Exploring the Effect of Specific Bacterial Strains on the Degradation and Production of Volatile Fatty Acids from Food Waste Leachate

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Abstract. Waste stream has well known as a best option for biorefinery process as it effectively mitigates the organic amount to produces biogas as a renewable and an alternative energy. Volatile Fatty Acids (VFAs) are huge valuable sources for the chemical industry as the precursors of reduced chemicals and derivatives. On the other hand, they have well known as the substrates for bio-fuel production like methane and hydrogen. Thus, the aim of this study is to isolate a pure specific bacterial strains from a variety of sources such as soil, wastewater and food waste leachate that play a crucial role in VFAs degradation and production. In this study, twenty bacterial strains isolated from soil and waste samples were tested in presence of VFAs components concluding acetic acid, butyric acid and propionic acid at 35 °C in four periods: 1-day, 5-day, 10-day and 15-day incubations. Consequently, eight strains illustrated strong growth in the mineral salt media containing VFAs components after 15 days. Four strains out of eight have been found as producing VFAs bacterial candidates. These strains later were tested back in the Food Waste Leachate as a substrate for carbon and energy sources at the same operational conditions. Moreover, there are other four strains which have been explored from the other group chosen for an examination of VFA degradation. After determining potential VFAs consuming and producing candidates, a consortium has been screening for accelerating and optimizing the gas production in the next study at variety of operation conditions.

1. Introduction

In The Paris Agreement (2016), to be able to limit the rise of global temperature to below 1.5 °C, more eco-friendly, efficient and commercially strategies are more necessary to increase the resource recovery. Therefore, technologies which can turn carbon-rich substrates or waste streams into chemicals and materials instead of petrochemical based processes have been seen as a high trend nowadays [1]. Anaerobic digestion of organic wastes has become a sustainable waste management strategy as biogas which is a source of renewable energy producing from various types of raw materials such as manure sewage sludge and food waste (FW).

In the case of FW, it has been recognized as a valuable source because of its benefits achieved from circular economy as for farming, agriculture, for producing biomaterials and other sectors for markets and
retail facilities [2]. VFAs are being concerned as a great potential, economically feasible and sustainable method for waste degradation, biomaterial sources and biogas production. Bhatia and Yang defined the importance of bio-based VFAs [3] which can be replaced the non-renewable petrochemical sources causing serious negative health and environmental effects [4]. Volatile fatty acids (VFAs) generated during treatment process at high or low level have the advantages and disadvantages depending on the desired products. However, an accumulation of VFAs will lead to an increase of the undissociated VFA species, which are well known to inhibit the anaerobic digestion process for biogas production [5]. Thus, these studies have been conducted both by pure culture of specific bacterial strains isolated from various source such as soil and wastes using it as an inoculums for anaerobic treatment process of FW which play a crucial role in both VFAs production and degradation.

2. Sampling and Methods

2.1. Sampling and Isolation

Soil sample was collected from the surface covering of plant roots in the forest at Kyonggi University, Suwon, Gyeonggi-do, South Korea and passed by a mesh sieve to remove gravel and plant debris. Food waste leachate was collected from Suwon Environment Affairs Agency, South Korea. Approximately 3 g of soil was added to each well of a transwell plate filled with 3mL of three various media (see components of each medium in the table 1). Similar steps were carried out with 3 mL of waste using the same media as above (Figure 1) [6]

![Figure 1. Structure of Transwell plate system](image)

(A) SEM of the surface of a 0.4 µm pore polycarbonate membrane. (B) Schematic diagram of transwell plate system. (C) Transwell plate with 6 wells

Three media were used in this study for isolation including media for Methanogens, Ethanoligenens, amylolytic which was used to culture a variety of fastidious organisms (Table. 1) [7]

| Components (mg L⁻¹)       | For methanogens | For Ethanoligenens | For amylolytic bacteria |
|---------------------------|-----------------|--------------------|------------------------|
| K₂HPO₄                    | 0.14            | 1.5                |                        |
| KCl                       | 0.34            |                    |                        |
| NaCl                      | 18              | 2                  |                        |
| NH₄Cl                     | 0.25            |                    |                        |
| CaCl₂.2H₂O                | 0.14            |                    |                        |
| MgCl₂.6H₂O                | 4               | 0.6                |                        |
FeSO₄·7H₂O 0.2
NH₄NO₃ 1
NaHCO₃ 5
Na₂S·9H₂O 0.5
Na-acetate 1
L-systeine HCl·H₂O 0.5
Fe(NH₄)₂SO₄·6H₂O (0.1% w/v) 2 mL
Glucose 10
Beef Extract 2
Peptone 2
Yeast Extract 2
Soluble Starch 2
Standard Salt Solution (mL L⁻¹) 50
Trace Element (mL L⁻¹) 10
Vitamin Solution (mL L⁻¹) 10

