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Production of Biofuels (H_2&CH_4) from Food Leftovers via Dual-Stage Anaerobic Digestion: Enhancement of Bioenergy Production and Determination of Metabolic Fingerprinting of Microbial Communities

Gamal K. Hassan*[1], Aly Al-Sayed[1], Ahmed A. Afify[2] , Mohamed Azab El-Liethy[1], Sherien Elagroudy[3], Fatma A.El-Gohary[1]

[1]*Water Pollution Research Department, National Research Centre, 33 Behooth St., P.O. Box 12622, Dokki, Giza, Egypt*

[2]*Canal Higher Institute of Engineering and Technology, Chemical Engineering Department, Suez, Egypt*

[3]*Egypt Solid Waste Management Center of Excellence, Ain Shams University, Cairo, Egypt*

Abstract

Proper management of food waste has become a major cause of concern over the past few years in both developed and developing countries. This work aimed to properly treat food leftovers through 2-stage anaerobic fermentation at mesophilic temperature for biofuel production (H_2 and CH_4) and correlate this with the metabolic fingerprints of the most dominant bacterial strains in the two biofermenters. The diversity of bacterial communities in the surface, middle and bottom levels of the hydrogen fermenter as well as in the methane fermenter was examined. Moreover, the phenotypic identification and metabolic fingerprints for the bacterial strains was carried out using Biolog GEN III. The dominant bacterial strain responsible for hydrogen production was *Bacillus amyloliquefaciens.* While, *Stenotrophomonas rhizophila* was the most dominant in the methane fermenter. The total energy production was improved by 22.2% in case of increasing HRT for the first fermenter from 17h to 34h.

Keywords: Food Leftovers; Bacterial Consortium; Bioenergy; Biohydrogen; Biomethane.

1. Introduction

According to Ministry of State of Environmental Affairs (MSEA) reports, the production of wastewater [1], especially industrial wastewater [2] and solid wastes (SW) in Egypt becomes a big problem and should be solved. SW in Egypt was around 22 million tons in year 2015. Cairo, Capital of Egypt, is considered one of the most air polluted cities over the entire world. This pollution could be revealed to open burning of the solid wastes including food leftovers (FL) [3–5]. SW production has been increasing due to population growth, industrialization and urbanization [6]. Worldwide, FL represents approximately 32% of all produced food and also about 1.3 billion tons of solid waste produced each year as a part of meats, dairy product, vegetables, bread and others [7]. In Egypt, no regular official data about the FL quantities is available. FL, among MSW, is typically being disposed in landfills due to the limited financial resources. The landfill disposal of these wastes have high environmental risk due to its high organic content which can cause health hazards problems, contributing to air, soil and groundwater pollution [38]. Several conventional methods for FL up-cycling exist such as animal fodder, composting, and anaerobic digestions (AD) [8,9]. The anaerobic digestion is considered one of the most suitable processes for FL treatment and its management as well [10,11,39]. This method has dual benefits through FL disposal by eco-friendly way and biogas production. This advantage can help the world for solving the problem of the energy scarcity [12]. FL co-digestion through addition of bio-solids under anaerobic condition is capable to enhance biogas production by 80% compared to mono-digestion [7]. FL contains proteins, carbohydrate, oil, mineral and

*Corresponding author e-mail: gk.hassan@nrc.sci.eg; gamal_kamel9@yahoo.com.; (Gamal K. Hassan).
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fat, all of them can be breakdown by a wide range of microbes through enzymatic hydrolysis [13]. Microorganisms particularly bacteria play a vital role in biogas production from anaerobic digestion [14]. That carried out through four successive phases by breakdown the complex organic molecules into simple molecules and producing CO_2, H_2 and CH_4 gases as an end product [15,16]. The initial phase starts by hydrolysis of complex organic matters such as starch, carbohydrate and proteins into simple monomers such as sugars and amino acids followed by fermentation phase of these monomers into alcohols and carboxylic acids combined with acetogensis phase of H_2 and CO_2. Finally, methane is produced through methanogensis phase from H_2 and CO_2 consumption under anaerobic condition [16,17]. The operation conditions of the fermenters such as microbial load, pH, temperature, mixing, retention time and organic loading rate (OLR) are very important parameters to enhance the degradation efficiency processes [18,19]. Fermentative hydrogen production has been performed using a variety of global fermentation systems [20] and could be dynamically produced from different co-digestion between different substrates [21]. In this study, Upflow Anerobic Biofilter Fermenter (UABF) as a simple technique for food leftovers (FL) treatment and bioenergy production using bacterial consortium as eco-friendly procedure depending on multi-stages fermentation.

