Tofu Processing Wastewater Treatment using Anaerobic Fixed Bed Reactor with Bamboo as the Biofilm Carrier

Dissertation

Tofu Processing Wastewater Treatment using Anaerobic Fixed Bed Reactor with Bamboo as the Biofilm Carrier

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Contents

Abstract

Wastewater generated during coagulation using acid and sulfate coagulant in tofu processing has high organic concentration, low pH, and sulfate concentration. Almost all tofu industries in Indonesia discharge such wastewater without treatment, necessitating economical treatments to control water pollution. In this study, the feasibility of methane production from the treatment of tofu processing wastewater was investigated. Herein, anaerobic treatment using a one-stage and multistage fixed-bed reactor employing cut bamboo as the carrier was examined and compared to an upflow anaerobic sludge blanket reactor as a control reactor. Without neutralization, the fixed-bed reactor outperformed the upflow anaerobic sludge blanket reactor. A one-stage and multistage fixed bed reactors are potential as a low-cost treatment for methane recovery from tofu wastewater. On treating acidic tofu wastewater, one-stage and multistage reactors achieved maximum methane yield of 0.3, and 0.29 NL/g COD added, respectively. On treating sulfate-containing tofu wastewater, sulfide inhibition affects methane production in one stage reactor, resulting in 0.26 NL/g COD added. Methane generation dominates by hydrogenotrophic methanogenesis as a major pathway.

Chapter 1

Introduction

1.1. Background

Tofu or bean curd is a common food in Asian countries, and global demand is increasing due to its health benefits and low price [1]. Tofu is made by coagulating soy milk. There are many different types of tofu, such as extra firm, firm, soft, and silken tofu, and they are processed in various ways [2]. The primary process consists of soybean grinding (a) soaking, grinding, and cooking the soybeans; (b) filtration; (c) protein coagulation; (d) pressing and molding; and (e) packaging.

During tofu production, large amounts of wastewaters are produced as it is a water-intensive process. Wastewater from tofu production is generated from the soaking, washing, coagulation–pressing processes, and housekeeping [3]. The coagulation process is the most important step in the tofu-making processes that can influence the chemical composition of the wastewater. The types of coagulants usually used in tofu processing such as calcium sulfate, calcium chloride, magnesium sulfate, and magnesium chloride. Coagulation occurs due to the cross-linking of protein molecules in soymilk with the divalent cations [4].

In Indonesia, tofu is produced locally by approximately 84,000 small and medium-sized industries throughout the country. To produce 80 kg of tofu, approximately 2,700 L of water is required, which results in about 2,610 L of wastewater [5]. Many tofu industries in Indonesia use acid coagulants such as fermented whey or vinegar and calcium sulfate [6]. Therefore there are two types of tofu wastewater characteristics based on the type of coagulant; low pH wastewater produces from industries using an acid coagulant and sulfate-containing wastewater from industries using calcium sulfate coagulant. The COD of tofu wastewater ranged from 5,000-8,500 mg/L with a pH value of 3.6-5.5 [7][8]. There is no available

information about wastewater characteristics from tofu processing using sulfate as a coagulant in Indonesia. While in Taiwan, tofu processing wastewater containing sulfate concentration around 3,400 mg/L, COD 36,000 mg/L, and pH 5.8 [9].

Many tofu industries in Indonesia are home industry and tofu produced using traditional technology (as described in Fig. 1.1). A large volume of wastewater is generated from tofu industries and generally discharged directly into the environment without being fully treated, thereby emitting offensive odors, greenhouse gas emissions, pollution in water and soil. This condition represents a significant loss of resources and causes serious pollution problems since the wastewater has a high strength of organic pollutants.

Figure 1.1 Tofu factory condition in Sumedang Regency, West Java-Indonesia

The management of wastewater from tofu industries in Indonesia is currently needed. Improved environmental protection through the optimization of waste management practices focuses on waste management policies and technologies. Achieving reductions of organic

pollutants and maintaining economic competence are particularly challenging for small and medium-sized industries.

Biological treatment processes that can simultaneously exploit energy in biogas such as methane might solve both economic and environmental challenges for small and medium-sized industries. Due to tofu wastewater characteristics, the anaerobic digestion process is an attractive treatment solution for high-strength wastewater. Anaerobic wastewater treatment presents several advantages over conventional aerobic systems: minimum sludge production, low energy requirements (no aeration is required), and energy recovery from the methane gas produced in the process. Because many tofu industries in Indonesia still use biomass as fuel (wood, rice husks, and sawdust), the methane recovered from the industry can be used directly on-site as an alternative source of renewable energy for tofu processing.

In anaerobic treatment, it is necessary to cultivate immobilized biomass to increase the stability and performance of the reactor. Two of the most promising treatments are the upflow anaerobic sludge blanket (UASB) reactor and the anaerobic filter reactor implemented in the fixed-bed reactor (FBR). The granular sludge in the UASB reactor and the packing medium in the FBR serves as a filter that prevents bacterial washout and provides a larger surface area for faster biofilm development and improved methanogenesis [10][11]. UASB reactors have been used extensively by agro-food industries to treat various types of waste such as sugar, maize starch, wheat starch, breweries, slaughterhouses, dairy, and vegetable canning [10]. In a UASB reactor, a dense sludge bed is established at the bottom, where all biological transformations occur. Under favorable conditions, bacteria aggregate in flocs and granules with good settling properties so such that they are not washed out from the system [12]. However, a negative characteristic of the UASB reactor is its too long start-up period compared to FBR; typically 2–8 months are required for the development of an anaerobic-granular sludge [13]. FBRs have been used to treat different kinds of wastewater in the beverage, food processing,

pharmaceutical, and chemical industries due to their biosolid retention capacity [14].

However, high biofilm carrier costs prevent the use of this technology in developing countries. A bamboo carrier is easy to obtain in most tropical countries and has been utilized in the anaerobic treatment of slaughterhouse and cassava-starch wastewater [15][16]. The cost of the bamboo carrier is approximately 5–10% of that of commercial media. Since botanical carriers have a high affinity for biofilms compared to plastic media, a faster reactor start-up is expected, and both acid fermentation and methanogenesis may occur without a decrease in pH.

1.2. Literature review

1.2.1. Anaerobic digestion

Anaerobic digestion is a multistep process in terms of chemistry and microbiology. Organic material is degraded to primary constituents, finally to methane gas under the absence of an electron acceptor such as O_2.

Anaerobic digestion has been largely used in treating solid wastes, including agricultural wastes, animal excrements, sludge from sewage treatment plants and urban wastes, and it is estimated that millions of anaerobic digesters have been built through all the world with this purpose. Anaerobic digestion has also mainly been used to treat effluents from agricultural, food and beverage industries, both in developed and developing countries.

Compared to conventional aerobic methods, the anaerobic wastewater treatment concept indeed offers fundamental benefits as illustrated in Table 1.1

Table 1.1 Advantages and disadvantages of the anaerobic processes [17]

Advantages	Disadvantages
• Low production of solid, about 3 to 5 times lower than aerobic processes • Low energy consumption	• Anaerobic microorganisms are sensitive to inhibition by a large number of compounds

• Low land requirements • Low costs for construction • Produce methane gas, as a high calorific fuel • High organic loads tolerant • Can be applied on a small and large scale • Low consumption of nutrient	• The process start-up take along time • Post-treatment is usually needed • Generate bad odors, although they are controllable • Possible generation of effluents with unpleasant aspect • Unsatisfactory removal of nitrogen, phosphorus and pathogen

In anaerobic digestion, several microorganism groups work interactively to convert complex organic matter into final products (methane, carbon dioxide, hydrogen sulfide, water and ammonia, besides bacterial cells).

Although anaerobic digestion is generally considered a two phase process, it subdivided into several metabolic pathways, by the participation of several microbial groups, each with a different physiological behaviour, as illustrated in Fig. 1.2.

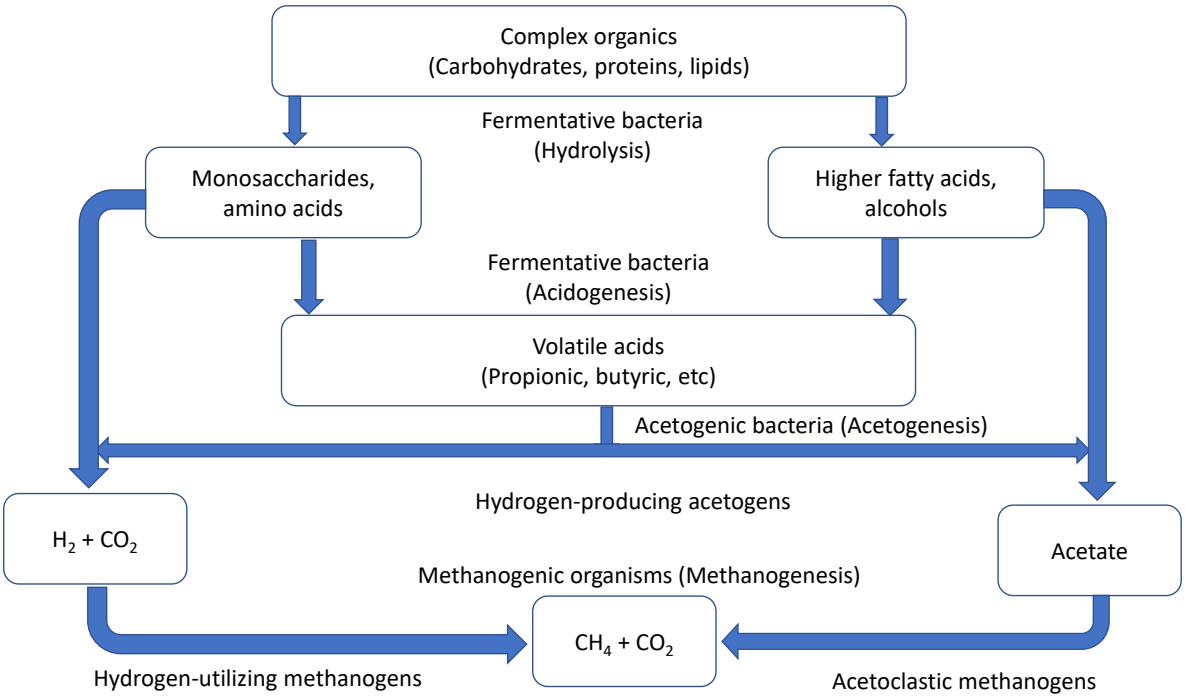


Figure 1.2 Metabolic pathways and microbial groups involved in anaerobic digestion [17]

The first step of anaerobic is hydrolysis and follows with acidogenesis, acetogenesis, and methanogenesis, respectively. The steps of anaerobic is described below in details [18]:

Hydrolysis: is the first phase of anaerobic digestion. It involves in the digestion of complex carbohydrates proteins and lipids into simpler substrates (break down process) such as sugars, amino acids and fatty acids. It is analogous to the functions carried out by the stomach in mammalian digestive systems. Hydrolysis bacteria include both facultative anaerobic microorganisms and strictly anaerobic microorganisms (obligate microorganisms). Hydrolysis bacteria are likely to resist environmental fluctuations such as temperature and pH changes, which strive in an acidic environment and have high reproductive rates and growth rates. It is not usually adversely affected by toxins and heavy metals which may be present in the feedstock. Since the hydrolysis step is required to treat raw particulate matter, it often is a rate-limiting step in the anaerobic process due to the difficulty of digesting these often complex substrates

The acidogenesis: is the second phase in the anaerobic food chain. Hydrolysis is followed by the acid-forming step of acidogenesis. Soluble organic components, including hydrolysis products, are converted into VFAs (propionic acid, lactic, butyric, succinic acids), ketones, aldehydes, formate, acetate, CO_2 and H_2 by the action of acid-forming (fermentative) bacteria known as acidogens. These organisms comprise a wide variety of different bacterial genera representing both obligate and facultative anaerobes. In a stable anaerobic digester, the main degradation pathway is through acetate, CO_2, and H_2, and the reduced fermentation intermediated play a minor role. This degradation pathway also gives a higher energy yield for the microorganisms and the products, which can be used directly as substrate by methanogenic microorganisms. The accumulation of products such as lactate, ethanol, propionate, butyrate and higer VFAs is the bacteria's response to increased hydrogen concentration. These products can be used directly by methanogens and must be degraded further by the obligate hydrogen-

producing bacteria in a process that refers to acetogenesis.

The products rely on the type of bacteria and environmental conditions such as temperature and pH. Microorganisms responsible for fermentation are *Bacteroids succinogens*, *B.fibrisolvens*, *rumen spirochete*, *Acetivibrio celluloyticus*, *clostridium, thrmocellum*, *clostridium butyricum,* etc. It relates to another group of both facultative and strictly anaerobic bateria that utilize the simple substrates provided by hydrolysis bacteria, metabolize these secondary compounds into water soluble organic acids, alchohols, and CO_2 and H_2 .

The acetogenic phase: is third phase in the anaerobic food chain. Both long-chain fatty acid (hydrolysis products) and volatile fatty acid (acidogenesis products) are converted to acetic acid, formate, H_2 and CO_2 by obligate Hydrogen Producing Acetogenic bacteria (OHPA). Acetogens are slow-growing and suffer from a thermodynamic product inhibition by H_2 or formate. Their growth rate depends on simultaneous removal of their own metabolic products that usually depend on the activity of methanogens. The reaction will proceed if the hydrogen partial pressure is low enough thermodynamically to allow the conversion. The presence of H_2 scavenging bacteria that consume H_2 thus lowering partial pressure, is necessary to ensure thermodynamic feasibility and the conversion of all acids. Therefore, the partial pressure of H_2 is an indicator of the performance of the digester.

The degradation of butyrate to acetate is not energetically feasible under standard conditions but is dependent on co-culture with a hydrogen-removing organism. The degradation of acetate to methane is thermodynamically feasible where H_2 serve as the metabolic link between a non-methanogenic and a methanogenic bacterium, as shown in the chemical reaction below:

$$CH_3CH_2COO^- + 4H_2O \leftrightarrow 2HCO_3^- + 4H_2 + H^+ \qquad \Delta Go' = +104.6\ kJ$$

$$4H_2 + CH_3COO^- + H^+ \rightarrow CH_4 + 3H_2O \qquad \Delta Go' = -135.6\ kJ$$

An increased hydrogen level inhibits the degradation of propionic and butyric acids due to its effect upon the thermodynamics of reaction, and therefore, can inhibit acetoclastic methanogens. Microorganism involved here are, *Syntrophomonas wolfei*, *Sytrophobacter wolinii*, Syntrophous *buswelii*, etc.

Acetogens that oxidize organic acids obligately use H_2 ions and CO_2 as an electron acceptor. The conversion of propionate and butyrate is important intermediate in the anaerobic fermentation of complex organic matter to the methanogenic substrates acetate and hydrogen. It is proven that bacteria can only derive energy for growth from these conversions if the concentration of the product is kept low. This results in an obligate dependence of acetogenic bacteria on methanogenic archaea or other hydrogen scavengers (e.g., sulfate reducers) for product removal.