| Components                          | Standard Salt Solution | Trace Element | Vitamin Solution |
|-------------------------------------|------------------------|---------------|-----------------|
| MnSO₄·7H₂O                           | 41.41                  | 0.01          |                 |
| ZnSO₄·7H₂O                           |                        | 0.05          |                 |
| H₃BO₃                               |                        | 0.01          |                 |
| N(CH₂COOH)₃                         |                        | 4.5           |                 |
| CoCl₂·6H₂O                           |                        | 0.2           |                 |
| AlK(SO₄)₂                            |                        | 0.01          |                 |
| NaCl                                |                        | 41.41         |                 |
| NH₄Cl                               |                        | 0.05          |                 |
| CaCl₂·2H₂O                           | 61.14                  | 0.01          |                 |
| MgCl₂·6H₂O                           | 84.02                  |               |                 |
| Biotin                              |                        |               | 0.002           |
| Folic acid                           |                        |               | 0.002           |
| Thiamine                             |                        |               | 0.005           |
| Riboflavin                           |                        |               | 0.005           |
| Nicotinic acid                       |                        |               | 0.005           |
| Calcium pantothenate                 |                        |               | 0.005           |
| Pyridoxolhydrochlorid                |                        |               | 0.01            |
| Vitamin B12                          |                        |               | 0.0001          |
| Lipoic acid                          |                        |               | 0.005           |
| P-aminobenzoic acid                  |                        |               | 0.005           |

After incubating at 35 °C and 55 °C for one week and two weeks under both aerobic and anaerobic conditions, a series of dilutions of each culture later was spread on the agar plate for incubating at the same previous conditions. Picked up pure colonies were used as inoculums for further experiments of waste treatment.
2.2 Analytical Methods

For volatile fatty acid degradation examination, the facultative bacterial strains with strong growth have been screened their ability of substrate consumption to degrade volatile fatty acids or accelerate biogas production.

Serum 150-mL bottles with 100-mL working volume were set-up as anaerobic batch reactors running in triplicates. pH values were adjusted by 1M HCl and 1M NaOH and monitored using an pH meter (HI 2210). Each reactor was flushed with N₂ for 10 minutes after filling to make sure anaerobic conditions. All samples were incubated at 35 °C for 30 days and checking the concentration of VFAs in each period of 1-day, 7-day, 15-day and 30-day.

For VFAs production screening, all samples were operated at initial pH 6, pH 8, pH 10 and pH no control (without an adjustment).

Concentration of each VFAs component was determined based on its compositions of food wastes. Total concentration of VFAs was 585 mg/L including the three most important organic acids (acetic, propionic and butyric) [8]. After a screening, the VFA consuming strains were underwent the next examination using food waste leachate as a carbon source and energy under anaerobic conditions.

After exacting by Ether, volatile fatty acids were determined using a gas chromatograph (GC-6890N, Agilent Inc., USA) equipped with a flame ionization detector and a 30 m x 0.25 mm x 0.25µm fused-silica capillary column (DB-FFAP). The temperatures of the injector and detector were 250 °C and 300 °C, respectively. The oven temperature was initially at 70 °C for 3 min, followed by a ramp-up of 20 °C/min for 5.5 min and held at a final temperature of 180 °C for 3 min. Nitrogen was used as the carrier gas with a flow rate of 2.6 mL/min. Hydrogen was fuel gas, and synthetic air was oxidizing gas. Acetic, propionic, isobutyric, butyric, isovaleric, valeric, isocaproic, caproic acids were those VFAs which were detected by this method of gas chromatograph.

TCOD/SCOD were measured with LCK 514 COD cuvette tests by Hach Lange DR 5000 spectrophotometer. Total solid content (TS) and volatile solid content (VS) of the food waste were measured according to the Standard Methods [9].

2.3 Phylogenetic Analysis

To determine partial 16S rRNA sequences, the genomic DNA from three effective strains in this study were extracted according to a method described by [10]. The 16S rRNA was then amplified by PCR using the universal bacterial primer set 27F and 1492R [11]. A multiscreen filter plate (Millipore Corp, Bedford, MA, USA) was used to purify the PCR product, which was then sequenced using primers 518F (5’-CCA GCA GCC GCG GTA ATA CG-3’) and 800R (5’-TAC CAG GGT ATC TAA TCC-3’) by a PRISM BigDye Terminator v3.1 cycle sequencing kit (Applied Biosystems, Foster City, CA, USA). This process was carried out at 95 °C for 5 min, cooled on ice for 5 min, and then analyzed using an ABI Prism 3730XL DNA analyzer (Applied Biosystems, Foster City, CA, USA). Finally, SeqMan software (DNASTAR Inc., Madison, WI, USA) was used to assemble the nearly full-length 16S rRNA sequence. This sequence was compared with that of other bacteria using the EZBioCloud server (http://ezbio cloud .net/) [12].