2. Materials and methods

2.1 Sources and characterization of food leftovers (FL)

The fermenter was continuously fed with FL that was collected from fast food restaurants around Dokki area, Giza Governorate, Egypt. Collected FL was checked to remove inorganic components (glass, metals and plastics), if any FL, consisting of bread, vegetables, fruits and meats, was well homogenized using a blender then sieved through 0.6 mm pore size. The homogenized FL was diluted with water according to the required OLR. Some physicochemical parameters of substrate were determined to stimulate the actual conditions. The average values of pH, COD_{tot}, BOD_{tot}, TSS, VSS, T.VFA and total carbohydrates were 4.0, 9.2 gO_2/L, 2.8 gO_2/L, 2.1 g/L, 276 mg-acetate/L, and 3.9 g-glucose/L, respectively.

2.2 Fermenter design

The Up-flow Anaerobic Bio-filter Fermenter (UABF) consists of dual stages with 4.6 L working volume for each fermenter in addition to feed supplies and temperature controllers (Fig. 1). This fermenter considered as a new version of w8-anaerobic digester. Fermenter number 1 was used for hydrogen production through a volumetrically hydrogen collector using CO_2 displacement scrubbing column. Fermenter number (2) was used for methane production through gas collector number 2 with 1 M NaOH to scrubbing CO_2. The collected gas was exhausted from the vessel and the volume refilled with water during operation. Sampling points for liquid and gases are located at strategic points around the fermenters. Non-return valves and liquid seal syphon breaks are included in the process pipe work to ensure the operation of each fermenter at constant volume, without the entrance of air or the danger of accidental symphonic action. The advantage of this system and its recommendation as well to use the two-stage fermentation, after the two-stage fermentation of the diluted food waste, the removal of the organics would be higher [22].

The dual-stage biofermenter was inoculated with anaerobic sludge collected from a local wastewater treatment plant. The total solids (TS) and total volatile solids (TVS) of the inoculated sludge were 10.0 and 8.2 g/L, respectively. The inoculated sludge was heated up to 100ºC for an hour to inactivate any hydrogen-consuming microbial agents in the hydrogen fermenter [23]. This thermal treatment will enrich the spore-forming bacteria.

2.4 Isolation of microorganisms

In addition, the most frequent bacterial strains from the two biofermenters were isolated and identified. This reactor is a fixed bed reactor and contains many non-homogenous layers therefore the bacterial strains were isolated from three different sampling sites of the hydrogen fermenter; bottom, middle and the surface level of the fermenter. In addition a composite sample was collected from the methane fermenter. The morphological characteristics of bacterial microorganisms were carried out using spread plate method onto plate count agar (PCA). The samples were collected from the fermenters daily for the first three days after the condition optimization then weekly for five weeks in sterile bottle and transferred to Microbiology Lab., National Research Centre within 15 min. The bacterial microorganisms were isolated onto plate count agar (Oxoid, UK) using spread plate method. Approximately, 100 µL from the tested sample was spread onto the plates. The plates were incubated at 37ºC for 48h under anaerobic condition in anaerobic jar. While the inoculated plate by the sample collected from the surface of hydrogen fermenter was incubated at 37ºC for 48h under aerobic condition. The most frequent bacterial isolates were

selected for identification depending on morphological features.