Methanogenesis: is the final stage of anaerobic digestion generating methane in two ways: by methanogenic archaea either using cleavage of acetic acid molecules to generate CO_2 and CH_4 or by reduction of CO_2 with H_2 to yield CH_4 and H_2O. The microorganisms responsible for these conversions are strict anaerobes called methanogens and are identified in the literature as methanogens. Methane producers are not true bacteria but belong to an ancient group of microorganisms called the Archaea. The most important methanogenic transformations in anaerobic digestion are acetoclastic reaction and the reduction of CO_2

Methanogens have very slow growth rates, so they are usually considered as rate-limiting in the anaerobic organic waste treatment. Waste stabilization in anaerobic digestion is accomplished when CH_4 and CO_2 are produced. However, the ultimate yield of biogas depends on the composition and biodegradability of the waste feedstock. Still, its production rate will depend on the population of bacteria and archaea, their growth conditions, and the temperature of the system. Methanogens are likely to be affected negatively by potential toxins such as heavy metals, solvents, pesticides, and herbicides. Only a limited number of compounds can

act as substrates in methanogenesis among these are formate, acetate, H_2/CO_2, and methanol.

1.2.2. Fixed bed reactor (FBR)

The anaerobic fixed-bed is a type of filter reactor that has been widely used as a high rate anaerobic reactor to treat high strength effluent. These system have several advantages over aerobic and conventional anaerobic reactors such as: rapid start-up with a minimum operational problem, ability to withstand shock loading without a significant decrease in digestion efficiency, ability to adapt intermittent feeding and rapidly of restart after lengthy shutdown periods, lower hydraulic retention times.

The anaerobic filter or fixed bed reactor, is composed of one or more vertical filter beds containing some inert material, such as rocks or plastic media, which acts as a stationary support surface for microbial film attachment (Fig. 1.3). Wastewaters are pumped upwards through the support media, allowing contact between the attached microorganism and wastewater.

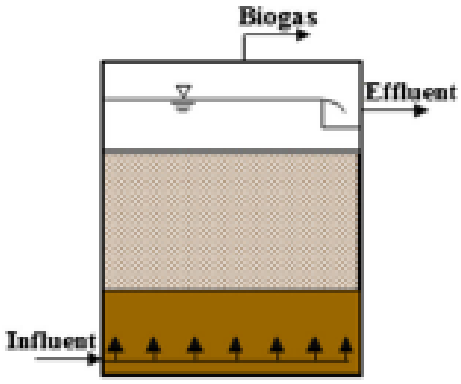


Figure 1.3. The fixed bed reactor

Microbial growth also takes place in the voids between the support media. This system permits an adequate mean cell residence time for the methane producing bacteria and still allows a short hydraulic retention time for system economy [19].

The FBR have been widely used for the treatment of high strength wastewaters. In the type

of anaerobic reactors, a large amount of biomass remains in the filter to secure solid retention despite short hydraulic retention times (HRT). These reactors have several advantages, such as higher organic loading, lower HRT and smaller reactor volumes. Lower sludge and suspended solid quantities can also be achieve in these reactors.

A fixed film digester is filled with an inert medium or packing providing an extensive surface area for microbial growth. The influent passes through the media and anaerobic microbes attach themselves to it, creating a thin layer of anaerobic bacteria called biofilm. This film gives the digester its name, a fixed film.

Compared to conventional units, fixed-film bioreactors perform efficiently at higher organic loading rates (OLR) due to more effective biomass retention in the reaction zone, yielding higher cellular retention times. Immobilized biomass anaerobic reactors also show better responses to organic shock loading and toxic inputs. In many cases, immobilized biomass reactors completely recover their performance after such deleterious occurrences.

1.2.3. Upflow Anaerobic Sludge Blanket (UASB)

The UASB reactor designed by Lettinga and his co-workers has made anaerobic digestion the most competitive and favorable treatment technology for high–strength organic wastewaters [20]. It has been widely employed to treat industrial and domestic wastes around the world due to features such as simple design, easy construction and maintenance, low operating cost, high removal efficiency, short retention time, stability, temperature and low energy demand [21]. A sludge blanket reactor is basically a dense blanket of granular or flocculated sludge placed in a reactor, which is designed to allow the upward movement of liquid waste through the blanket. The schematic diagram of a typical UASB reactor is shown in Fig. 1.4. In a UASB reactor, the influent enters through the bottom of the reactor, thereby helping in the aggregation of microbial biomass in the sludge bed and blanket to get in contact

with the influent.

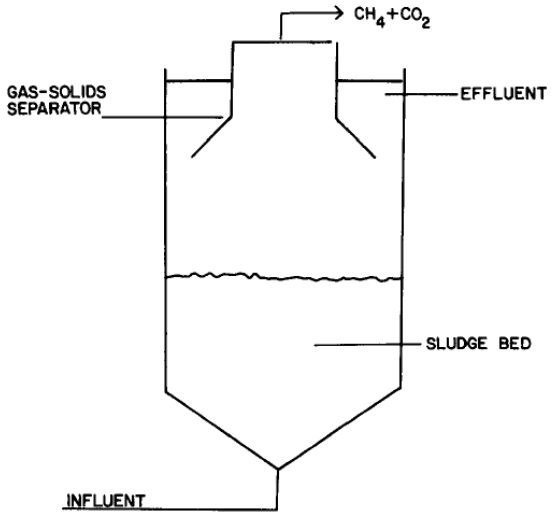


Figure 1.4 Schematic diagram of an upflow anaerobic sludge bed (UASB) reactor [19]

UASB reactors are highly dependent on its granular sludge as the core component during wastewater treatment for effective conversion of organic matter to biogas [22]. The key to successful operation of the UASB is to keep the sludge within the system (i.e. maintaining the solids without any support material). The active biomass is maintained within the reactor, and high solid retention time (SRT) values are maintained independently of HRT and without the need for support material. For proper operation, granular dense sludge particles must be developed with excellent settling properties. These particles are approximately 0.5-2.5 mm in diameter [19]. For these reasons and its ability to withstand the fluctuations in pH, temperature and influent composition that are so common in industrial wastewater, a full-scale USAB reactor has come into operation to treat various wastewaters since its introduction. UASB systems can achieve good settleability, low retention times, elimination of the packing material cost, high biomass concentrations (30,000-80,000 mg/L), excellent solids/liquid separation and operation at very high loading rates. This process's only limitation is related to the wastewaters having high solid content that prevents the dense granular sludge development. Design organic

loading rate is typically in the range of 4 to 15 kg COD/m^3.day.

1.2.4. Factors affecting performance anaerobic reactors and biogas production

- **Organic Loading Rate (OLR)**

The parameter that can affect affects the microflora and the performance of the anaerobic reactor is OLR. Fluctuations in organic load depend on the SRT, HRT, sludge properties, mixing intensity, duration of the variation, bacterial mass and activity. Different studies have shown that higher values of OLR can cause a reduction in COD removal efficiency in a wastewater treatment system have reported that a higher loading rate could cause unrecoverable acidification, suppression of the methanogenic activity due to serious imbalance between the methanogens and the acidogens, as well as inhibition of methanogens by VFA production.

- **Nutrients**

The ability of anaerobic microorganisms to grow depends on the availability of the essential nutrients that are present in the wastewater. Lack of these nutrients could negatively affect their growth and the efficiency of anaerobic degradation. The biochemistry of fermentation and CH4 production involves many enzymes that contain different trace elements that need to be supplied as nutrients. Each anaerobic microorganisms involved in the degradation of complex organic matter to simple components are trace element-specific, depending on the enzyme pathways.

Several studies on the impact of nutrients on the efficiency of AD and enhancement of granules in the bioreactors have been reported. Some bacteria, such as CH_4-forming bacteria in the reactors, have relatively high internal concentrations of iron, cobalt and nickel, which may not be present in sufficient concentrations in the wastewater produced from the industries. Therefore, the addition of trace elements prior to treatment to improved reactor performance is highly recommended. The optimum C: N: P ratio to enhanced CH_4 yield was reported to be

100:2.5:0.5. This could be calculated based on the wastewater biodegradable COD concentration, nutrient concentration in bacterial cells, and cell yield.

- **Hydraulic retention time**

The hydraulic retention time (HRT) has been defined as the average time that wastewater spends inside the reactor. The flow rate and wastewater composition entering the anaerobic reactor both affect the HRT. High HRT can increase the contact time of wastewater with the sludge, thus improving the effluent quality and biogas production rate. Therefore, a suitable HRT is important for proper wastewater treatment in a UASB reactor for better treatment efficiency as well as quality and quantity of biogas concentration.

Several studies have shown the effect of HRT on microbial degradation in a single UASB reactor treating different types of industrial wastewater.

- **Volatile Fatty Acids**

Volatile fatty acids (VFAs) are intermediate products in the formation of CH_4 that can determine the substrate removal efficiency from the reactor. The overload or sudden HRT variations could cause VFA accumulation and stressful conditions during the break down of complex organic matter. It can also affect the type of intermediates produced. This might cause a shift between acetogens and acidogens population (VFA producers), nitrogen reducing bacteria (NRB), sulphate reducing bacteria (SRB), and methanogens (consumers) leading to drastic changes in biogas production rates and compositions. The toxic effects of all VFAs in the AD process especially propionate, on acetogens and methanogens have been investigated. Therefore, VFAs should be monitored and parameters adjusted in order to avoid their accumulation in the UASB reactor to prevent the inhibition of methanogenic organisms, thus reducing biogas production.

- **Temperature**

In anaerobic degradation processes, the temperature is an important parameter that affects

microbial growth. The ideal operating temperature for an anaerobic reactor is in the range 30-35 ˚C. And another in the thermophilic range 50-55 ˚C). Most of the anaerobic reactors have been designed in the mesophilic range. In the case, this range is hardly reached, once the average temperature of the influent in warm temperature regions (20-28 ˚C). Under this sub-optimum temperature condition, the anaerobic reactor can be started up more easily with the inoculation of sufficient amounts of anaerobic sludge, preferably acclimatized to the type of sewage [23].

- **pH**

pH is an important parameter in the characterization and control of anaerobic digestion because of the inhibitory effects of low pH on the activity of anaerobic digestion bacteria. The optimum anaerobic operation in a pH range of 6.6 to 7.6. The standard operating method to keep the pH in this range has been the addition of lime and bicarbonate salts or reusing treated effluent in the reactor. Therefore, controlling the pH of the bioreactor is an essential factor for developing a diverse group of microorganisms and high reactor performance.

1.2.5. Substrate utilization in anaerobic sulfate-rich wastewater

The production of sulfide is the major problem associated with the anaerobic treatment of sulfate-rich wastewaters. The produced sulfide in an anaerobic reactor is distributed among S_2^-, HS^- and H_2S in solution, H_2S in the biogas, and insoluble metallic sulfides according to chemical and physical equilibria. Sulfide in solution is a weak acid and dissociates as follows:

$$H_2S(l) \leftrightarrow HS^- + H^+$$

$$HS^- \leftrightarrow S^{2-} + H^+$$

The pKa value of the dissociation equilibrium of hydrogen sulfide is estimated at 6.9 at 30 °C based on Van 't Hoff equation[24]. Consequently, small pH variations within the pH range (6.5-8) that is considered as optimal for anaerobic digestion can have a very significant effect on

the inhibition. The gas-liquid distribution coefficient is 2.27 at 30 °C [25].

Sulfate-reducing bacteria (SRB) in the presence of sulfate can use several intermediates of the anaerobic mineralization process (Table 1.2) [26]. SRB can use direct methanogenic substrates molecular hydrogen (H_2), formate, acetate, methanol, and pyruvate [27]. It can also use propionate, butyrate, higher and branched fatty acids, lactate, ethanol and higher alcohols, fumarate, succinate, malate, and aromatic compounds [28].

Table 1.2 Stoichiometry and Standard Free Enthalpy Change for Sulfate-Reducing, Acetogenic, and Methanogenic Reactions

Reaction		ΔG°′ (kJ/mol)
Sulfate reducing reactions		
$4H_2+SO_4^{2-}+H^+$	HS^-+4H_2O	-38.1
$Acetate^-+SO_4^{2-}$	$HS-+2HCO_3^-$	-47.6
$Propionate^-+3/4\ SO_4^{2-}$	¾ HS^-+Acetate+HCO_3-+1/4 H^+	-37.7
$Propionate^-+7/4\ SO_4^{2-}+1/4\ H_2O$	7/4 HS^-+3 HCO_3^-+1/2 H^++1/4 OH^-	NR
$Butyrate^-+1/2\ SO_4^{2-}$	½ HS^-+2 $Acetate^-$+1/2 H^+	-27.8
$Butyrate^-+5/2\ SO_4^{2-}$+1/4 H2O	5/2 HS^-+4 HCO_3^-+3/4 H^++1/4 OH^-	NR
Syntrophic reactions		
$Propionate^-+3\ H_2O$	Acetate+HCO_3^-+H^++3 H_2	+76.1
$Butyrate^-+2\ H_2O$	2 Acetate+H^++2H_2	+48.3
Methanogenic reactions		
4 H_2+HCO_3^-+ H^+	CH_4+3 H_2O	-33.9
$Acetate^-$ + H_2O	CH_4+HCO_3^-	-31.0

NR= not reported

In a sulfidogenic breakdown of volatile fatty acid (VFA), two oxidation patterns can be distinguished (Table 1). Some SRB can oxidize VFA to CO_2 and sulfide as end products completely. Other SRB lack of tricarboxylic acid cycle and carried out incomplete oxidation of VFA with acetate and sulfide as end products.

There are several steps for SRB to degrade organic compounds, involving the hydrolysis of large molecular compounds to lower molecular products (proteins, nucleic acids, carbohydrates

and lipids). These substances may then be fermented into VFAs (acetate, propionate and butyrate) and gases (H_2, CO_2). These substrates could be further degraded through terminal oxidative processes (Fig.1.5) [29]. During the anaerobic fermentation, a fermentative acidogenic bacterium (FAB) could produce a lot of small molecular substances, which could provide abundant electron donor and carbon source for SRB [30]. By using metabolites from FAB, substrate degradation efficiency could be promoted. Meanwhile, there are many similarities in the ecology and physiology of methanogens (MB) and SRB [31], they have overlapping niche, showing strong competition, so the addition of SRB could inhibit the methane production to some extent [32], and lead anaerobic fermentation to acetic acid fermentation, and further increase the amount of VFAs produced.

In the presence of sulfate, competition between sulfate reducers and the anaerobic bacteria involved in methanogenesis can occur in the several stepwise degradation process:

-competition between sulfate reducers and fermentative bacteria for monomeric starting compounds, such as sugars, amino acids, etc.,

- competition between sulfate reducers and hydrogen-producing acetogenic (OHPA) species for intermediate fermentation products, such as propionate, butyrate, ethanol, etc.,

- competition between sulfate reducers and homoacetogenic bacteria for H_2, and

-competition between sulfate reducers and methanogens for direct methanogenic substrates, such as H_2 and acetate [28].

