The fate of carbon in two-stage anaerobic digestion of vegetable waste

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Abstract. This study aims to investigate the fate of carbon in two-stage anaerobic digestion (TAD) of vegetable waste. The TAD including a hydrolytic reactor and a methane reactor were controlled at mesophilic temperature (36±1°C) with a retention time (RT) of 9 days and 20 days, respectively. Carbon tracking was conducted step by step throughout the system. Non-hydrolysable carbon accounted for a significant proportion of total initial carbon (25%). Meanwhile, a large amount of carbon in the feedstock (23.5%) was hydrolysed but remained in the effluent including water and suspended solids. It was mostly inorganic carbon which is not harmful to the environment. The only 41.3% initial carbon was converted to biogas in both reactors. In the hydrolytic reactor, biogas was mainly carbon dioxide (99%), accounted for 11.3% of total biogas and 35.8% of total CO$_2$ product. In the methane reactor, biogas was 373.9 NmL/g-VS including 73.3% CH$_4$, 21.9% CO$_2$ and 4.8% others. Non-hydrolysable materials can be a source of thermal energy. Meanwhile, a large amount of hydrolysed carbon was not converted into biogas, was still in the effluent, it was a significant energy loss. Therefore, how to further increase the effectiveness of TAD is an issue that needs to study.

1. Introduction
Vegetable waste (VW) is generated daily with enormous amounts everywhere in the world as a natural consequence of agricultural activity and the food industry [1; 2]. Unfortunately, It has a high biodegradability feature. Pham Van et al. (2018a) [3] reported that hydrolyzable rate and ultimate biodegradable rate of VW were 74% and 49-69%, respectively. Meanwhile, Li et al. (2013) [4] and Lin et al. (2011) calculated biodegradability based on the theoretical methane yield and showed that biodegradable rate of VW was 59.3 and 71.7 %, respectively. Therefore, disposal of VW into landfills or dumping sites inevitably causes the various environmental problems by the occurrence of anaerobic digestion (AD) naturally. AD is a series of bio-degradation processes in the condition of oxygen absence by activities of anaerobic microorganism [5; 6]. These processes create both intermediate products (soluble materials, volatile fatty acids) and biogas products (CH$_4$, CO$_2$, H$_2$S, NH$_3$) [7; 5; 8]. This leading to negative effects are separated into two categories: atmospheric effects and hydrological effects [9; 1]. The atmospheric effects include odour pollution and greenhouse gas emission. The hydrological effects are mainly groundwater contamination, surface water pollution. Thus, controlling the AD processes of VW in the reactor systems has attracted attention as one of the best sustainable solutions [6]. AD system does not only solve the problem of solid waste pollution that
humanity is facing, but also contribute to guarantee energy security while fossil fuel energy is decaying out.

The most used anaerobic digestion systems include the wet single-stage, the dried single-stage, and two-stage systems. In which, the dried system is applied for high total solids (TS) concentration of feedstock (TS>20%) [10]. Meanwhile, VW was often reported having the characteristic of low TS concentration (<20%) [1]. Moreover, the wet single-stage systems have a series of disadvantages compared to the two-stage systems such as short-circuiting, sensitive to shock load and inhibitors, sink and float phases [10]. Therefore, the two-stage anaerobic digestion system is the most effective for the treatment of VW. However, the information in applying the two-stage digestion system to deal with VW is still limited.

The anaerobic digestion pathway of the feedstock to biogas includes hydrolysis, acidogenesis, acetogenesis, and methanogenesis, has been studied particularly and well known in the literature. In recent years, a large number of articles has been focusing on the possibility of conversion to biogas of substrates through the biomethane potential tests. Others have investigated the rate of transformation through kinetic equations. In brief, most study in the research area in anaerobic digestion has made conduct to find the relationship between feedstock (input) and biogas (output products). A series of papers performed AD of vegetable in the same conditions of temperature, pH, total solids but biogas yields were very different. So there are many questions left open with limited information in mass flow of the anaerobic process. Chernicharo and Augusto (2007) presented a very simple scheme of biological conversion in the anaerobic system through chemical oxygen demand (COD) values. In which, 100% COD of feedstock is distributed to biogas (70-90%), biomass sludge (5-15%) and effluent (10-30%). Meanwhile, van Lier et al. (2008) [11] assumed that in 100% COD of feedstock is converted into biogas (75-85% COD), biomass sludge (5% COD), and effluent (10-20% COD). And both groups of authors did not show proof of their assumptions. Moreover, the feedstock is not only biodegradable materials but also non-biodegradable fractions, and the effluent is not only wastewater but also suspended solids. Furthermore, the fate of feedstock in two-stage digestion is unknown in the literature. Therefore, the current literature does not make satisfaction in considering the fate of feedstock in anaerobic digestion.

