature data. The volatile solids to total solids ratio (VS/TS) of the food waste is 85.04% when it is usually above 80% [4]. The VS/TS ratio for the cow rumen is at 85.04% when 84% can be

expected, 72% for AD sludge for 71% expected and 65.92% for UASB for 63% expected [5]. One can say that the food waste and the *inocula* completely have similar parameters value than the literature data, which is an important parameter for the reproducibility of the experiment.

3. RESULTS AND DISCUSSIONS

Figure 2 shows the methane production for all the digesters. It can be noticed that digesters 10, 11, 12, 16, 17, 25, 26 and 27 have a negative production or really close to zero. They did not achieve to produce more gas than the endogenous production of their *inoculum*. Leakages checks have been confirmed. They have therefore been set aside.

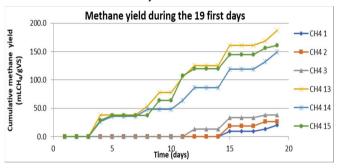


Figure 2. Methane production during the 19 first days

During the 19 first days, the methane production in most of the digesters is really limited. According to Figure 2, only digesters 1, 2, 3, 13, 14 and 15 have produced a consequent amount of biogas (only production over 100mL/gVS has been plotted) and they are among the few digesters to be in the optimal pH range on day 19 (Figure 3). It should be noted that the methane production in Figure 2 also contains the endogenous production.

As explained in the state of the art, a too low pH value prevents the acetogenesis and the methanogenesis to efficiently occur. And as we can see in the Figure 3, the majority of the digesters, which experienced a serious pH drop during the 19 first days, had a limited methane production over this time.

The chosen control strategy was to maintain a low concentration of VFA and a pH range of 6.5 < pH < 7.5. Sodium bicarbonate has been added in the digesters with a pH lower than 5.5 in order to level up the pH. Table 6 shows that experiments 6, 7, 8 and 10 that benefits from the pH correction have seen their methane production increase. However, experiment 9, which also did benefit from the pH correction looks unchanged. After the pH-correction, most of the experiments have kept a pH close to the optimal range until the end of the experiment (Figure 3). From the same figure, it can be noted that the experiments: 4, 6, 9 and 10, which have the lowest pH at the end of the 50 days also have the highest VFA level at the end of the experiment (Figure 4).

All the digesters of experiment 2 experienced a pH drop to 4.5 or less during the 19 first days. The digesters did not recover from the pH-drop even after the pH-adjustment. Small quantity of gas has been measured few days after the pH-adjustment, but this is all. The pH-drop experienced by the digesters has been too severe to be fixed with a pH adjustment.

Table 3 shows the percentage of the total methane produced by the endogenous production. The results are particularly high; they are in the range 17 - 40%, which are generally the values for a low S/I ratio set-up. However, in this study a rather high S/I ratio has been used. This might be explained by the fact that the study has been stopped after 50 days of experiments, which might not have been long enough for some digesters (Table 4). The digesters in grey in Table 3 have produced until the last day of experiment.

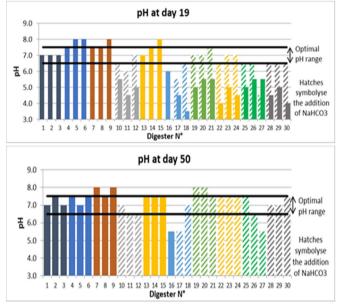


Figure 3. Changes in pH

Table 3. Percentage of endogenous production

Digester N°	13	14	15	18	19	20	21	22	24	28	29	30
% of CH4 produced by the endogenous production (%)	17	17	19	30	18	22	17	22	21	30	40	34