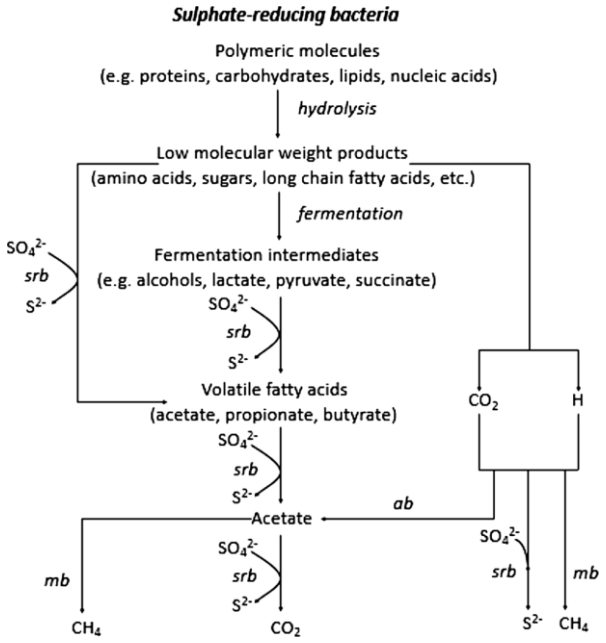


Figure. 1.5 Pathway of the anaerobic degradation of organic matter, showing potential interactions of sulphate-reducing bacteria.(srb=sulphate-reducing bacteria; mb=methanogenic bacteria; ab=acetogenic bacteria).

The sulfide levels reported as being inhibitory to methane formation may be summarised as being in the range of 100-800 mg/L dissolved sulfide or approximately 50-400 mg/L undissociated H_2S [33]. In the unacclimated batch digester, Speece & Parkin (1983) found that methane production was inhibited by a sulfide level as low as 50 mg S^{2-}-S/L (1.6 mM). By contrast, Kroiss and Wabnegg (1983) reported that an unionized H_2S level of 50 mg/L inhibited acetoclastic methanogenesis by about 50%, with complete inhibition only occurring free H_2S level of ca. 200 mg/L [34].

1.3. Objectives

In this study, we developed tofu wastewater treatment applicable for small-medium scale industries by using FBR packed with cut bamboo as a biofilm carrier to produce methane. Also, to investigate the microbial interaction relating to the reactor performance.

1.4. Thesis organization

This thesis is structured:

Chapter 1 introduces the background, literature review and objectives of the thesis

Chapter 2 describes the results of methane recovery from acidic tofu wastewater by using one-stage FBR with bamboo as a biofilm carrier. Evaluation of microbial interaction relating to reactor performance is also included.

Chapter 3 describes the results effect of sulfate on anaerobic treatment of sulfate-containing tofu wastewater by using one-stage FBR with bamboo as biofilm carrier

Chapter 4 describes a laboratory-scale treatment of acidic tofu wastewater by using three-stage FBR. Reactor performance and microbial interaction in the system are described.

Chapter 5 describes the performance of the pilot plant six-stage FBR with bamboo as a biofilm carrier. Microbial interaction was evaluated related to reactor performance.

Chapter 6 Conclusions of this research and recommendations for future research

Chapter 2

Methane recovery from acidic tofu wastewater using a one-stage anaerobic fixed-bed reactor with bamboo as the biofilm carrier

2.1. Background

In Indonesia, many tofu industries using an acid coagulant (fermented whey and acetic acid) in tofu processing, especially in Java Island. These industries produce low pH and high organic concentration of wastewater. The wastewater characteristics in several tofu industries, especially in West Java, Indonesia, are shown in Table. 2.1.

Table 2.1 Tofu wastewater characteristics of several industries in West Java, Indonesia

Location	Parameter	Concentration
Sumedang	NH_3-N (mg/L)	23.3-23.5
	NO_2-N (mg/L	0.1-0.5
	NO_3-N (mg/L)	3.5-4
	pH	4-6
	BOD (mg/L)	6000-8000
	COD (mg/L)	7500-14,000
	TSS (mg/L)	635-660
	TS (mg/L)	203-688
Bogor	BOD (mg/L)	3,095.4
	COD (mg/L)	12,293
Bandung	pH	3.3-5.1
	BOD (mg/L)	3750-4200
	COD (mg/L)	7800-8600
Jakarta	BOD (mg/L)	33,300
	PO_4 (mg/L)	4.7
	TSS (mg/L)	900

Source:Sriharti et. al, 2004 [35]

Most of the tofu industries still discharge the wastewater directly into the environment. In this study, the utilization of acidic tofu wastewater by anaerobic treatment using a fixed bed reactor to produce methane is proposed. Cut bamboo is used as a biofilm carrier in the treatment

to achieve a low-cost operation of the reactor. Rapid acidification that would prevent methane production is a major challenge in methane production from tofu wastewater because of its low pH, high carbohydrate. pH is an important parameter for successful biogas production that utilizes the anaerobic digestion process; the optimum range is 6.5–7.5 [36]. To control the pH and increase the methane yield, a low pH can be adjusted using various chemicals: NaOH [37], urea [38], and $Ca(OH)_2$ [39]. However, adjusting the pH may represent 40% of the total operational cost [38]. Alternative methods to reduce the base addition have been proposed for tofu wastewater treatment by using an anaerobic filter reactor with soft, fibrous media carriers and effluent recycling [40]. Previous results have shown that effluent recycling could increase the biogas production rate, but does not significantly affect the methane production rate. However, the effluent recycling system can increase energy consumption and cost due to additional equipment requirements. Therefore, a process to treat acidic wastewater without the addition of bases or effluent recycling would prove beneficial to small and medium-scale treatment plants that wish to treat their highly acidic wastewater on-site.

The purpose of this study is to develop a one-stage FBR packed with cut bamboo as a biofilm carrier for acidic tofu wastewater treatment to produce methane without pH adjustment method. Microbial interaction related to reactor performances was also investigated in this study.

2.2. Materials and methods

2.2.1. Experimental reactor design and operation

Three lab-scale anaerobic column reactors (shown in Fig. 2.1) were operated in this study. Each reactor was made of a plastic column (diameter 65 mm, height 320 mm) with a working volume of 0.6 L. In the experimental FBR reactor, 83 pieces of cut bamboo (inner diameter of 7–10 mm, outer diameter of 12–16 mm, and length of 18–23 mm) were randomly packed into

the plastic column. The cut bamboo had a surface to volume ratio of 909±103 m^2/m^3, and total solid (TS) of 90.3%. The total volume of the cut bamboo and the effective volume of the FBR were 0.18 L and 0.42 L, respectively, resulting in 30% porosity, and the total surface area in the FBR was 223 m^2/m^3.The other reactor was UASB with and without recycling system acted as control for the non-carrier reactor. All reactors were operated continuously at 35°C. Up velocity of 1 m/h was applied to UASB with recycling system. The substrate was added to the bottom of the reactors by a peristaltic pump. A U-shaped tube was used to discharge the effluent and to prevent the entrainment of ambient air into the reactor. The generated biogas was collected by a gas bag connected to the reactor. The reactors were inoculated with 420 mL of mostly digested sludge from a high-solids mesophilic co-digester of sewage sludge and fried tofu. The TS, volatile solids (VS), and pH of the seed sludge were 39.1 g/L, 30.6 g/L, and 8.45, respectively. The seed sludge was purged with nitrogen gas to ensure anaerobic conditions.

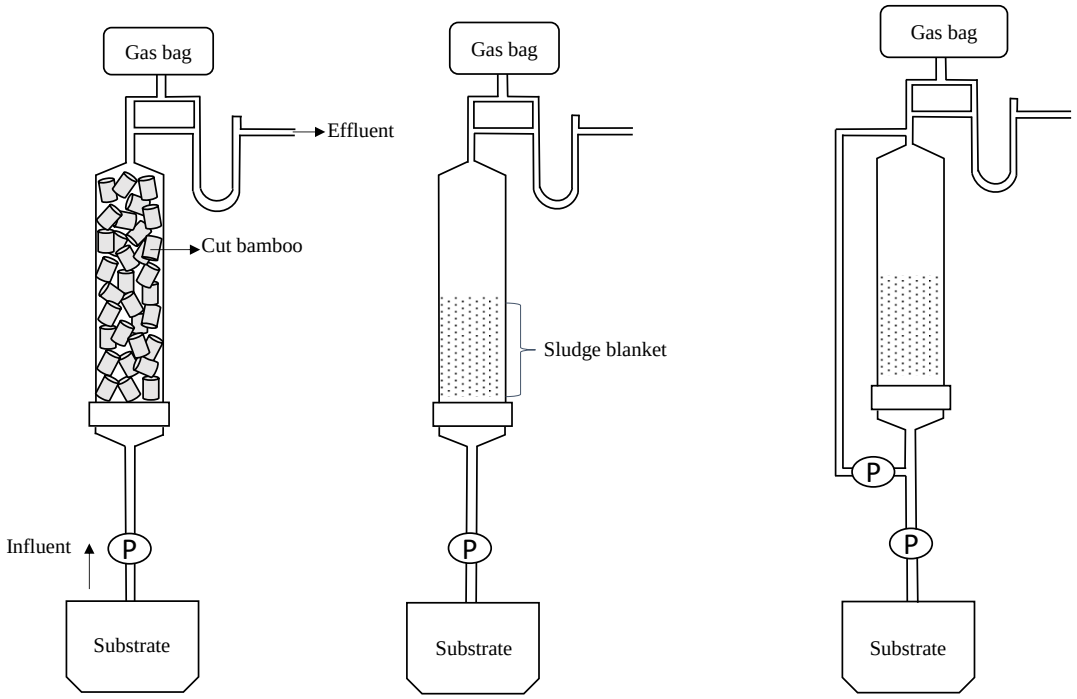


Fig. 2.1. Reactors utilized in the experiment: a) FBR b) UASB without recycling system c) UASB with recycling system

The soybean wastewater used in this study was prepared in the laboratory. Boiled soymilk was coagulated with acetic acid (5 mL acetic acid/1 L soymilk). The soy curd formed in the coagulation process was then filtered by non-woven fabric and compressed to release the excess liquid (tofu wastewater). The tofu wastewater was diluted by approximately 4 times to obtain a COD of approximately 10,000 mg/L. The tofu wastewater used in this study had characteristics similar to tofu wastewater in Indonesia, based on a preliminary study (Bandung City and Sumedang Regency, West Java Province), as shown in Table 2.1.

Table 2.1. Characteristics of tofu wastewater in the Sumedang Regency and Bandung City, West Java Province, Indonesia. TCOD is the total chemical oxygen demand. SCOD is the soluble chemical oxygen demand

Parameter	Sumedang Regency			Bandung city		Average
	A	B	C	D	E	
TCOD (mg/L)	8,400	8,600	10,600	11,800	12,800	10,440
SCOD (mg/L)	5,500	5,540	6,925	7,700	8,380	6,809
Total Nitrogen (mg/L)	498	446	302	359	340	389
Acetate (mg/L)	126	93	122	353	189	176
pH	5.42	5.52	5.61	5.00	5.00	5.31

*A, B, C, D, and E = tofu factories

The main characteristics of the wastewater used in this study are listed in Table 2.2 The wastewater contained high concentrations of organics and a low pH. The principal organic substances were carbohydrates, and the C/N ratio was relatively low. The wastewater contained acetate because acetic acid was used in the tofu production process. The reactors were operated for 230 days under operational conditions shown in Table 2.3.

Table 2.2. Composition of the substrate used in this study

Parameter	Concentration
Chemical Oxygen Demand (COD) mg/L	10,000
Soluble Chemical Oxygen Demand (SCOD) mg/L	9,720
Total Organic Carbon (TOC) mg/L	3,328
Dissolved Organic Carbon (DOC) mg/L	3,224
Total Nitrogen (TN) mg/L	219
Dissolved Total Nitrogen (DTN) mg/L	165
Suspended Solid (SS) mg/L	316
Acetate (mg/L)	388
Carbohydrate (mg/L)	5,415
Protein (mg/L)	239
pH	5.52

Table 2.3. Operating conditions of the continuous anaerobic digestion experiment

Period No.	1	2	3
Apparent HRT (h)[a]	72	48	55
COD loading rate (kg COD/m^3 day)	3.3	5.0	4.3
TOC loading rate (kg TOC/m^3 day)	1.1	1.7	1.4

[a] HRT of FBR based on void volume (h): Period 1 = 50.4, Period 2 = 33.6, and Period 3 =38.9

The organic loading rate (OLR) increased from 3.3 to 5.0 kg COD/m^3 day (Period 1 to Period 2). Due to the acidification of the UASB reactor without recycling system and decreasing organic removal in the FBR and UASB without recycling system at the end of Period 2, the organic loading decreased to 4.3 kg COD/m^3 day in Period 3 to recover the reactor.

2.2.2. Analytical Methods

A supernatant for the analysis was prepared by centrifuging effluent samples at 10,000 rpm for 10 min and filtering the resultant sample through a 0.2 μm filter (Omnipore^{TM} PTFE Membrane, Merk KGaA, Darmstadt, Germany). Concentrations of total organic carbon (TOC), dissolved organic carbon (DOC), total nitrogen (TN), and dissolved total nitrogen (DTN) were measured using a TOC/TN analyzer (TOC-V CPH/TNM-1, Shimadzu, Japan). Ammonium nitrogen (NH_4^+-N) concentration was determined using ion chromatography (HIC-SP equipped with an IC-C4 type column, Shimadzu, Japan). Volatile fatty acids (VFAs) (acetate, propionate, lactate, iso-butyrate n-butyrate iso-valerate and n-valerate) concentrations were measured with a High Performance Liquid Chromatograph Post-Column, pH Buffering (Organic Acid Analysis System with a SCR-102 H type column, Prominence, Shimadzu, Japan). The suspended solids (SS) and VS of the samples were measured according to standard methods [41]. Biogas yield was measured using a wet-gas meter (W-NK type, Shinagawa, Japan). Methane content was determined with gas chromatography (GC-8A, Shimadzu, Japan). COD_{cr} was measured using COD digestion vials, and the concentration was read by a portable colorimeter (DR890, HACH, USA). The pH of the sample was measured using a pH meter (HM-21P, TOA-DKK, Japan).

2.2.3. Microbial Community Analysis

Biofilm attached to the bamboo carriers (BF) and suspended biomass from the bottom of the reactor (SB) were collected from the FBR at the end of Period 3 (day 230) to investigate the microbial community structure. DNA was extracted from the samples using a DNeasy PowerSoil Kit (Qiagen, Germany). The 16S rRNAs amplicons were amplified from the extracted DNA utilizing a polymerase chain reaction (PCR) method that implemented a 515F [42] and 806R [43] primer set that target the V3-V4 regions of bacteria. A 340F and 806Rb [44] primer set was used to target the V4-V5 regions of archaea. The PCR was carried out using

a HotStarTaq DNA Polymerase (Qiagen, Hilden, Germany). The PCR conditions were as follows: denaturation at 95 °C for 15 min, annealing at 94 °C for 30 s, 55 °C for 30 s, and 72 °C for 60 s. This was performed for 25 cycles for bacteria and 37 cycles for archaea. The initial PCR products were purified using Agencourt AMPure XP (Beckman Coulter, Pasadena, USA), while following the manufacturer's standard protocols. The second PCR was conducted to attach Illumina Adapters for DNA sequencing using the KAPA HiFi HotStart PCR Kit (Kapa Biosystems, Inc, Wilmington, USA), while following the manufacturer's protocols. The amplified DNA was purified using Agencourt AMPure XP. The 16S rRNA amplicon sequencing was performed by Illumina MiSeq platform.