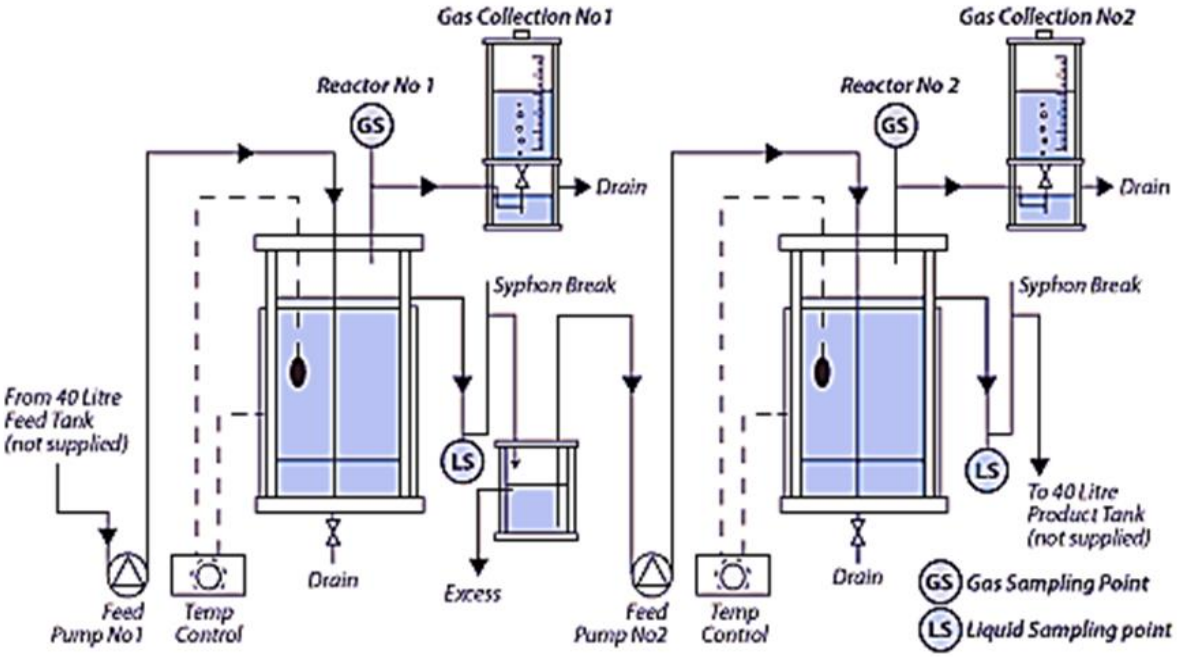


Fig. 1. Schematic Diagram for the multi-stages fermenters

2.3 Inoculation of microorganisms

2.5 Identification of microorganisms

The phenotypic identification for the bacterial isolates was carried out using Biolog GEN III (Biolog Inc, USA).The pure bacterial colony was streaked onto tryptic soy agar (BD, Germany) and incubated at 37ºC for 24h. A loopful of the single bacterial colony was inoculated into 10 ml of inoculating fluid (IF) according to protocols A (for aerobic isolates) or B (for anaerobic isolates). Then 100 µl from IF was dispensed onto each well of a 96 wells microplate. The inoculated microplate was incubated at 37ºC for 24h under anaerobic condition except the bacterial isolates originated from the surface of hydrogen fermenter was incubated at 37ºC for 24h under aerobic condition. The reading was carried out automatically by the computerized MicroStation™ system (Biolog Inc, USA) with the fingerprint data which was previously fed into the software (OmniLog® Data Collection) [24].

2.6 Packing Materials and Inoculum

Fig. 2 shows the packing material with the shape of bio-balls inside the fermenter with diameter 25mm each. The capacity of the empty fermenter was 5.5L and that of the packed fermenter was 4.6L. The packing material constituted 16.4% of the working volume of each fermenter.

Fig. 2. Bio-balls configured as Packed Bed (PB) for the fermenter

2.7 Experimental phases

During fermentation three distinct experimental phases were evaluated as below:

- The pH of the FL used in the fermenter after dilution by factor (1 FL: 20 water) was found to be around 4.0. To simulate existing conditions, the

first run was operated without changing the pH of the feed entering the hydrogen fermenter. The pH of the effluent of the hydrogen fermenter was elevated to pH 8.0 by using 1M NaOH prior to entering the methane fermenter. To study the impact of increasing the pH on hydrogen production and consequently on the energy yield, the second run was operated at pH 5.5 for the first fermenter and the pH of the second fermenter was constant (8.0). The temperature used was 35°C±0.5°C to stay within the mesophilic condition and the HRT was constant (17h).

- The objective of the third run is to study the impact of increasing the HRT on hydrogen production and consequently on the energy yield for 35 days. The first fermenter was operated at HRT 34h while the second fermenter was operated at constant HRT (17 h). The temperature used was 35°C±0.5°C and the pH was kept constant at 5.5 for the first fermenter based on the results from the previous runs. The three runs were a consecutive manner.

2.8 Analytical methods

Determination of COD, BOD, TSS, VSS and total volatile fatty acids (T.VFA) for the substrate before and after treatment were carried out according to [25]. Carbohydrates were measured using the phenol–sulfuric acid method, within preparation glucose as standard [26]. Biogas composition including H_2, CH_4, H_2S, CO_2 and O_2 was determined using portable biogas instrument entitled 5000 gas analyzer (Geotech, Geotechnical Instruments (UK) Ltd, England).