Table 4. Methane production along the time and BMP

Experiment	Total CH₄	Total (H		% of total CH₄	BMP ₅₀
•	-	-	-	-	50
N°	Produced in	Produced	Produced	production in	(mL
	the 17 first	in the 25	in 50	the first 25	CH₄/gVS)
	days	first days	days	days	
1	103.2	185.3	217.4	84%	-
2	45.3	106.5	153.8	70%	-
3	178.4	358.5	425.5	84%	-
4	130.8	159.1	176.4	93%	-30.9
5	968.5	1313.3	1353.1	96%	321.5
6	185.6	327.3	391.7	84%	18.0
7	128.8	344.5	1497.4	25%	243.3
8	114.3	192.1	920.4	32%	156.9
9	135.2	135.2	266.7	53%	-7.4
10	180.7	356.5	919.0	39%	90.9

The use of a mixed *inoculum* as in the experiments 7 to 10 slows down the AD. According to Table 4, these experiments produce 25 - 53% of their methane in 25 the first days, whereas

experiments 4 to 6, which are using only one *inoculum*, produce 84 - 93% of their methane in the same time.

Difficulties in VFA and alkalinity measurements have been observed during the first three weeks of the experiment due to a heterogeneous feedstock and the detection of VFA accumulation has not been possible. The VFA and alkalinity measurements have finally only been conducted for the start and the end of the experiment.

The Figure 4 shows the change in VFA between the start and the end of the experience compared with methane production. Experiments 5, 7 and 8 have ended with small VFA quantity and produced a significant amount of methane, while experiments 4, 6, 9 and 10 have seen their VFA been multiplied up to 7 times their initial value and they hardly produced any methane. As explained in the state of the art, VFA are a product of the acidogenesis, they are then used by the next reactions to produce methane. However, when too much VFA are produced, inhibitions of the methane production may occur. The amount of remaining VFA is as many methane that as not be produced.

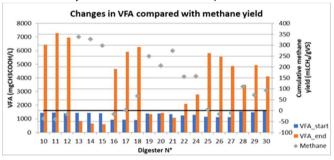


Figure 4. How VFA impact methane production

According to Figure 5, the methane represents 55% to 75% of the volume of biogas. It is slightly higher than the range of value from the literature data, which can be explained by the assumption that biogas is only made of methane and carbon dioxide. The solution of hydroxide sodium only removes the carbon dioxide, thus other gas remains in the volume considered as methane.

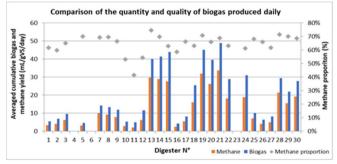


Figure 5. Daily methane and biogas production

The experiments have been sorted along their methane production in a decreasing order:

- Experiment 5: food waste + AD sludge.

- Experiment 7: food waste + Cow rumen + AD sludge.

- Experiment 8: food waste + AD sludge + UASB sludge.

- Experiment 10: food waste + Cow rumen + AD sludge + UASB sludge.

- Experiment 6: food waste + UASB sludge.

The results obtained in experiment 5 (Figure 6) using the AD sludge provided by a running AD plant gave results close to the literature data - represented by the two horizontal lines - which indicates that the experimental setup works well.

An analysis of variance was initially planned for all the experiments; however, because some digesters have been set aside, analysis of variance can only be conducted between experiments 5, 7 and 10.

For those experiments:

- The homogeneity of variances and the normal distribution of the variable do not need to be checked, because the samples have the same size.

- Independence of observation is checked by the study design.

The analysis of variance gives us the following results:

- The use of only AD sludge as *inoculum* produces significantly more biogas and methane than the use of a mixed of AD sludge and cow rumen (p < 0.05).

- The use of only AD sludge as *inoculum* produces significantly more biogas and methane than the use of a mixed of AD sludge, cow rumen and UASB sludge (p < 0.01).

- The use of a mixed *inoculum* of AD sludge and cow rumen produces significantly more biogas and methane than the use of a mixed of AD sludge, cow rumen and UASB sludge (p < 0.01).