Raw sequence reads were filtered from the adapter contaminant using the Trimmomatic [45]. After quality trimming, sequence reads were clustered using Usearch at 97% similarity [46]. Clustered reads were then classified into operational taxonomical units (OTU) using the UPARSE pipeline [47]. The taxonomic classification of OTUs was performed using QIIME with SILVA_128 as the reference database [48]. The microbial community abundances were generated using Microsoft Excel™.

2.3. Results and Discussion

2.3.1. Process Performance

The performances of the FBR and UASB reactors in terms of pH, TOC, DOC, VFAs, ammonium, methane yield, and SS are shown in Fig. 2.2. At an OLR of 3.3 kg COD/m^3 day and an HRT of 72 h (Period 1), similar performances were observed for both FBR and UASB reactors. Although an accumulation of organic acids and a decrease in pH were observed during days 37–44 in FBR and UASB without recycling system, and during the first 74 days in UASB with recycling system, both the TOC and DOC concentrations in the effluent gradually decreased. Without the addition of an alkaline solution, the pH in three reactors could be

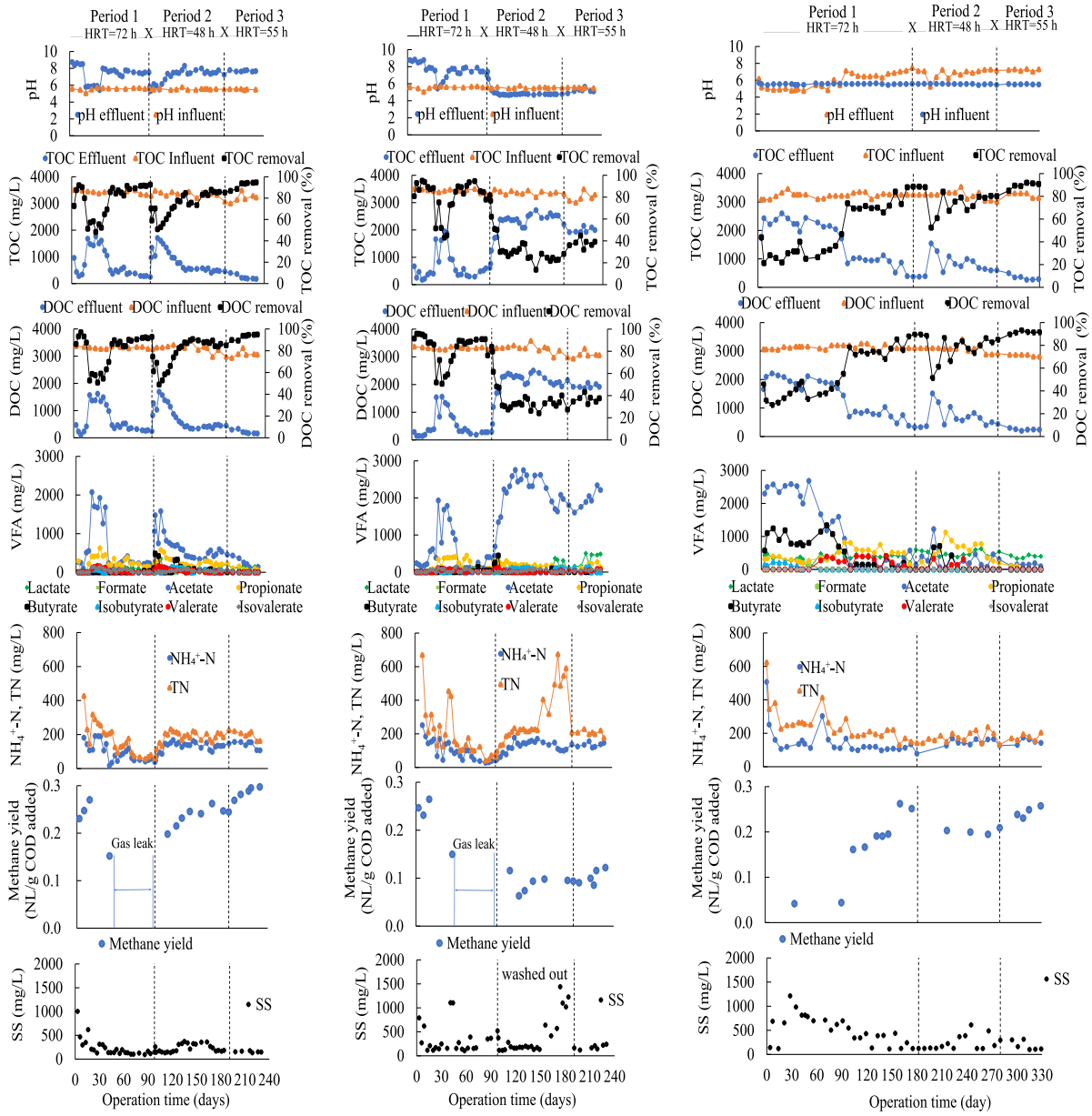
maintained at the desired value (greater than 7), and most of TOC was removed at the end of Period 1. However, the SS concentrations of effluent in the UASB without recycling and with recycling system reactor increased up to 1107 mg/L and 1038 mg/L, respectively by solids transferring from the sludge to the effluent.

In Period 2, the COD loading rate increased from 3.3 to 5 kg COD/m^3 day, with an HRT of 48 h (Period 2). In the UASB without recycling system, the acetate concentration immediately increased to over 2,000 mg/L and the pH dropped to 4.6–4.95. Meanwhile, the FBR and UASB with recycling system exhibited a better performance. Although in FBR and UASB with recycling system an accumulation of VFA (up to 2652 mg/L and 3076 mg/L, respectively) and a decrease in the pH under 6 was observed in the first 2 weeks, the VFA concentrations gradually decreased and the pH increased to 7.2. Daily methane production increased in FBR and UASB with recycling system up to 0.73 NL/day and 0.63 NL/day, respectively. Although the SS concentration decreased in both reactors, the concentrations of TOC, DOC, and VFAs in the effluent were higher than those in Period 1. These results indicate that the OLR in Period 2 was too high to maintain a superior reactor performance.

In Period 3, the OLR decreased from 5 to 4.3 kg COD/m^3 day, with an HRT of 55 h. However, the UASB without recycling system reactor was unable to prevent an accumulation of volatile acids, which resulted in a loss of pH control. High concentrations of TOC (1,918–2,092 mg/L) remained in the effluent and low daily methane production values (0.22–0.32 NL/day) were detected. Meanwhile in the UASB reactor with recycling system showed better performance, pH could be maintained in neutral value, and methane production can be achieved in period 3 up to 0.67 L/day.

The protein present in the substrate used in this study can potentially produce ammonia when degraded and reduce methane production. It has been reported that when the total

ammonia nitrogen concentration exceeds 3000 mg NH_4^+–N/L, anaerobic digestion processes are inhibited at any pH [49].



(a) (b) (c)

Figure 2.2 Overall performance during continuous operation of the (a) FBR, (b) UASB without recycling system and (c) UASB with recycling system

Because the NH_4^+-N concentrations of the UASB with and without recycling system effluent during Periods 1–3 were 34–130 mg/L and 78- 165 mg/L, respectively (in steady state condition), inhibition of ammonia did not occur throughout the experiment. TN is occasionally

high due to biomass being washed out. UASB with recycling system seemed could retain more biomass than UASB without recycling system and caused methane production still occurred until Period 3. Although sludge floc formed in both UASB reactors, no strong aggregates of granules were observed in the reactors during Periods 1–3. It has been reported that a stable, granular sludge cannot be cultivated in a UASB reactor fed with acidic wastewater (acetate-propionate mixture as substrate) at pH 6 [50]. The failure of the UASB without recycling system reactor enhanced methane production in Period 2 and 3 might be due to the wastewater characteristics and might require a longer startup time to achieve strong granules. The lowest effluent TOC and DOC concentrations were obtained in the FBR system by decreasing the OLR to 4.3 kg COD/m^3 day. The lowest TOC and DOC concentrations were under 200 mg/L. In addition, VFA concentration of 344 mg/L (mainly, acetate, lactate, and propionate), SS concentration of 145 mg/L, and daily methane production of 0.77 NL/day were also observed. An accumulation of VFA, which can lower the pH, did not occur during this period; instead, a stable pH of 7 was observed. These results indicate that the metabolic processes of acetogenesis and methanogenesis were balanced in the FBR during Period 3. Inhibition of ammonia also did not occur in FBR at NH_4^+-N concentrations of 48–138 mg/L. The biofilm mass attached to the bamboo carrier and sludge solids deposited on the bottom FBR might have been factors in the successful FBR performance. Fig. 2.3 shows the bamboo carrier before and after treatment in the FBR reactor. Biofilms were not only attached to the surface but had also formed inside the bamboo rings. These results indicate that the bamboo ring could provide adequate conditions for biomass adherence. During the experiment, the withdrawal of sludge from the FBR was not required.

(a) (b)

Figure 2.3 Bamboo carrier (a) before anaerobic treatment and (b) after anaerobic treatment

2.3.2. Organic Removal Rate and Methane Yield

The organic removal rates and the methane yields of each period after the effluent water quality became stable (at least ten days) were calculated, and the results are summarized in Table 3.

The performance of UASB without a recycling system was better than UASB without a recycling system. At OLR 4.3 kg COD/m^3 day and an HRT of 55 h UASB with recycling system achieved the highest methane yield of 0.25±0.01 NL/g COD added and organic removal rate 1.2±0.01 NL/g TOC removed. The improvement of UASB with the recycling system was associated with higher biomass retaining in the reactor. However, the retained biomass in the reactor did not form a strong granule and susceptible easy to washed out.

The inferior performance showed by FBR. At an OLR of 4.3 kg COD/m^3 day and an HRT of 55 h, the FBR achieved the highest organic removal rate (1.3±0.02 kg TOC/m^3 day), with methane yields of 0.92±0.01 NL/g TOC added and 0.98±0.01 NL/g TOC removed. The organic carbon removal efficiency was 95±0.27% (effluent had a soluble chemical oxygen demand (SCOD) of 240 mg/L), with a methane conversion rate of 0.30±0.01 NL/g COD added (86% of the maximum theoretical conversion). Similar excellent performances of upflow anaerobic filters packed with various media have also been reported for dairy wastewater

treatment under varying OLR (0.5–10.2 kg COD/m^3 day) and HRT values (0.83–15 day) [51]. When utilizing an upflow filter reactor packed with FLOCOR for the anaerobic digestion of cheese whey wastewater (COD concentration of 15 g COD/L), the highest levels of COD removal efficiency (95%) and methane yield (0.28–0.38 L/g COD removed) were achieved at an OLR of 3 kg COD/m^3 day [52]. Meanwhile, synthetic dairy wastewater treatment using PVC rings as packing material showed that at an OLR of 0.302 kg COD/m^3 day, a COD removal efficiency of 97.9%, a methane yield of 0.39 L/g COD removed, and an SCOD effluent concentration of 572 mg/L were achieved [53]. Other research regarding the treatment and processing of soybean wastewater using a filter reactor packed with soft-fibrous media at an OLR of 8.16 g/L day without and with recycling yielded COD removal efficiencies of 89.2% and 90.6–92.5%, respectively [40]. Using bamboo as a packing material has also been applied in a pilot-scale anaerobic fixed bed reactor to treat slaughterhouse wastewater, which resulted in a maximum 95% COD removal efficiency at an OLR of 1 kg COD/m^3 day and HRT of 7.5 days. At a higher OLR of 4.0 kg COD/m^3 day (HRT 2 days), the same reactor achieved a COD removal efficiency of 75% [15]. In this study, although the maximum OLR was 4.3 kg COD/m^3 day, this value was in the range of a previous study that occasionally achieved higher removal efficiencies. These results indicate that the FBR system utilizing a bamboo carrier was useful for soybean wastewater treatment.

Table 2.4. Organic removal, methane yields, and methane concentrations of the FBR and UASB reactors in each period

	Period 1 HRT=72 h (OLR=3.3 kg COD/m^3 day, 1.1 kg TOC/m^3 day)			Period 2 HRT=48 h (OLR=5.0 kg COD/m^3 day, 1.7 kg TOC/m^3 day)			Period 3 HRT=55 h (OLR=4.3 kg COD/m^3 day, 1.4 kg TOC/m^3 day)		
	FBR	UASB without recycling	UASB With recycling	FBR	UASB without recycling	UASB With recycling	FBR	UASB without recycling	UASB With recycling
TOC removal efficiency (%)	93±0.59	82±2.03	88±0.43	85±1.60	28±2.39	80±0.87	95±0.27	39±1.65	91±0.55
TOC removal rate (kg TOC/m^3 day)	1.0±0.01	0.9±0.02	0.9±0.03	1.3±0.04	0.4±0.02	1.2±0.01	1.3±0.02	0.6±0.09	1.2±0.01
CH_4 yield (NL/g TOC added)	-	-	0.79±0.02	0.78±0.03	0.30±0.04	0.63±0.09	0.92±0.01	0.35±0.01	0.79±0.05
CH_4 yield (NL/g TOC removed)	-	-	1.0±0.1	0.92±0.06	1.15±0.09	0.8±0.1	0.98±0.01	0.97±0.16	0.90±0.04
CH_4 yield (NL/g COD added)	-	-	0.24±0.04	0.25±0.01	0.09±0.002	0.20±0.01	0.30±0.01	0.11±0.01	0.25±0.01
CH_4 concentration (%)	-	-	60±0.44	59±1.11	53±0.08	61±0.17	60±0.15	53 ±0.64	60±0.49

2.3.3. Bacterial Community

Bacterial and archaeal communities from the BF and SB were analyzed. The 15 most abundant bacterial taxa are shown in Fig. 2.4. In general, the microbial diversity in the BF was low compared to that of the SB. *Bacteriodetes* were the most abundant bacteria in the BF and SB. Within these phyla, the genus *Paludibacter* and unclassified *Bacteriodetes* belong to the family *Porphyromonadaceae* and were the most abundant in the BF (9.1% and 8.7%, respectively) and SB (10.2% and 10.4%, respectively). Members of the *Bacteroidetes* are known as acidogenic, sugar fermenting, saccharolytic, and proteolytic bacteria that produce propionate, acetate, and succinate as their primary byproducts [54]. The fermentative-related species (Paludibacter) can degrade organic pollutants to smaller molecular compounds (such as acetate), which may enhance the acetate production in the system [55]

Within the phyla *Actinobacteria*, most of the bacteria were from the genus *Sanguibacter* (order *Actinomycetales*), accounting for 24.5% and 16.9% in the BF and SB, respectively. This genus has also been observed in the glucose-base wastewater that was treated in an anaerobic reactor [56]. Furthermore, unclassified *Actinobacteria* belong to the family *Coriobacteriaceae* (BF=1.0%, SB=1.1%) and the unclassified phyla *Firmicutes* belong to the family *Veillonellaceae* (BF=2.3%, SB=3.6%). The latter might contribute to the decrease in lactate concentrations in this study because of its ability to convert lactic acid into acetic and propionic acids [57], [58].