To clarify the mentioned issue above this study was conducted to investigate two-stage anaerobic digestion of vegetable. The system including hydrolytic and methanogenic reactors was operated at mesophilic temperature (36±1°C). Carbon from feedstock, intermediate product (hydrolysate), and final products (such as biogas, biomass, and effluent water) were determined to track the input and output of reactors. This study was performed at Okayama University, Japan in 2018.

2. Materials and methods

2.1. Feedstock

Vegetable waste was collected at an agricultural field of Okayama University where the activity is not only limited in producing vegetable products but also like a small vegetable supermarket. Thus, VW was fresh at collection time. The vegetable was cut into small size particles by manual then blended by a household blender. Finally, VW was mixed with horse dung by the ratio of 10:1 to form the feedstock. In which, horse dung played the role of the bacterial source for the hydrolysis process. The feedstock had characteristics of 7.4% TS, 6.1% VS, and C/N ratio of 15.7.

2.2. Analytical methods

Gas content (carbon dioxide, methane, and others) was determined according to the method described by Pham Van et al. (2018a). Carbon (C) in the solid samples was analysed using a CHN analyser (2400 II, PerkinElmer, USA). Meanwhile, the liquid samples were filtered through a 0.45 µm microfilter paper before measuring by using TOC analyser (Shimadzu, 2010). TS, VS, and pH were carried out in accordance with APHA standard methods [12]. A brief description was presented by Pham Van et al. (2018b) [13] and Dinh et al. (2018) [14]. In which, the TS content was calculated after drying the samples at 105°C for 24 hours to reach the constant weight. The VS was determined
after drying the total solid at 550°C for an hour to reach the constant weight. The pH-value was detected using the LAQUAtwin (Horiba, Japan).

2.3. Experimental setup
The scheme of the two-stage digestion system is presented in Fig. 1. In which, the hydrolytic process employed a continuously stirred tank reactor (CSTR). This reactor was operated in batch mode with pH of 6.5, a retention time of 9 days, and at a temperature of 36 ± 1°C. After hydrolysed, the substrate was filtered through a sieve (pole of 1mm) to remove non-hydrolysable materials before being stored in a buffer tank. In the buffer tank, the liquid was diluted (five folds) with tap water and mixed by a magnetic stirrer. The temperature was not controlled in this tank. The liquid from the buffer tank was pumped into a methane reactor by using a peristaltic pump. The methane reactor was an upflow anaerobic sludge blanket reactor (UASB) with the temperature maintained (using a hot water jacket) at 36 ± 1°C. The UASB was operated with continuous mode and a hydraulic retention time of 20 days. Before the current experiment, the UASB reactor had been warmed up nearly five months with anaerobic bacterial cultures originated from horse dung.

Fig. 1. Diagram of the experimental model

2.4. Diagram of carbon sampling
The carbon metabolism was evaluated through the input materials and output products of each process in the two-stage digestion system. Among which, the products of hydrolysis include carbon dioxide, non-hydrolysable fraction and hydrolysate. The products of the methanogenic process contain biogas, wastewater and suspended fraction in the wastewater. Details of carbon sampling are shown in Fig. 2.

Fig. 2. Diagram of carbon sampling
3. Results and discussion

Data of carbon in the feedstock, hydrolysate, and final products of the two-stage anaerobic digestion was observed, and the flow of carbon is shown in Fig. 3. In which, carbon of the non-hydrolysable fractions and the effluent were a high proportion of 25.0% and 23.5%, respectively. The only 41.3% of the total initial carbon was converted to biogas. The rest of carbon including biomass sludge and lost of mass accounted for 10.2%. Compared to the aerobic process, it was often reported that 50-60% mass of feedstock converted into sludge [7]. This residual sludge has to be treated, and it is a cost burden for the treatment plant [15]. Thus, less biomass sludge production is one of the big advantages of anaerobic processes compared to aerobic processes. It was often reported that biomass sludge was generated from about 5% of feedstock [11]. Therefore, the loss of mass could be approximately 5%.