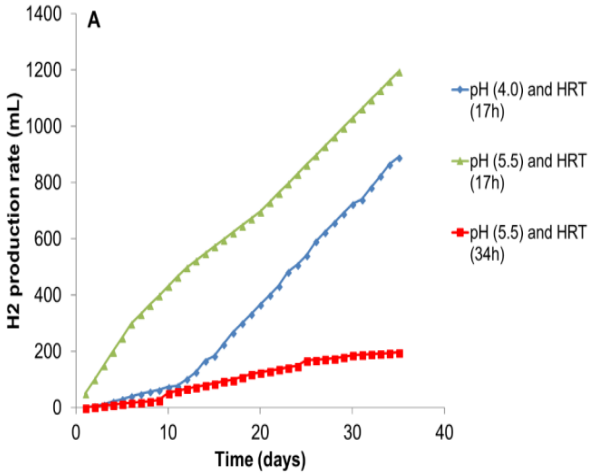
3. Results and discussions

3.1 Hydrogen and energy yields at different pH and HRT for UABF

3.1.1 Performance of the first bio-stage: Hydrogen fermenter

Fig. 3 shows the measured cumulative hydrogen and methane gases for the first fermenter at different operating conditions. The system was operated continuously for a period of 35 days for the three runs in a successive mode. Daily observation of the biogases produced indicated that total biogas production varied according to the operating conditions. Analysis of the biogas produced, during the first phase (pH=4.0 and HRT 17h conditions), indicated that up to the ninth day no methane gas was detected and the biogas produced consisted mainly of hydrogen. The hydrogen percent reduced during the experiment and this reduction due to formation of methanogenic bacteria inside the cake which formed around the packing materials [27]. During this period, average hydrogen production (HP) and hydrogen production rate (HPR) were 25.14 mL/d and 3.9 mL/L.d and average methane production (MP) and methane production rate (MPR) were 11.4 mL/d and 1.8 mL/L.d for the same fermenter**.**

Following this experiment, the pH of the substrate entering the hydrogen fermenter raised up to 5.5. The results of measurement of the accumulative gas are overlaid on the same Fig. 3. A gradual increase in hydrogen production up to 50% of the total biogas produced is noticed. During this 2nd experimental setup, average HP and HPR reached 34.1mL/d and 5.7 mL/L.d and average MP and MPR reached 34.2 mL/d and 5.3 mL/L.d which is lower than 45 mL/d reported by Li et al. who used CSTR for hydrogen production from diluted FL with kitchen wastewater at the same pH and concentration [28]. Based on the results of the first experimental run, the hydrogen fermenter was operated at pH 5.5. To assess the effect of increasing the HRT on hydrogen yield, the HRT in the first fermenter was increased from 17h to 34h.This lead to a reduction in HP and HPR to 6.0 mL/d and 1.9 mL/L.d. However, an increase in MP and MPR to 70 mL/d and 21.9 mL/L.d respectively was noticed. It is important to emphasize that the average hydrogen content in the total produced biogas was 8% at (HRT=34h) which is noted to be inferior to that produced at the shorter HRT that accounted of 34%. A similar trend of hydrogen production efficiency has been reported by Farghaly et al. after increasing HRT from 24h to 48h [29].



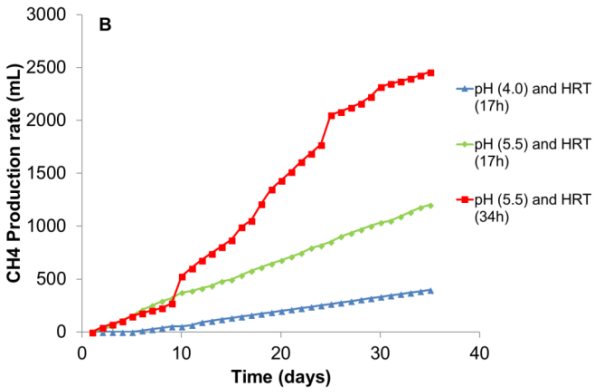


Fig. 3. Impact of pH and HRT on A- hydrogen and B-methane production from the first stage (hydrogen fermenter)

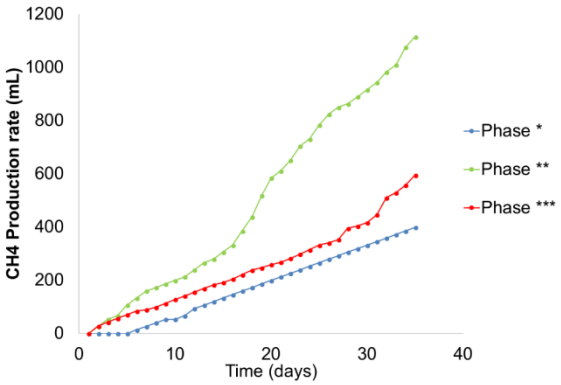


Fig. 4. Impact of changing the pH and HRT in the first fermenter on the performance of the second fermenter.