The remaining bacteria from the phyla *Firmicutes* were classified into SHA-98 (BF=3.4%, SB=2.7%), the family *Ruminococcaceae* (both BF and SB=1.4%), the genus *Syntrophomonas* (BF=1.1%, SB=1.0%), the genus *Clostridium* (BF=1.0%, SB=1.1%), and the unclassified *Clostridiales* (both BF and SB=1.0%). These bacteria might be responsible for syntrophic

acetogenesis, which is the oxidation of fatty acids to produce H_2, which is an important substrate for hydrogenotrophic methanogens [59], [60], [61].

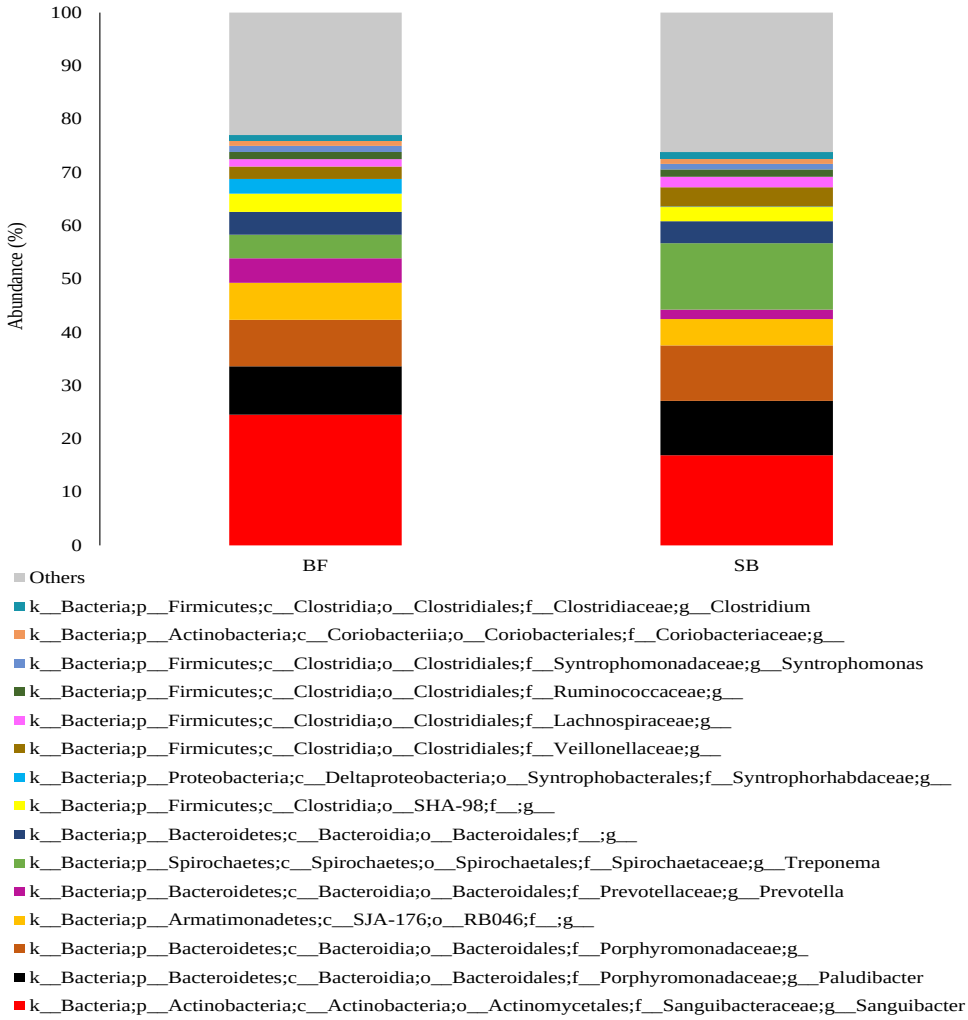


Figure 2.4. Distribution of the 15 most abundant bacteria in the bamboo carriers (BF) (attached to carrier) and in the suspended solids (SB) (bottom reactor)

The unclassified genus belong to the phyla *Armatimonadetes* and were found in the BF (6.9%) and SB (4.9%). These phyla are expected to be chemoheterotrophs that possess a carbohydrate-based primary metabolism [62].

The phyla *Spirochaetes* were dominated by the genus *Treponema* (BF=4.4%, SB=12.5%). The *Treponema* affiliated bacteria were likely homoacetogens, which can consume H_2 and CO_2 to produce acetate [63].

Proteobacteria was the sixth most common phyla in all samples and is represented by the unclassified genus belonging to the family *Syntrophorhabdaceae* (BF=2.8%, SB=0.1%). The higher observed abundance of this family in the BF compared to that of the SB was expected because the SB environment was more acidic than that of the BF and *Syntrophorhabdaceae* grow optimally at a neutral pH. In addition, this family forms a syntrophic relationship with its hydrogenotrophic partners to oxidize short and long-chain fatty acids [64].

2.3.4. Archaeal Community

The distributions of the 10 most abundant archaeal populations in the BF and SB are shown in Fig. 2.5. All samples were dominated by *Euryarchaeota*, which are known methanogenic microorganisms. The major methanogenic archaea were found in the BF and SB and were dominated by hydrogenotrophic methanogens, which included *Methanoculleus* (31.8% and 6.3%, respectively), *Methanobacterium* (19.8% and 58.5%, respectively), *Methanobrevibacter* (0.2% and 7.2%, respectively), *Methanospirillaceae* (4.2% and 3.0%, respectively), and *Methanoregulaceae* (0.8% and 2.2%, respectively). The primary substrates for methane production by hydrogenotrophic methanogens were CO_2 and H_2.

Furthermore, *Methanosaeta* and *Methanosarcina* are typical acetoclastic methanogens and were also found in the BF and SB, but in lower abundances than hydrogenotrophic methanogens. Acetate is directly transformed to methane and carbon dioxide by acetoclastic methanogens. Besides utilizing acetate as a growing substrate, *Methanosarcina* is also capable of utilizing methanol, methylamines, and H_2/CO_2 [65]. Abundance of *Methanosaeta* was higher in the BF (19.3%) than in the SB (0.02%).

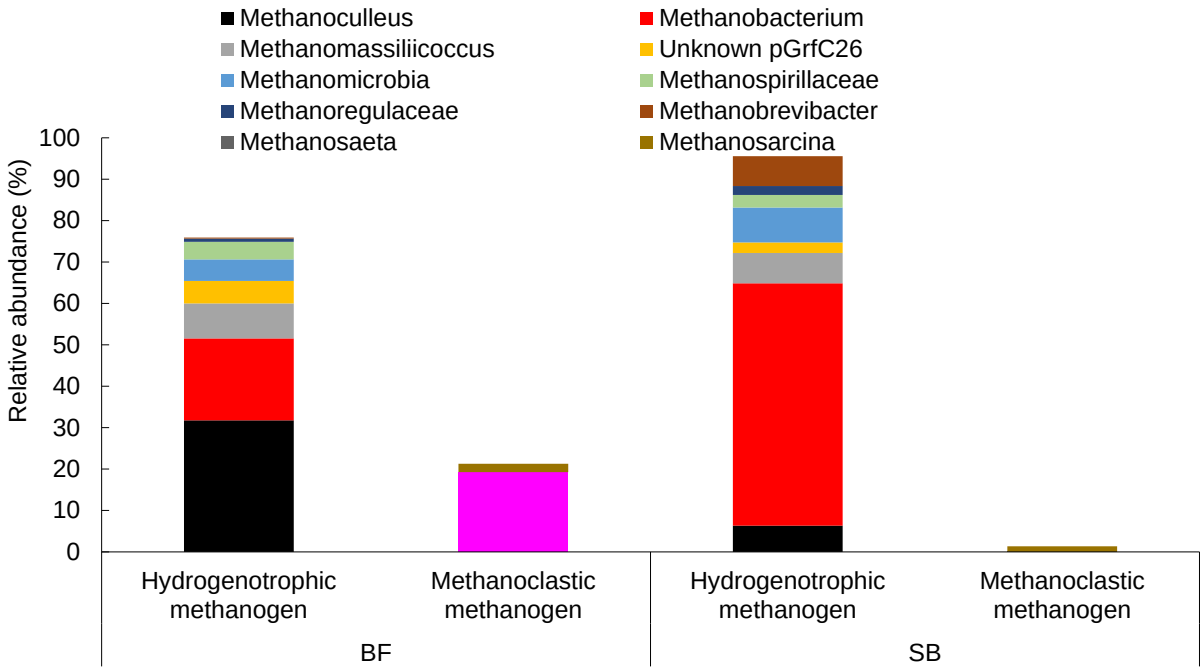


Fig. 2.5. Distribution of the 10 most abundant archaea in the bamboo carriers (BF) and bottom reactor (SB) at the genus level.

This may be due to the higher acetate concentration at the bottom-site reactor than at the upper-site reactor. In particular, *Methanosaetaceae* was more sensitive to high acetate concentrations than *Methanosarcina* [66]. The genus *Methanomassiliicoccus* (order *Methanomassiliicoccales*, class *Thermoplasmata*) was found in the BF (8.4%) and SB (7.3%) and may indicate H_2-dependent methylotrophic methanogens [67]. This genus can reduce methanol with hydrogen and can use methylamines as a methanogenic substrate [68], [69].

The predominance of hydrogenotrophic methanogens strongly suggests that methane production in the FBR mainly utilized CO_2 as an electron acceptor and hydrogen as an electron donor via the hydrogenotrophic metabolic pathway. The presence of syntrophic hydrogen

suppliers in the bacterial community had positive correlations with hydrogenotrophic methanogens. Because the influent in this study had a low pH and hydrogenotrophic methanogens are less sensitive to unfavorable pH levels than acetoclastic methanogens, hydrogenotrophic methanogens were dominant and adapted throughout the experiment [70].

2.4. Conclusions

An FBR was operated and maintained to treat acidic tofu wastewater and produce methane at a high OLR value under continuous operation, in contrast with a UASB reactor. Maximum methane yield of 0.30 ±0.01 NL/g COD added was attained at an OLR of 4.3 kg COD/m^3 day and HRT of 55 h in the FBR. FBR treatment without neutralization or a recycling system facilitated operational cost reductions. Cut bamboo was utilized as the biofilm carrier and can be considered as an appropriate support material for cell immobilization. Moreover, the cut bamboo provided stable treatment under continuous operation. The methane formation pathways in the FBR were dominated by hydrogenotrophic methanogenesis.

Chapter 3

Sulfate-containing tofu wastewater treatment by using one stage-fixed bed reactor

3.1. Background

In the previous chapter, acidic wastewater produced from tofu processing using acid coagulant was treated by FBR. The results show that FBR successfully could treat acidic tofu wastewater to produce methane. In this chapter, FBR was used to treat sulfate-containing wastewater produced from the tofu industry that using sulfate coagulant in tofu processing. It has been known that besides acidic coagulant, the two most commonly used in tofu processing are salt coagulants such as calcium sulfate ($CaSO_4$/gypsum) and nigari salt (magnesium chloride combined with calcium chloride).

In Indonesia, many tofu industries use sulfate coagulant, such as in Padang city, West Sumatera. Tofu processing still runs with simple technology. Untreated wastewater from tofu industries using sulfate coagulant becomes an environmental problem in Padang city since it discharges directly into the environment. The condition tofu industry in Padang city, Indonesia, describes in Fig. 3.1. However, there is still no available wastewater characteristic from tofu industries in Indonesia using sulfate coagulant. We expected that sulfate is contained in the wastewater since $CaSO_4$ is used as a coagulant.

In this study, we proposed to solve the problem by treating the wastewater using an anaerobic process to produce methane. Methane generation is expected can be used as renewable energy for tofu processing by the industry. In anaerobic treatment, the up-flow anaerobic sludge blanket (UASB) is an established anaerobic reactor. Compared to other anaerobic reactors, its advantages include low investment and energy costs and short hydraulic retention time with no support medium required [71]. However, it has some disadvantages

regarding sulfate reduction, such as mixing is provided solely by the flow rate because gas production is low or inexistent and SRB does not granulate as well as methanogenic microorganisms. This may cause prevent methane production in the anaerobic treatment.

(a)

(b)

(c)

(d)

(e)

Figure 3.1 The condition of a tofu industry in Padang city, Indonesia which using sulfate as a coagulant (a) simple equipment for tofu processing (b) soft tofu produced by using sulfate

coagulant (c-e) wastewater produce by tofu industry discharge directly to the river

In this study, to overcome this disadvantage, we propose anaerobic treatment by using FBR. In FBR, biomass retention by immobilization on inert support materials is a very efficient and economical method for achieving high space-time yields of biogas with simultaneous high COD and BOD removal efficiency [72].

All bioreactor designs developed for methanogenic wastewater treatment can be applied to treat sulfate-rich wastewaters, where organic matter is removed both via sulfate reduction and methanogenesis [73]. However, it has long been recognized that a high concentration of sulfate in wastewater will interfere with methane-producing bacteria (MPB) in anaerobic treatment. Sulfate reducing bacteria (SRB) use sulfate as their terminal electron acceptor and can outcompete methanogenic archaea for carbon and electrons [74]. SRB may also compete with syntrophic bacteria (e.g. acetogens) for short-chain volatile fatty acids such as propionate and butyrate, while hydrogen sulfide production by SRB can inhibit both methanogens and SRB [75]. Hydrogen and acetate are the key precursors to methane formation during normal anaerobic wastewater treatment. Hydrogen and acetate may also serve as electron donors for sulfate reduction. Therefore MPB and SRB must be considered as competing for these available substrates in anaerobic wastewater treatment systems where significant sulfate is present. SRB can use sulfate as a terminal electron acceptor in the oxidation of organic matter, resulting in hydrogen sulfide (H_2S) [76].

To overcome the development of strategies to improve methane production from anaerobic reactors, the effect of sulfate on methane recovery from sulfate-containing tofu wastewater needs to be addressed. In this study, FBR with bamboo as a biofilm carrier treating sulfate-containing tofu wastewater was evaluated by applying various organic load and HRT.

3.2 Material and methods

3.2.1. Experimental reactor design and operation

Two lab-scale anaerobic column reactors (shown in Fig. 3.1) were operated in this study. Each reactor was made of a plastic column (diameter 65 mm, height 320 mm) with a working volume of 0.6 L. In the experimental FBR reactor, 128 pieces of cut bamboo (inner diameter of 7–10 mm, outer diameter of 12–16 mm, and length of 18–23 mm) were randomly packed into the plastic column. The cut bamboo had a surface to volume ratio of 909±103 m^2/m^3 and a total solid (TS) of 90.3%. The total volume of the cut bamboo and the effective volume of the FBR were 0.15 L and 0.45 L, respectively, resulting in 30% porosity, and the total surface area in the FBR was 223 m^2/m^3. The other reactor was UASB with recycling system acted as a control for the non-carrier reactor. The reactors were operated continuously at 35 °C. Up velocity of 1 m/h was applied to UASB with a recycling system. The substrate was added to the bottom of the reactors by a peristaltic pump. A U-shaped tube was used to discharge the effluent and to prevent the entrainment of ambient air into the reactor. The generated biogas was collected by a gas bag connected to the reactor. The reactors were inoculated with 420 mL of mostly digested sludge from a high-solids mesophilic co-digester of sewage sludge and fried tofu. The TS, volatile solids (VS), and pH of the seed sludge were 39.1 g/L, 30.6 g/L, and 8.45, respectively. The seed sludge was purged with nitrogen gas to ensure anaerobic conditions.