![Fig. 3. The fate of carbon in the two-stage anaerobic digestion](image)

Hydrolysis of fibrous materials is a relatively slow process. Meanwhile, the materials used in this study were often reported containing a large amount of fibre. In particularly, Kafle and Chen (2016) [16] analysed characteristics of HD and detected a very high crude fibre concentration up to 38.5% TS. Abbasi et al. (2011) [17] reported that vegetable also containing a high lignocellulose composition up to 30.2% of TS. That explains why this study found a high proportion of non-hydrolysable carbon in the feedstock (accounted for 25% total initial carbon). Likewise, Pham Van et al. (2018a) also investigated the co-hydrolysis of HD and VW through carbon metabolism, reported that non-hydrolyzable rate of HM and VW were 39% and 26%, respectively. Wu et al. (2016) [18] performed vegetable digestion by a two-stage digestion system and reported that solid fraction after hydrolysis was 30.5% TS.

**Biogas production**: the only 41.3% of initial carbon was converted to biogas equivalent to the biogas yield of 419.4 Nml/g-VS. In which, biogas from the hydrolytic reactor was mainly carbon dioxide (99%), accounted for only 11.3% of total biogas and 35.7% of total CO₂ product. Pham Van et al. (2018a) also observed a high proportion of CO₂ in hydrolysis (12-44% of total CO₂). Fortunately, hydrolysis does not generate poison gas such as H₂S and NH₃ [5]. So biogas from hydrolysis can be discharged directly without any treatment process. Therefore, the idea of TAD not only helps digestion system more stable but also reduces a significant cost of biogas upgrading. Meanwhile, biogas was mainly produced in the methane reactor (373.9 Nml-biogas/g-VS) including 73.3% CH₄ (equivalent to 274.1 Nml-CH₄/g-VS), 21.9% CO₂, and 4.8% others. Related to the use of the two-stage system for digestion of vegetable waste, the result in this study could be compared to the report of Wu et al. (2016) who also used a configuration of CSTR for hydrolysis and UASB for methanogenesis, observed methane yield of 244.2 ml/g-VS. Meanwhile, Zuo et al. (2014) [19] received a higher methane yield (290-310 ml/g-VS) when using FBR for methanogenesis.

**Effluent water**: The effluent from the methane reactor contained a large amount of carbon (23.5% of total initial carbon). In which, the main proportion was inorganic fractions (21.5%). Organic carbon was
only 0.365 mg/l equivalent to 2.9% of total initial carbon. Therefore, the effluent will release very low greenhouse gas emission values, even not. Meanwhile, a huge amount of nitrogen, phosphorous, and minerals are not converted into biogas remaining in the effluent [20]. Therefore, the effluent can be a potential source of fertiliser which can replace conventional chemical source. Suspended solids were a low concentration of 350 mg/l equivalent to 1.63% of initial carbon.

4. Conclusions and Recommendations
Anaerobic digestion of VW was conducted successfully by the two-stage digestion system with a configuration of SCTR-UASB. In the hydrolytic reactor, non-hydrolysable carbon accounted for a significant proportion of total initial carbon (25%). It could be originated from fibrous materials which have a very low rate of degradation. This amount of non-hydrolysable substance can be a potential source for composting or burning. Biogas in this reactor was mainly carbon dioxide (99%) which was accounted for 11.3% of total biogas. Meanwhile, carbon dioxide has no energy values and does not contain poison gas then it can be discharged directly. In the methanogenic reactor, biogas yield was produced 373.9 Nml/g-VS including 73.3% of CH₄ (equivalent to 274.1 Nml· CH₄/g-VS), 21% of CO₂ and 4.8% of others. One major of material flows was effluent with 23.5% of carbon in feedstock remained in wastewater including water and suspended solids. However, almost carbon in the effluent was inorganic carbon and not harmful to the environment. Thus, the effluent can be a potential source of fertiliser which can replace conventional chemical source.

5. References
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