3.1.2 Performance of Second bio-stage: Methane fermenter

The methane (CH_4) production rate from the second fermenter at different operating conditions is illustrated in Fig. 4. The effluent of the hydrogen fermenter, operated once at pH 4 and then at pH 5.5 with the same HRT (17h) were fed continuously to the second fermenter after pH adjustment at 7.5. When the hydrogen fermenter was operated at pH 4, average MP and MPR in the methane fermenter were 11.4 mL/d and 1.8 mL/L.d which accounts for 85% of the total biogas produced. Increasing the pH of the hydrogen fermenter from 4 to 5.5 increased the average MP and MPR in the methane fermenter to 31.8 mL/d and 4.9 mL/L.d respectively. This increase in methane gas production is attributed to the increase in hydrogen and VFAs production at pH 5.5 because it acts as a substrate for methane production. This is in agreement with the findings of Zhu et al. [30]. Furthermore, doubling the HRT in the first fermenter did not improve methane production rate in the methane fermenter due to the lower amount of the hydrogen production in this stage however, MP and MPR in this phase were 17 mL/d and 2.7 mL/L.d.

3.2 Performance of the dual stages UABF fed with FL at different pH and HRT

Characteristics of the two fermenters effluents at different operating conditions are presented in Table 1. The second fermenter operated at constant HRT (17h) in all phases and the differential operating conditions was subjected to the only first fermenter.

Under the same dilution of the real FL, the results show that the COD values were reduced by 58.3% for the first fermenter with operating conditions pH 4.0 and HRT 17h and this reduction increased to 62% after changing pH to 5.5. For the third phase, the COD was reduced by 72.8% after changing HRT to 48h at the same pH. Switching to increasing HRT from 17h to 34h in the first fermenter, the reduction of all parameters was similar. Over 90% of the remaining COD was removed in the methane fermenter for all phases. The extent of carbohydrates degradation was not affected by changing the operating conditions of the two fermenters (pH and HRT) as indicated in Table 3. Residual carbohydrates in the final effluent stage from the methane fermenter indicated that more than 90% of the carbohydrates have been consumed in the methane fermenter. The TSS and VSS concentrations also noticed a reduction 75% as lower as in the hydrogen fermenter that continued in the methane fermenter. The consumption and large reduction in these characteristic parameters in the two fermenters strongly assert the efficiency of the undertaken essay of the anaerobic process. The VFAs concentrations of the first fermenter were 817 and 837 mg/L, for pH 4.0 and 5.5 respectively, with same HRT. When increasing the HRT to 34h, VFAs increased to 1093 mg/L .Application of the second fermenter did not only increase the energy production but also reduced the accumulation of the VFAs in the fermenter. The VFAs concentration in the second fermenter in the first, second and third phases decreased to 434, 102 and 226 mg/L, respectively. Previous investigations reported similar trends [31].

3.3 Identification of the most frequent bacterial isolates in the biofermenters

The breakdown of the organic substances to methane from organic waste during biological processes is facilitated through sequential processes by three bacterial communities [32]. The first bacterial community (*Burkholderia vietnamiensis*) is able to ferment the organic substances into fatty acids by producing extracellular enzymes. The second bacterial community (*Bacillus amyloliquefaciens*) is acidogenic bacterial group and able to oxidize the fatty acids into formic acid, acetic acid, H_2 and CO_2. The third bacterial community (*Stenotrophomonas rhizophila*) is methane producing bacterial group and capable to convert formic acid, acetic acid, H_2 and CO_2 into methane (50-75%) and CO_2 (19-34%) in anaerobic digestion [33]. Therefore in this study it was remarkable to identify the bacterial strains responsible for hydrogen and methane production in the two stage fermenter. Figure 5 shows that 84% of the isolated bacteria from the biomass samples collected from the surface of hydrogen fermenter were identified as *Burkholderia vietnamiensis*. This bacterial strain is able to destroy sugar and amino acid with the end products of hydrogen, CO_2, butyrate, and propionate. *Burkholderia vietnamiensis* is Gram negative, motile and utilize wide range of sugar, organic and carbohydrate as substrate and produce hydrogen and also able to reduce nitrate to nitrite [34]. Moreover it well known that *Burkholderia vietnamiensis* is responsible for the acidogenesis process in anaerobic digestion [35]. In the present study *Bacillus amyloliquefaciens* was the most frequent bacteria in the biomass samples which collected from middle level of the hydrogen fermenter with incidence percentage reached to 43% (Fig. 2). In previous study carried out by Liliane et al. found that *Bacillus amyloliguefaciens* was competent to produce biohydrogen yield with values of 0.50 ± 0.20 mol H_2/mol from glycerol [36]. In this study Stenotrophomonas rhizophila was the most dominant bacterial strain in the biomass samples collected from the bottom of the hydrogen fermenter with percentages reached up to 64% (Fig. 5).