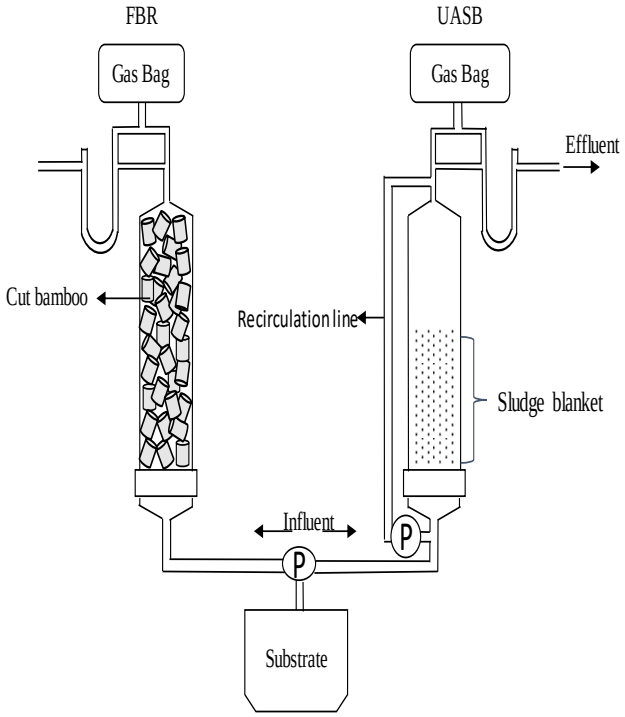


Figure 3.1. Reactors utilized in the experiment: FBR and UASB with recycling system

The soybean wastewater used in this study was prepared in the laboratory. Boiled soymilk was coagulated with calcium sulfate (18 g $CaSO_4$/1 L soymilk). The soy curd formed in the coagulation process was then filtered by non-woven fabric and compressed to release the excess liquid (tofu wastewater). The tofu wastewater was diluted by approximately 4 times to obtain a COD of approximately 10,000 mg/L. The reactors were operated for 385 days (FBR) and 329 days (UASB with recycling system) under continuous operational conditions. The reactor operation is shown in Table 3.1.

Table 3.1. Operating conditions of the continuous anaerobic digestion experiment

Parameter	Period 1	Period 2	Period 3
HRT (h)	72	48	55
COD/SO_4^{2-} ratio	14		
Influent flow (L/day)	0.2	0.3	0.26
Temperature (°C)	35±1		
pH	5.97±0.08		
Sulfate (mg/L)	692 ± 61		

COD (mg/L)	10,000		
OLR (kg COD/m³ day)	3.3	5	4.3
Sufate Loading Rate (SLR) (kg SO_4^{2-} /m³ day)	0.23	0.36	0.32

The organic loading rate (OLR) increased from 3.3 to 5.0 kg COD/m^3 day (Period 1 to Period 2). Then due to decreasing in reactor performance in Period 2, the organic loading decreased to 4.3 kg COD/m^3 day in Period 3.

3.2.2. Analytical Methods

The samples of influent and effluent from each reactor are taken. A supernatant for the analysis was prepared by centrifuging effluent samples at 10,000 rpm for 10 min and filtering the resultant sample through a 0.2 μm filter (Omnipore^{TM} PTFE Membrane, Merk KGaA, Darmstadt, Germany). Concentrations of total organic carbon (TOC), dissolved organic carbon (DOC), total nitrogen (TN), and dissolved total nitrogen (DTN) were measured using a TOC/TN analyzer (TOC-V CPH/TNM-1, Shimadzu, Japan). Sulfate and Ammonium nitrogen (NH_4^+-N) concentration was determined using ion chromatography (HIC-SP equipped with an IC-C4 type column, Shimadzu, Japan). Volatile fatty acids (VFAs) (acetate, propionate, lactate, iso-butyrate n-butyrate iso-valerate and n-valerate) concentrations were measured with a High-Performance Liquid Chromatograph Post-Column, pH Buffering (Organic Acid Analysis System with a SCR-102 H type column, Prominence, Shimadzu, Japan). The suspended solids (SS) and VS of the samples were measured according to standard methods [19]. Biogas yield was measured using a wet-gas meter (W-NK type, Shinagawa, Japan). Methane content was determined with gas chromatography (GC-8A, Shimadzu, Japan). COD_{cr} was measured using COD digestion vials, and the concentration was read by a portable colorimeter (DR890, HACH, USA). The pH of the sample was measured using a pH meter (HM-21P, TOA-DKK, Japan).

Percent electron flow by SRB and MPB. In the anaerobic digestion of media rich in sulfate, the substrate electrons (in terms of COD) are normally partitioned between the SRB and MPB. The electron flow by the SRB and MPB can be calculated from the following equations [77]:

(a) By the SRB

Sulfate reduction: $4H_2 + H^+ + SO_4^{2-} \rightarrow HS^- + 4H_2O$ (1)

$CH_3COO^- + SO_4^{2-} \rightarrow HS^- + 2HCO_3^-$ (2)

The COD of the H2S produced is given by:

$H_2S + 2O_2 \rightarrow H_2SO_4$ (3)

Thus, 1 mol of sulfate reduced =1 mol of H_2S produced =2 mol of COD =64 g of COD.

Electron flow by the SRB = moles of sulfate S reduced x 64

g = A g.

(b) By the MPB

Methane production: $4\ H_2 + HCO_3^- + H^+ \rightarrow CH_4 + 3H_2O$ (4)

$CH_3COO- + H_2O \rightarrow CH_4 + HCO_3-$ (5)

The COD of the CH4 produced is given by:

$CH_4 + 2O_2 \rightarrow CO_2 + 2H_2O$ (6)

Thus, 1 mol of CH4 produced 2 mol of COD = 64 g of COD.

Electron flow by the MPB = moles of CH4 produced x 64 g = B g.

Therefore:

Percent electron flow by SRB = [A/(A + B)] x 100

Percent electron flow by MPB = [B/(A + B)] x 100

3.3. Results and Discussion

3.3.1. Process Performance

The reactor performance in each operation period in terms of pH, TOC, DOC, VFAs, ammonium, methane yield, and SS are shown in Fig. 3.2. From period 1 until Period 3, the FBR achieve a higher organic removal and methane yield than UASB with the recycling system. Although in Period 1, FBR needs along time (approximately 3 months) to achieve the optimum pH of 6.5-7.5 for methane production, FBR could maintain pH higher than 7 and produce methane until Period 3. Meanwhile, in UASB with a recycling system as a control reactor, a low pH of 5.74±0.22 and low methane production was observed (Period 1-3). This condition leads to the failure of UASB in treating sulfate-containing wastewater to produce methane.

By increasing OLR to 5.0 kg COD/m³ day (Period 2), a decreased in methane yield and accumulated organic acid were observed in both reactors. Also, the SS concentration in both reactors increased, which indicated some of the biomass washed out from the reactors. These results indicate that the OLR in Period 2 was too high to exhibit a superior reactor performance.

To avert the inhibition of methane production which occurred in Period 2, then OLR decreased to 4.3 kg COD/m³ day (Period 3). In FBR, methane production increased concerning the decrease in the OLR from 5.0 to 4.3 kg COD/m³ day (Period 3). However, although the methane production was higher from Period 2, but the organic acid in the effluent was higher than Period 1. These results showed that at OLR in this Period was still too high to achieve a superior reactor performance. While the UASB reactor failed to recover, low pH and biomass washed out even occurred. Complete failure of a UASB system is likely to occur. The strong granule did not form in UASB throughout the experiment.

Sulfate reduction occurred in FBR and UASB reactors in Period 1-3. By increasing OLR and accompanying decrease in HRT, caused a decrease in sulfate reduction in both reactors. These results showed that sulfate reduction was HRT dependant.

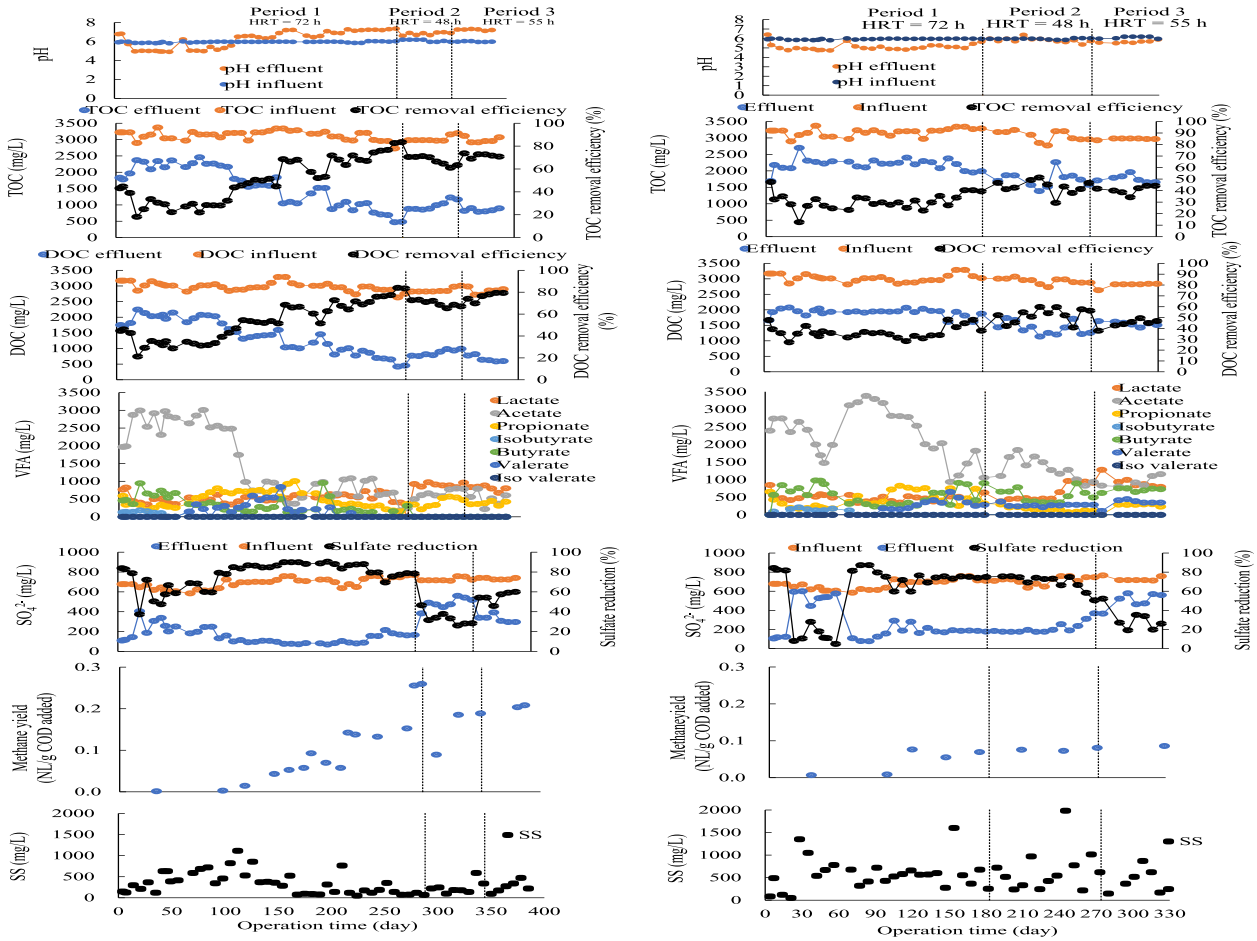


Figure 3.2 Overall performance during continuous operation of the (a) FBR, (b) UASB with recycling system

A summary of FBR and UASB with recycling system performance in each period are shown in Table 3.2. The results show that FBR has better performance than the UASB reactor. At OLR =3.3 kg COD/m^3 day and SLR= 0.23 kg SO_4^{2-}/m^3 day, FBR achieved a maximum methane yield of 0.26±0.002 NL/g COD added and sulfate-reducing rate of 0.2±0.01 kg SO_4^{2-}/m^3 day. Besides, at the same loading rate, COD and TOC removal rate of 2.85±0.03 (kg COD/m^3 day) and 0.8±0.0.3 (kg TOC/m^3 day) can be achieved, respectively.

Table 3.2 Organic removal, methane yields, and methane concentrations of the FBR and UASB reactors in treating sulfate-containing tofu wastewater

	Period 1 HRT=72 h (OLR=3.3 kg COD/m^3 day, SLR=0.23 kg SO_4^{2-} /m^3 day)		Period 2 HRT=48 h (OLR=5.0 kg COD/m^3 day, SLR= 0.35 kg SO_4^{2-} /m^3 day)		Period 3 HRT=55 h (OLR=4.3 kg COD/m^3 day, SLR= 0.30 kg SO_4^{2-} /m^3 day)	
	FBR	UASB	FBR	UASB	FBR	UASB
TOC removal efficiency (%)	81±3.70	40±0.55	63±1.80	42±3.00	72±1.0	43±1.20
COD removal efficiency (%)	86±0.84	41±0.79	64±0.18	45±1.23	74±0.24	46±0.63
TOC removal rate (kg TOC/m^3 day)	0.8±0.0.3	0.4±0.44	1±0.03	0.6±0.64	0.9±0.01	0.6±0.56
COD removal rate (kg COD/m^3 day)	2.85±0.03	1.36±0.02	3.21±0.01	2.28±0.06	3.24±0.02	2.04±0.03
Methane yield (NL/g TOC removed)	0.98±0.26	0.69±0.17	0.93±0.05	0.58±0.05	0.97±0.001	0.71±0.01
Methane yield (NL/g COD added)	0.26±0.002	0.07±0.011	0.19±0.003	0.08±0.006	0.21±0.003	0.09±0.006
Methane concentration (%)	59±0.71	31±0.62	54±1.41	42±0.46	51±0.89	36±0.14
Sulfate removal efficiency (%)	78±0.82	75±0.66	28±1.24	51±0.20	59±1.26	29±4.43
Sulfate reducing rate (kg SO_4^{2-}/m^3 day)	0.2±0.01	0.18±0.001	0.1±0.004	0.2±0.008	0.19±0.01	0.09±0.022
H_2S (g) (%)	1.2±0.07	1.1±0.07	2.2±0.12	2±0.14	1.6±0.14	0.5±0.35

The abundant of acetate as a primary end product (Fig. 3.2) seemed beneficial to the methanogenesis efficiency but limited the sulfate reduction efficiency in utilizing COD. The amount of COD in the reactor consists of the COD effluent, recovered CH_4-COD by MPB and COD utilized sulfate reduction (ΔSO_4-COD). The portion of electron flow used by SRB and MPB in FBR are shown in Figure 3.3.