3 Result and discussion

Sixty-two strains can grow well under both anaerobic and aerobic conditions at 35 °C but only ten of them can grow strongly at both 35 °C and 55 °C. After 15 days of incubation without pH control during the period of anaerobic digestion, seven strains illustrated their degradation of VFAs over 60% and reached the highest rate of 74.2% by strain VSE-1T isolated from soil, following by VSE-2T, VSE-3T, VSE-4T isolated from soil and digested sludge, with 71.4%, 70.7% and 66.7% respectively (Figure 2). From a result of sequencing, strain VSE-1T and strain VSE-2T have been found that they belong the genus
Bacillus which are closest to *Bacillus* CP003056-s 36D<sup>T</sup> and *Bacillus paralicheniformis* KJ-16<sup>T</sup> respectively. Strain VSE-3<sup>T</sup> is one member of the *Bhargavaea* genus which is closest to *Bhargavaea beijingensis* GE10<sup>T</sup>. Strain VSE-4<sup>T</sup> is closest to the *Bacillus coagulans* ACTT 7055<sup>T</sup>. *Bacillus* genus is not only abundant in the environment but the functions of its member also still open questions to attract interest in science.

![Figure 2](image-url)

**Figure 2.** The change of VFAs concentration and composition after 1-day, 7-day and 15-day incubation

In term of VFA production, strain WM3<sup>T</sup> illustrated the best candidate with VFA production yield of 13,000 mg. L<sup>-1</sup>, following by strain WM2<sup>T</sup>, WM4<sup>T</sup> and WM1<sup>T</sup> with 10,195 mg. L<sup>-1</sup>, 9,979 mg. L<sup>-1</sup> and 4125 mg. L<sup>-1</sup>, respectively. Phylogenically, they are closest to *Bacillus toyonensis* BCT-7112<sup>T</sup>, *Bacillus smithii* DSM 4216<sup>T</sup>, *Enterobacter* KK736235<sup>T</sup> and *Vagococcus acidifermentans* AC-1<sup>T</sup>, respectively.

pH has been investigated as a key parameter on VFA production in both mixed and pure culture [13]. Thus, a range of pH value was set up to 6, 8, 10 and pH no-control with incubation time (day 1, 5, 10 and 15). The effect of pH on performance and composition of VFA was given in the Figure 3 and Figure 4. In previous study, alkali pH enhances the hydrolysis of complex substrates [14-15]. However, in the other research, it revealed that the performance metabolism is higher under neutral pH [16]. In our study, the highest VFA was obtained when using an initial pH 8, following by the value of pH 6 and pH 10. pH-no control (without adjusting pH), pH 5 and pH 10 showed the lower VFA production in all strains. Especially, there is no VFA production after one day of incubation at pH 10 compared to the other pH
values under the same other operational conditions but VFA started to produce from the second day. Moreover, our results showed that initial pH value has an important determination on VFAs compositions as other research mentioned also [17]. The production of total fatty acids in all cultures at different pH achieved a difference in acid domination. In our research, acid acetic was dominant at all pH values. At pH 8, acetic acid was account for 78.05% as a highest value, following by propionic acid with 15.85% and 4.15% of butyric acid (Figure 3).

![Figure 3. VFA production of potential bacterial strains at pH 8](image)

Base on literature, acetic acid can be directly utilized by aceticlastic methanogens while other acids need a collaboration of three groups including syntrophic bacteria, hydrogenotrophic and aceticlastic methanogens to produce methane.
Figure 4. VFA production of effective bacterial strains at a variety of pH values

While a mixture and butyric only started to produce and increased dramatically at alkali condition. Under acidic condition (pH 5) just one or a mix of two compositions were produced. After 15 days in the rest of experimental period, there was no significant pH change without effect on VFA production. Thus, we suggest that pH was the responsible parameter for not only the VFA production but VFA compositions also. Even the previous study investigated that the pH was negatively correlated with VFA concentration [18] or some ones mentioned its affect on VFA concentration only.

4 Conclusion
The results of this study illustrated that the dependent-culture approach of the isolation has given a valuable source for exploring the functions of individual microorganism in lab-scale. These effective bacterial strains will be examined in further studies for optimizing the VFA degradation and production. It is difficult to define a definite optimal operation parameter for all case due to the complex relationship of microbial community. In the next studies, each of these strains will be tested its ability in a variety of operation conditions to investigate the optimal the domination of target VFA compositions which enhance the hydrogen and methane production as to complete an anaerobic digestion of waste stream.

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