Table 1. Performance of the two stage UABPF fed with FW at the same dilution (1 FW: 20 Water) with different pH and HRT

A- First phase***

Parameter	Unit	Influent	Effluent-R*	%Removal*	Effluent-R**	% Removal**
pH	-	5.5	5.4	-	7.5	-
COD	gO_2/L	7.2 ± 1.02	2.9 ± 0.96	58.3 ± 13.9	1.53 ± 0.31	47.5 ± 25.9
BOD	gO_2/L	3.4 ± 0.79	1.6 ± 0.96	54.7 ± 16.9	0.84 ± 0.39	48.5 ± 26.5
TSS	g/L	1.6 ± 0.23	0.58 ± 0.29	61.3 ± 20.9	0.38 ± 0.24	43.2 ± 19.4
VSS	g/L	1.3 ± 0.22	0.43 ± 0.23	63.3± 23.5	0.24 ± 0.20	53.0 ± 13.3
VFA	mg-acetate/L	449 ± 105	817 ± 281	-	434 ± 150	42.1 ± 8.7
Carbohydrates	g-glucose/L	3.5 ± 0.54	1.9 ± 0.55	43.4 ± 14.4	1.12 ± 0.61	51.6 ± 16.0

*First fermenter; **Second fermenter; ***pH =4.0 and HRT = 17h for first fermenter

B- Second Phase***

Parameter	Unit	Influent	Effluent-R*	%Removal*	Effluent-R**	% Removal**
pH	-	5.5	5.1	-	7.5	-
COD	gO_2 /L	9.03 ± 2.4	3.3 ± 0.72	62 ± 4.3	0.19 ± 0.05	93 ± 3.0
BOD	gO_2 /L	5.9 ± 2.0	2.35 ± 0.45	55.9 ± 19.2	0.16 ± 0.04	92 ± 2.3
TSS	g /L	2.2 ± 0.52	0.94 ± 0.18	57.5 ± 2.8	0.03 ± 0.02	96 ± 2.7
VSS	g /L	1.7 ± 0.77	0.9 ± 0.26	51 ± 6.9	0.02 ± 0.01	96 ± 3.4
VFA	mg-acetate/L	126 ± 7.3	837 ± 82	-	102 ± 13.5	87.8 ± 2.0
Carbohydrates	g-glucose/L	3.6 ± 0.46	1.9 ± 0.61	45.7 ± 12.4	0.15 ± 0.06	92.2 ± 5.0

*First fermenter; **Second fermenter; ***pH =5.5 and HRT = 17h for first fermenter

C-Third phase***

Parameter	Unit	Influent	Effluent-R*	%Removal*	Effluent-R**	% Removal**
pH	-	5.5	5.1	-	7.5	-
COD	gO_2 /L	11.4 ± 2.5	3.1 ± 0.63	72.8 ± 5.1	0.25 ± 0.07	94 ± 2.0
BOD	gO_2 /L	5.6 ± 1.4	2.0 ± 0.45	64.3 ± 3.3	0.13 ± 0.06	92 ± 4.5
TSS	g /L	4.5 ± 2.5	1.4 ± 0.4	64.6 ± 15	0.16 ± 0.07	87.3 ± 7.9
VSS	g /L	3.1 ± 2.4	1.05 ± 0.3	56.7 ± 19	0.08 ± 0.02	91 ± 4.6
VFA	mg-acetate/L	170.6 ± 77	1093± 300	-	226 ± 68	78.5 ± 3.0
Carbohydrates	g-glucose/L	4.3 ± 1.3	1.8 ± 0.16	55.6 ± 12	0.20 ± 0.05	88.7 ± 2.8

*First fermenter; **Second fermenter; ***pH =5.5 and HRT = 34h for first fermenter

Stenotrophomonas rhizophila also was the most dominate (64%) in methane fermenter which is known as acetate-degrading denitrifiers and is playing an important role in methane production [35].

Metabolic fingerprints of the most dominant bacterial strains in the two biofermenters are shown in table (2). Hydrolysis of the dominant bacterial strains for the 71 different carbon and 23 chemical sources were varied between each strain. *Burkholderia vietnamiensis*, *Bacillus amyloliquefaciens* and *Stenotrophomonas rhizophila* were able to grow at pH 5 and 6, 1% NaCl,1%Sodium Lactate,L-Mallc Acid, Tetrazollum Violet and blue, GuandineHCl and Aztreonam. Moreover, they intermediate hydrolysis for N-Acetyl-D-Glucoseamine, A-D-Glucose, D-Mannose, D-Fractose, L-Serine, L-Lactic Acid and Tween 40 (Table 2).