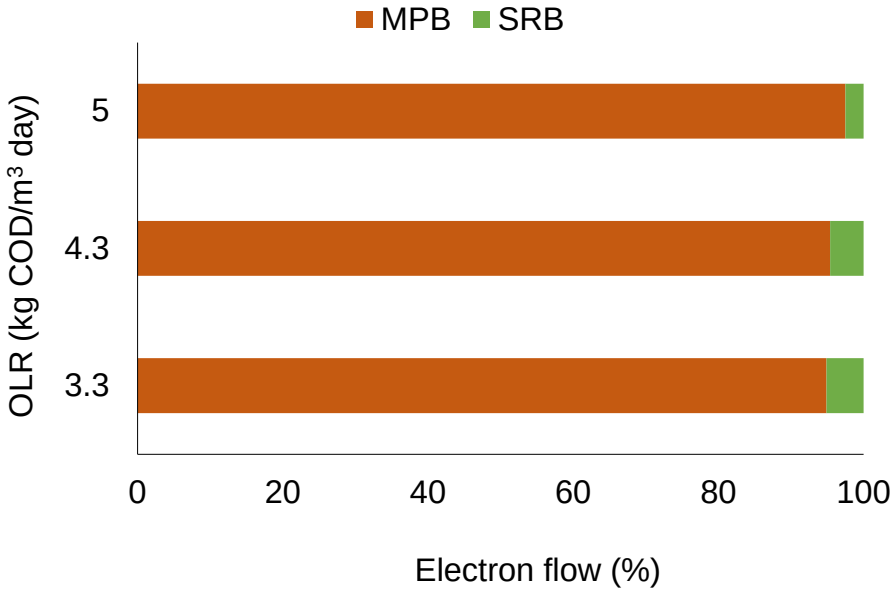


Figure 3.3 The portion of electron flow used by SRB and MPB in FBR

By increasing OLR, increased the electron flow by MPB. This study showed that sulfate concentration of 692 ± 61 mg/L has no significant effect on methane production from sulfate-containing tofu wastewater in FBR. At maximum, approximately 5.1% was inhibited methane production. These results might be at low sulfate levels; less hydrogen can be used by the SRB due to the lack electron acceptor [78].

However, FBR treating sulfate-containing wastewater resulted in a lower methane yield than treating acidic tofu wastewater as shown in Figure 3.4.

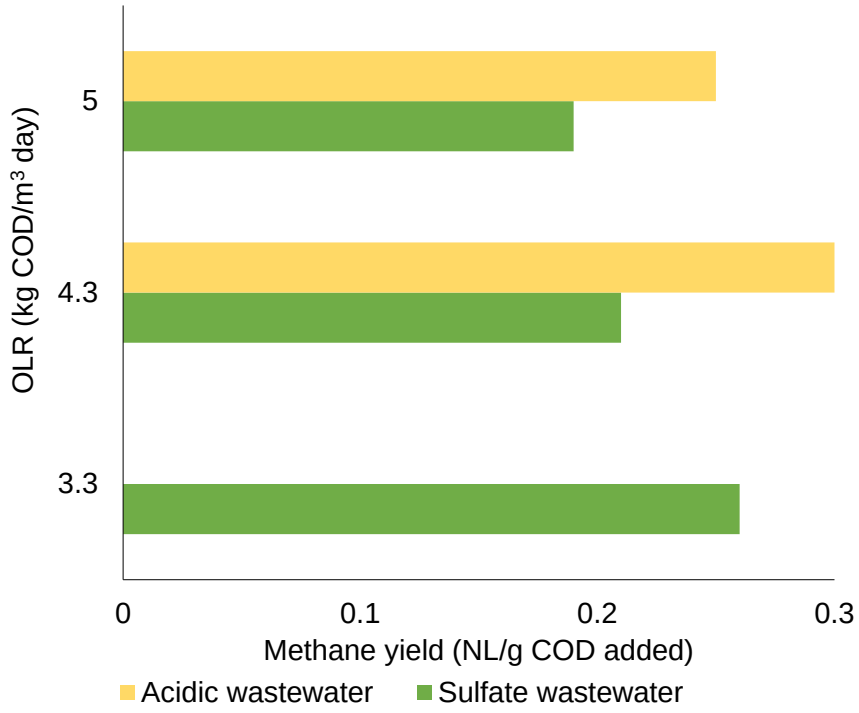


Figure 3.4 Comparison of methane yield produced by FBR in treating acidic and sulfate containing tofu wastewater

At higher OLR of 4.3 kg COD/m^3 day, FBR treating acidic tofu wastewater resulted in higher methane yield of 0.30±0.01 NL/g COD added than FBR treating sulfate-containing wastewater. This might be due to the sulfide inhibition on treating sulfate-containing wastewater which sulfide in the range 66-189 mg/L was observed during the reactor operation. It has been reported that sulfide can inhibit methanogen from concentration 55 mg/L, and at 250 mg/L inhibit 50% activity of MPB [34].

3.4. Conclusions

Methane recovery from sulfate-containing tofu wastewater can be achieved maximum at OLR 3.3 kg COD/m^3 day; 0.23 kg SO_4^{2-} /m^3 day. Sulfide inhibition is a limiting factor that affects the decrease in methane recovery from sulfate-containing wastewater.

Chapter 4

Laboratory scale treatment of acidic tofu wastewater by using three-stage fixed bed reactor

4.1. Background

Acidic tofu wastewater was successfully treated by one stage FBR to produce methane and bamboo used as biofilm carrier in the previous chapter. The FBR was operated under a controlled optimum temperature for methanogenesis in mesophilic temperature (T=35˚C). For the application of FBR Indonesia, low temperature and fluctuating flow rate operation should be taken into consideration. Indonesia as a tropical country; the temperature range is approximately 20-35 ˚C. However, at low temperatures, chemical oxygen demand (COD) removal efficiency is lower, a longer hydraulic retention time (HRT) is needed, and the amount of accumulated suspended solids (SS) in the anaerobic reactor increases due to the lower hydrolysis rate [79]. Besides, since the tofu industries in Indonesia are mostly home industries that generate fluctuating wastewater, the treatment process needs to be against this parameter. Accumulation of volatile fatty acids (VFAs), caused by an imbalance of the methanogenesis steps, is common in single-stage anaerobic systems operated at high organic loadings. VFA accumulation could reduce the reactor pH, inhibit methanogenic activity and subsequently cause system failure

To overcome these parameters, we developed a multistage FBR instead of one stage FBR for the tofu wastewater treatment application in Indonesia. Multistage reactor promotes the separation of the acidogenic and methanogenic phases of the anaerobic process. The acidogenic bacteria and methanogenic archaea show different characteristics, particularly concerning their nutrition, physiology, growth pH, and ability to withstand the environmental changes [80]. The separation stage alleviates metabolites' accumulation by buffering the loading rate and organic

matter in the first stage, allowing healthier methanogenesis in the next stage. These methods allow increased protection against toxic materials and higher resistance to changes in environmental parameters such as pH and temperature [81].

Lab-scale test is an essential preliminary in assessing a multistage FBR feasibility before large scale construction. The aim of this study was to evaluate the performance of a multistage FBR by using three stages of FBR for treating acidic tofu wastewater at different organic load and HRT. The corresponding bacterial and methanogen communities were also characterized.

4.2. Material and methods

4.2.1. Experimental reactor design and operation

The lab-scale reactors comprised three identical cylindrical PVC FBR reactors (R1-R3) with efective volumes of R1, R2 and R3 are 10.59 L, 10.45 L, and 10.36 L, respectively (Fig. 4.1). The reactors were filled with cut bamboos as biofilm carrier at the height of 300 mm (outer diameter of 30-45 mm, and length of 45 mm). A plate was placed at 100 mm from the bottom to support the bamboo. The reactor was fed continuously from the feeding tank using a peristaltic pump to R_1; the flow from R_1 to R_2 and R_2 to R_3 was gravitational. The liquid sampling ports were 0, 1, 2, and 3 for feeding, R_1, R_2, and R_3, respectively. The gas produced in each reactor was collected through a Tedlar bag.

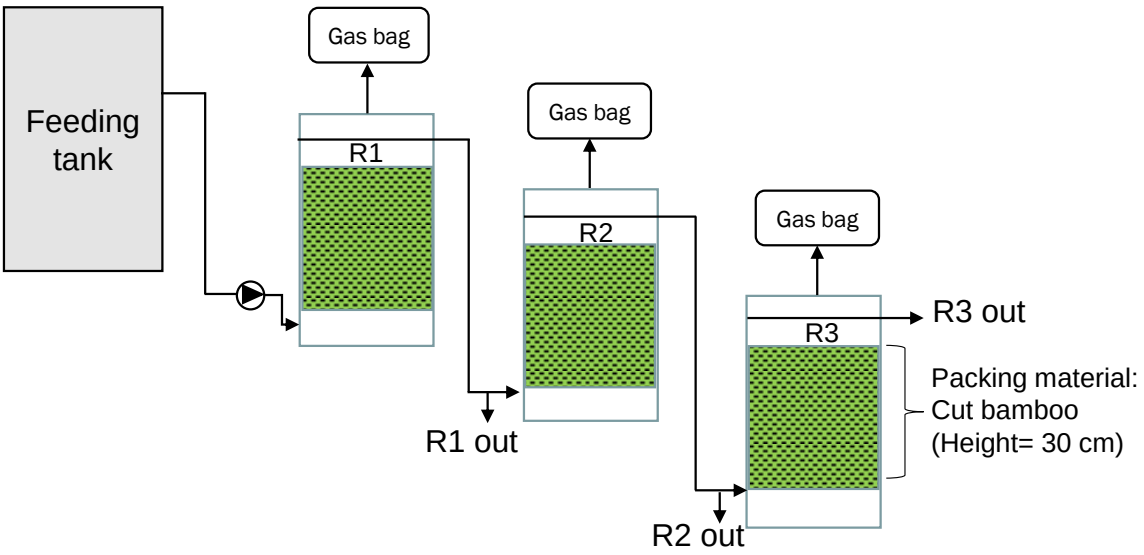


Figure 4.1 The three stages of FBR

The temperature and pH were uncontrolled; the average ambient was temperature is shown in Fig. 4. The temperature ranged from 20.3-29.6 ˚C. The experiment was conducted at Research Unit for Clean Technology LIPI in Bandung city, West Java, Indonesia.

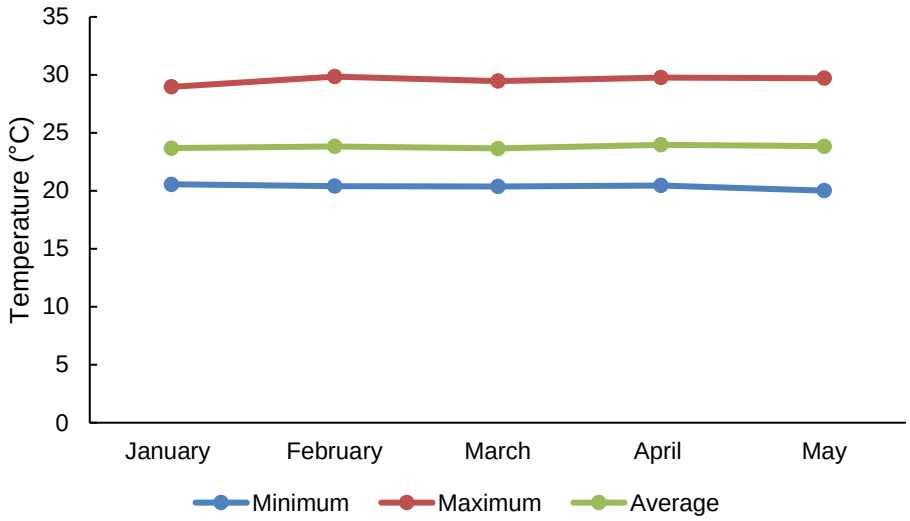


Figure 4.2 The ambient air temperature in the study area

The reactor was operated continuously by using different OLR and HRT (as shown in Table 4.1).

Table 4.1 Operating conditions of the continuous anaerobic digestion experiment

HRT (day)	8	6	4
OLR (Kg/m^3 day)	0.88±0.2	1.73±0.14	2.43±0.28

4.2.2. Substrate and Seed Sludge

Tofu wastewater from the tofu industry (in Bandung city, West Java, Indonesia) was used as the substrate. The characteristics of the substrate is shown in Table 4.2.

Table 4.2 Characteristics of the substrate using in this study

No	Parameter	Concentration mg/L
1	Soluble COD	6597±3142
2	Total COD	8091±4034
3	Total N	314±236
4	Total P	41.74±22.74
5	SO_4	5.18
6	TS	7041±2654
7	TSS	1380±883
8	pH	4.8±0.63

The seed sludge was using cattle rumen, taken from the slaughterhouse industry in Bandung city, West Java, Indonesia. The seed was diluted and added molasses, then left for six months. The mixed culture of 10 L seed sludge was fed into each reactor without any dilution.

4.2.3. Analytical Methods

Liquid samples were collected from the effluents port of each stage. The tofu wastewater and effluents of each stage were analyzed for total COD, total suspended solids (TSS), volatile

suspended solids (VSS), and pH. COD was analyzed using the potassium dichromate method measured with a UV-VIS Spectrophotometer (Shimadzu GC14-A, Japan) at 615 nm using acetic acid standard. TSS and VSS were analyzed using the gravimetric method. Total N, P, and sulfate were analyzed using standard methods [41].

The gas volume and composition were analyzed by the gas meter (Ritter, Germany) and biogas analyzer (CombimassGA, Germany, and Geotech Biogas 5000, UK).

4.2.4. Microbial Community Analysis

Biofilm attached to the bamboo carriers (BF) and suspended biomass from the bottom of the reactor (SB) were collected from each reactor at the end of reactor operation to investigate the microbial community structure. DNA was extracted from the samples using a DNeasy PowerSoil Kit (Qiagen, Germany). The 16S rRNAs amplicons were amplified from the extracted DNA utilizing a polymerase chain reaction (PCR) method that implemented a 515F [42] and 806R [43] primer set that target the V3-V4 regions of bacteria. A 340F and 806Rb [44] primer set was used to target the V4-V5 regions of archaea. The PCR was carried out using a HotStarTaq DNA Polymerase (Qiagen, Hilden, Germany). The PCR conditions were as follows: denaturation at 95 °C for 15 min, annealing at 94 °C for 30 s, 55 °C for 30 s, and 72 °C for 60 s. This was performed for 25 cycles for bacteria and 37 cycles for archaea. The initial PCR products were purified using Agencourt AMPure XP (Beckman Coulter, Pasadena, USA), while following the manufacturer's standard protocols. The second PCR was conducted to attach Illumina Adapters for DNA sequencing using the KAPA HiFi HotStart PCR Kit (Kapa Biosystems, Inc, Wilmington, USA), while following the manufacturer's protocols. The amplified DNA was purified using Agencourt AMPure XP. The 16S rRNA amplicon sequencing was performed by Illumina MiSeq platform.

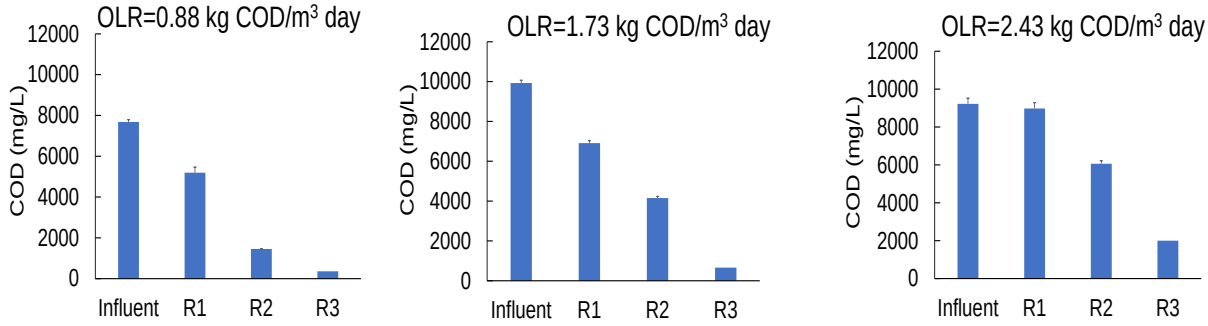
Raw sequence reads were filtered from the adapter contaminant using the Trimmomatic [45]. After quality trimming, sequence reads were clustered using Usearch at 97% similarity

[46]. Clustered reads were then classified into operational taxonomical units (OTU) using the UPARSE pipeline [47]. The taxonomic classification of OTUs was performed using QIIME with SILVA_128 as the reference database [48]. The microbial community abundances were generated using Microsoft Excel™.