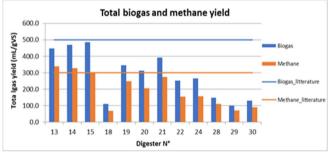


Figure 6. Total biogas and methane yield

Even if no analysis of variance could have been done on experiment 8 because it ended up containing only 2 digesters, it shows interesting results. The use of a mixed *inoculum* of AD sludge and UASB sludge seems to give results between only AD sludge and a mix of AD sludge and cow rumen. Cow rumen and UASB sludge are not as suitable choice for an *inoculum*, indeed experiments 4 and 6 using as *inoculum* respectively cow rumen and UASB sludge did both fail except digester 18 but its results are not conclusive. The largest biogas and methane production have been obtained while using a single *inoculum*: AD sludge. Any mix of AD sludge with another *inoculum* has only lower the gas production as it can be seen in experiments 7, 8 and 10.

4. CONCLUSIONS

The goal of the study was to determine the impact of the use of a mixed *inoculum* on the methane yield. The use of a mixed *inoculum* was expected to provide more diverse nutrients and microorganism to achieve anaerobic digestion than one *inoculum*. For the chosen *inoculum* and food waste, the use of a mixed *inoculum* did not lead to any improvement in the methane production and even slow down the kinetic of the reaction.

The use of a single *inoculum* AD sludge gave better results, while the cow rumen and UASB sludge seems to be inadequate *inoculum* for food waste AD. This AD sludge came from a homemade AD plant, where the feedstock is based on pig manure and human feces. However, this study presents some bias, only one type of food waste has been used, which can lead to different results in methane production due to different affinities between the food waste and the *inocula*. Moreover, strong assumptions have been made for the S/I ratio and for the simplification of the biogas composition.

Further researches must be conducted to focus on the identification of the reasons for the differences in methane yield between the digesters. They should investigate the inhibitions occurrences and use a microbiological approach. Anaerobic digestion must be considered as one of the few technologies that can both produce energy and reduce environmental pollution,

ACKNOWLEDGEMENTS

This research was financed by Hanoi University of Science and Technology (HUST), Vietnam, in a project name T2018-PC 080.

REFERENCES

[1]. Gustavsson, Jenny, Christel Cederberg, Ulf Sonesson, 2011. *Global Food Losses and Food Waste: Extent, Causes and Prevention; Study Conducted for the International Congress Save Food!* At Interpack 2011, [16 - 17 May], Düsseldorf, Germany. Rome: Food and Agriculture Organization of the United Nations.

[2]. André, Laura, 2016. Étude de Verrous Scientifiques et Technologiques Pour La Compréhension et l'optimisation Du Procédé de Méthanisation Voie Sèche Discontinu de Sous-Produits d'origine Agricole. Compiègne. http://www.theses.fr/2016COMP2279.

[3]. Buchauer K., 2007. A Comparison of Two Simple Titration Procedures to Determine. n.d., 8.

[4]. Zhang, Ruihong, Hamed M. El-Mashad, Karl Hartman, Fengyu Wang, Guangqing Liu, Chris Choate, Paul Gamble, 2007. *Characterization of Food Waste as Feedstock for Anaerobic Digestion*. Bioresource Technology 98, no. 4, 929–35. https://doi.org/10.1016/j.biortech.2006.02.039.

[5]. Capson-Tojo, Gabriel, Maxime Rouez, Marion Crest, J-P Steyer, J-P Delgenes, Renaud Escudié, 2017. *Stabilization of Anaerobic Digestion of Food Waste for Methane Production*. Conference: 1st International ABWET Conference: Waste-to-bioenergy: Applications in Urban areas, At Paris, FRANCE.

THÔNG TIN TÁC GIẢ

Đinh Quang Hưng¹, Đỗ Trọng Mùi¹, Damien Lagar², Mai Thị Linh¹, Hoàng Thị Linh¹

¹Viện Khoa học và Công nghệ môi trường, Trường Đại học Bách khoa Hà Nội ²Viện Nghiên cứu ứng dụng quốc gia Lyon