3.4 Hydrogen, Methane and Energy yields from fermentation of dual stages UABF at different pH and HRT

Table 3 shows the average energy yield of FL using the heating values of 120 kJ/g and 50.0 kJ/g for hydrogen and methane [31]. The best Hydrogen yield was at pH 5.5 and HRT 17h with value of 4.2 ml/g-$COD_{consumed}$ from FL. Increasing pH from 4.0 to 5.5 led to 42.7% increase in the energy yield produced from the first fermenter due to the increase in hydrogen and methane production.

Table 2. Metabolic fingerprints of the most dominant Burkholderiavietnamiensis(S1), Bacillus amyloliquefaciens (S2) and Stenotrophomonasrhizophila (S3) bacterial strains in two fermenters

Properties	Results			Properties	Results			Properties	Results			Properties	Results		
	S1	S2	S3		S1	S2	S3		S1	S2	S3		S1	S2	S3
Negative Contro	-	-	-	A-D-Glucose	+/-	+/-	+/-	Gelatin	-	+/-	+	P-Hydroxy-Phenylaceti c Acid	+/-	-	-
Dextrin	-	+/-	+/-	D-Mannose	+/-	+/-	+/-	Glycyl-L-Prolline	-	+/-	+/-	Methyl Pyruvate	-	+/-	+/-
D- Maltose	-	+/-	+/-	D-Fractose	+/-	+/-	+/-	L-Alanine	+	+/-	-	D-Lactic Acid Methyl Easter	-	+/-	-
D-Trehalose	-	+/-	-	D-Galactose	+	+/-	-	L-Arginine	+	+/-	-	L-Lactic Acid	+/-	+/-	+/-
D- Cellobiose	-	+/-	+/-	3 Methyl Glucose	-	+/-	-	L-Aspartic Acid	+/-	+	-	Citric Acid	+	+/-	+/-
Gentiobiose	-	+/-	-	D-Fucose	-	+/-	-	L-Glutamic Acid	+	+	-	A-Keto-Glutaric Acid	-	+/-	-
Sucrose	+/-	+/-	-	L-Fucose	+/-	+/-	-	L-Histidine	+	+	-	D-Mallc Acid	+/-	+/-	-
D- Turanose	-	+/-	-	L-Rhamnose	-	+/-	-	L-Pyroglutamic Acid	+/-	+/-	-	L-Mallc Acid	+	+	+
Stachyose	-	+/-	-	Inosine	-	+/-	-	L-Serine	+/-	+/-	+/-	Bromo-Succinic Acid	-	+/-	-
Positive control	+	+	+	1%Sodium Lactate	+	+	+	Lincomycin	+	-	+	Nalidixic Acid	+	-	-

pH 6	+	+	+	FusidicAcid	-	-	-	Guanidine HCl	+/-	+	+	Lithium Chloride	-	+	+/-
pH 5	+	+/-	+	Serine	-	+	-	Niaproof 4	+/-	-	+	Potassium Tellurte	+	+	-
D-Raffinose	-	+/-	-	D-Sorbitol	+/-	+/-	-	Pecin	-	+/-	-	Tween 40	+/-	+/-	+/-
α-D-Lactose	-	+/-	-	D-Mannitol	+/-	+/-	-	D-Galacturonic	+/-	+/-	-	γ-Amino-Butryric Acid	+	+/-	-
D-Mellbiose	-	+/-	-	D-Arabitol	+/-	-	-	L-Galactonic Acid Lactone	-	-	-	α-Hydroxy-Butyric Acid	-	+	+/-
β-Methyl-DGlucoside	-	+	-	Myo-inostol	+	+/-	-	D-Gluconic Acid	+	+/-	-	β-Hydroxy-D,L-Butyric Acid	+/-	+	-
D-Sallcin	-	+/-	-	Glycerol	+/-	+/-	-	D-Glucuronic Acid	+/-	+/-	-	A-Keto-Butyric Acid	+/-	+	-
N-Acetyl-D-Glucoseamine	+/-	+	+/-	D-Glucose 6-PO_4	+/-	+/-	-	Glucuronamide	+/-	+/-	-	Acetoacetic Acid	-	+/-	+/-
N-Actyl-β-D-Mannosamine	-	+/-	-	D-Fructose 6-PO_4	+/-	+/-	-	Mucic Acid	+	-	-	Propionic Acid	+	-	+/-
N-Actyl-D-Galactosamine	-	+/-	+/-	D-Aspartic Acid	-	+/-	-	Quinic Acid	+	-	-	Acetic Acid	+	+/-	+/-
N-Acetyl Neuraminic Aci	-	-	-	D-serine	-	-	-	D-Scchric Acid	+	-	-	Formic Acid	+/-	-	-
1%NaCl	+	+	+	Troleandomycin	+	-	+	Vancomycin	+	-	+/-	Aztreonam	+/-	+	+
4%NaCl	+/-	+	-	Rifamycin SV	+	-	+	Tetrazollum Violet	+	+/-	+	Sodium Butyrate	-	+	-
8% NaCl	-	+	-	Minocycllne	+/-	-	-	Tetrazollum Blue	+	+/-	+	Sodium Bromate	-	+/-	-

(+): Positive hydrolysis. (+/-): intermediate hydrolysis. (-): Negative hydrolysis

In the third phase, in which HRT was increased from 17h to 34h with same pH, the hydrogen yield decreased to a very low limit of 1.0 ml/g-$COD_{consumed}$. In this phase, the total energy yield from the first stage fermentation increased by 55.5% due to the large amount of methane production and decreased to 42.5% for the second fermenter due to the lower amount of hydrogen produced from the first fermenter. This finding shows the success of increasing hydrogen yield by increasing pH from 4.0 to 5.5 and increasing total energy yields by increasing HRT and using fermenter with double-stage fermentation as well. Similar trends for increasing energy yields after using different double-stage systems were reported in literature [37].

Table 3. Hydrogen, methane and energy yields from FW by two stages fermentation of UABF at different pH and HRT

First Stage					Second stage	
Parameter	H_2 yield	EY[1]	CH_4 yield	EY[1]	CH_4 yield	EY[1]
Unit	ml/g-$COD_{consumed}$	kJ/g-$COD_{consumed}$	ml/g-$COD_{consumed}$	kJ/g-$COD_{consumed}$	ml/g-$COD_{consumed}$	kJ/g-$COD_{consumed}$
First phase*	4.1	38.6	1.9	59.6	5.7	178.8
Second phase**	4.2	39.5	4.2	131.7	7.4	232.2
Third phase***	1.0	9.4	12.0	376.4	4.3	133.3

[1] Energy yield (the yield was calculated at 35 °C and 1 atm).

* pH 4.0 and HRT 17 h for first fermenter - pH 8.0 , HRT 17h for second fermenter.

** pH 5.5 and HRT 17 h for first fermenter - pH 8.0 , HRT 17h for second fermenter.

*** pH 4.0 and HRT 17 h for first fermenter- pH 8.0 HRT 17h for second fermenter.

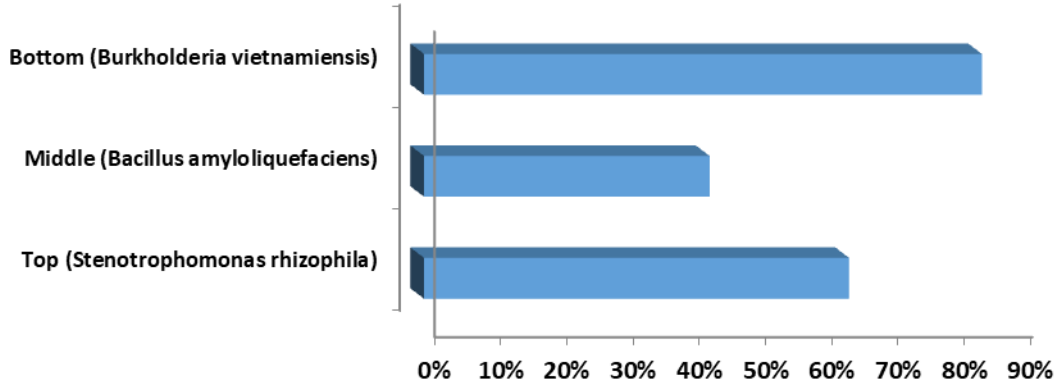


Fig. 5: The most dominant bacterial strains in samples collected from three levels of hydrogen fermenter.

4. Conclusions

- It is could concluded from this study that food waste could be treated by up-flow anaerobic bio-filter reactor and produce biofuels represented as hydrogen and methane.
- Increasing the pH form 4.0 to 5.5 in the hydrogen fermenter lead to increasing the methane production in the methane fermenter by a factor of 2.7.
- *Bacillus amyloliquefaciens* was isolated and identified in the hydrogen fermenter which confirms that FL as substrate have strong potential for producing the hydrogen gas.
- The total energy yields increased by 22.2 % after using double-stage fermentation system.

5. Conflicts of interest

There are no conflicts to declare.

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