4.3. Results and discussions

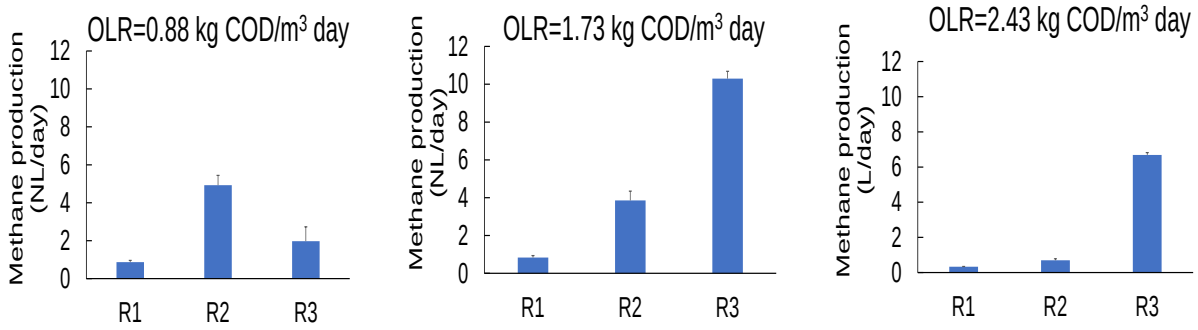
4.3.1. Reactor performance

The COD concentration of the influent and effluent in each reactor at different OLR is shown in Fig. 4.1. The results show that COD concentration gradually decreases from R1 to R3 at different OLR. This indicated the occurrence of methanogenesis. Methane production has occurred in all stages of reactors, as shown in Fig. 4.2.



(a) (b) (c)

Figure 4.1 The COD concentration of influent and effluent in each reactor at a) OLR= 0.88 kg COD/m^3 day b) OLR= 1.73 kg COD/m^3 day c) OLR= 2.43 kg COD/m^3 day



(a) (b) (c)

Figure 4.2 The methane production in each reactor at a) OLR= 0.88 kg COD/m^3 day b) OLR= 1.73 kg COD/m^3 day c) OLR= 2.43 kg COD/m^3 day

Fig. 4.2 shows that at a low OLR of 0.88 kg COD/m^3 day, R2 can achieve the highest methane production. This indicates that most of the COD substances were reduced and converted to methane in the initial methanogenesis stage of R2. Meanwhile, by increasing the OLR, the most increased methane production occurs in R3. Methane production in R1 is lower than in R1 and R2, demonstrated that hydrolysis and acidogenesis are the main biochemical activities in the first stage. This result in line with the pH value of R1, which lower than R2 and R3 at OLR= 0.88 and 1.73 kg COD/m^3 day. But when OLR increased to 2.43 kg COD/m3 day, low pH was observed in R1 and R2, then pH increase over 7 in R3. Thus, it can be concluded that separating the digestion phases can increase the distance between syntrophic bacteria, not hinder methanogenesis [82]. This provides an advantage, particularly for treating a high organic load.

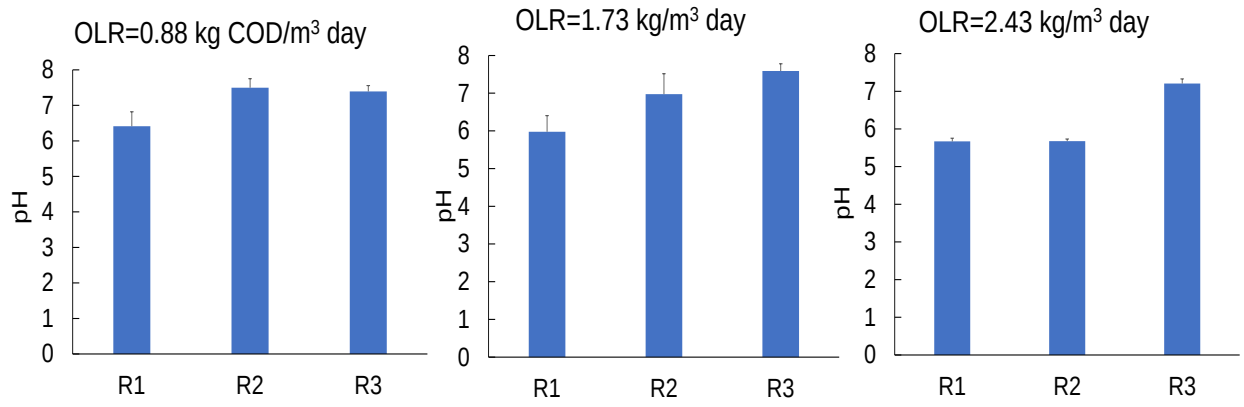


Figure 4.3 The pH in each reactor at a) OLR= 0.88 kg COD/m^3 day b) OLR= 1.73 kg COD/m^3 day c) OLR= 2.43 kg COD/m^3 day

However, compared to one stage FBR, three-stage of FBR show a similar performance. The comparison results between both reactors are shown in Table 4.2. At OLR 1.73 kg COD/m^3

day, three-stage FBR achieved maximum methane yield of 0.29±0.01 NL/g COD added and 93±0.88 % of COD removal. Meanwhile, the maximum methane yield of 0.3±0.01 NL/g COD added, and COD removal of 96±1.70 can be achieved by one-stage FBR at OLR= 4.3kg COD/m^3 day. The results indicated that although at room temperature, the three-stage reactors could be operated and produce methane, thus reducing energy expenses. Lower organic load than one-stage FBR arguably because of the low temperature may affect the methanogenesis.

Table 4.2. The comparison performance between three-stage and one stage FBR

	Three-stages FBR			One-stage FBR
OLR (kg COD/m^3 day)	0.88	1.73	2.43	4.3
HRT (day)	8	6	4	2.3
COD removal efficiency (%)	93±1.35	93±0.88	64±2.04	96±1.70
COD removal rate (kg COD/m^3 day)	1.39±0.01	1.53±0.01	1.42±0.05	4.26±0.02
Methane yield (NL/g COD added)	0.27±0.01	0.29±0.01	0.15±0.01	0.3±0.01

4.3.2. Bacterial community

Fig. 4.4 and 4.5 show the relative abundance of bacterial phyla in the bottom reactor (SS) and attached to the bamboo carrier (BF) at R1-R3. *Bateriodetes* and *Firmicutes* formed the most abundant phyla detected in all the reactors. Members of the *Bacteroidetes* are known as acidogenic, sugar fermenting, saccharolytic, and proteolytic bacteria that produce propionate, acetate, and succinate as their primary byproducts [54]. *Firmicutes* are involved in the process of hydrolysis, acido and acetogenesis [83]. The bacterial community at the phylum level show that *Bacteroidetes* and *Firmicutes* were gradually decreased from R1 to R3.

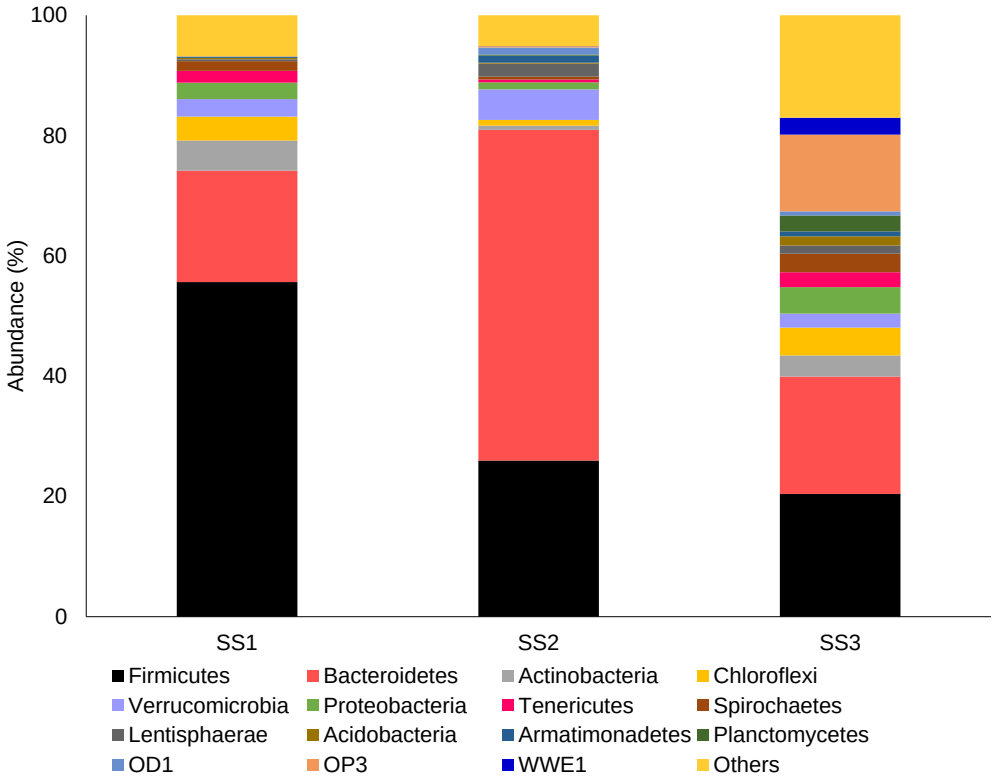


Figure 4.4 Distribution of bacteria in the bottom reactor at the phylum level

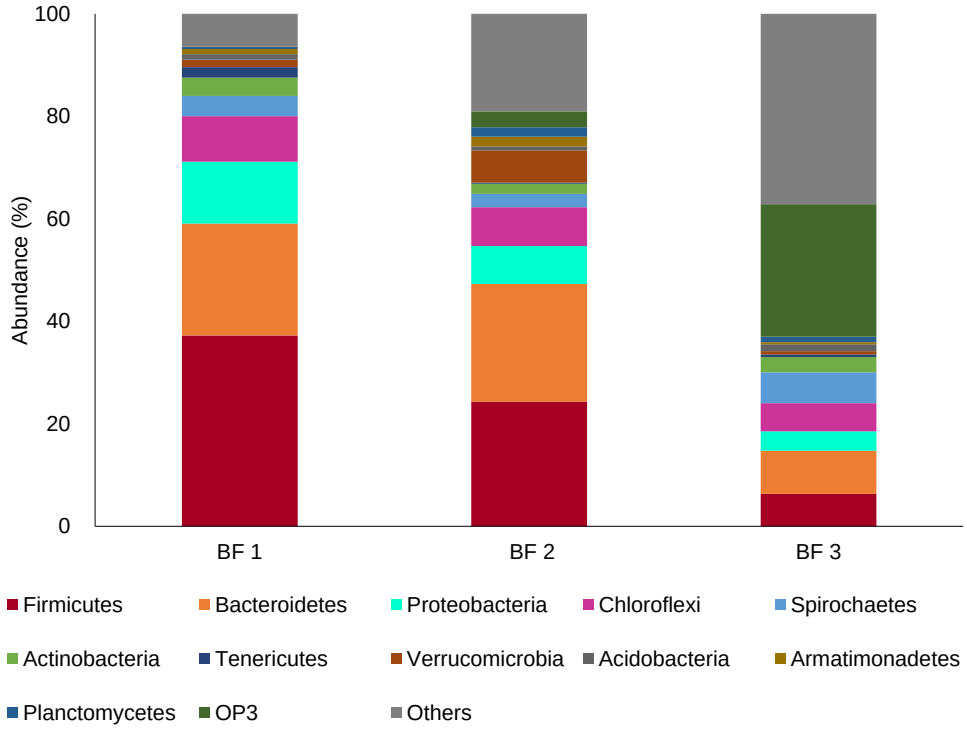


Figure 4.5 Distribution of bacteria in the bamboo carrier at the phylum level

We categorized the bacterial community at the family level into two groups: hydrogen producer and acetate producer afterward, as shown in Fig 4.6. and 4.7. In the methane formation, the primary stage of fermentation is done by hydrolyzing bacteria which can convert the macromolecules to simple sugars, amino acids, and fatty acids. Further degradation, organic matter will convert into propionic acid, butyric acid, pentanoic acid, other volatile fatty acids (VFAs), and alcohols by bacterial acidogenesis. The second stage, bacterial acetogenesis, will convert VFAs into acetic acid, H_2, and CO_2 [84]. The maximum conversion of organic matter into methane is required the synergistic action of at least three groups of above microorganisms. Fig 4.6 and 4.7 show that hydrogen-producing bacteria and acetate-producing bacteria existed in all reactors of three-stages of FBR at the bottom reactor and bamboo carrier.

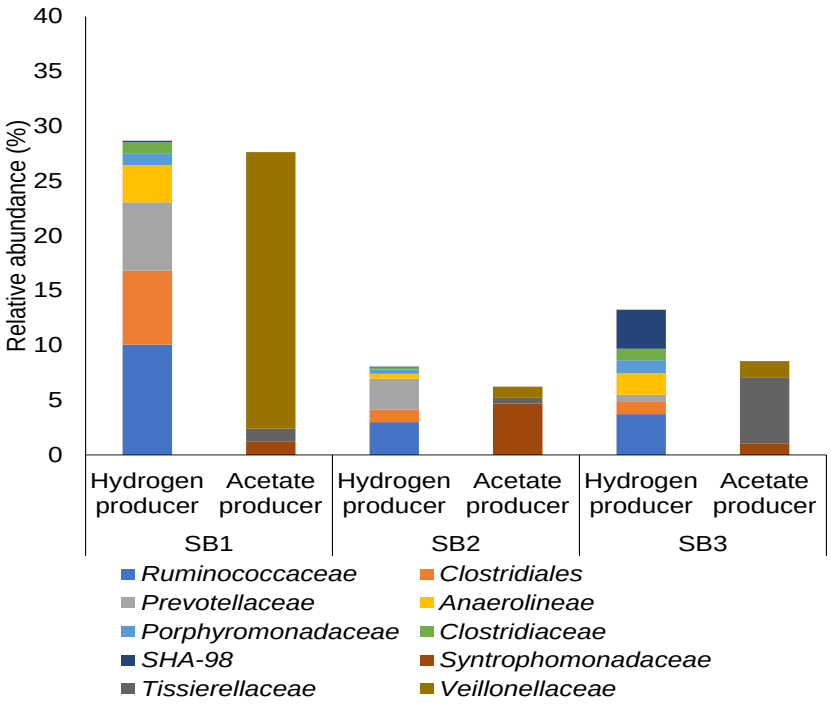


Figure 4.4 Distribution of bacteria in the bottom reactor at family level

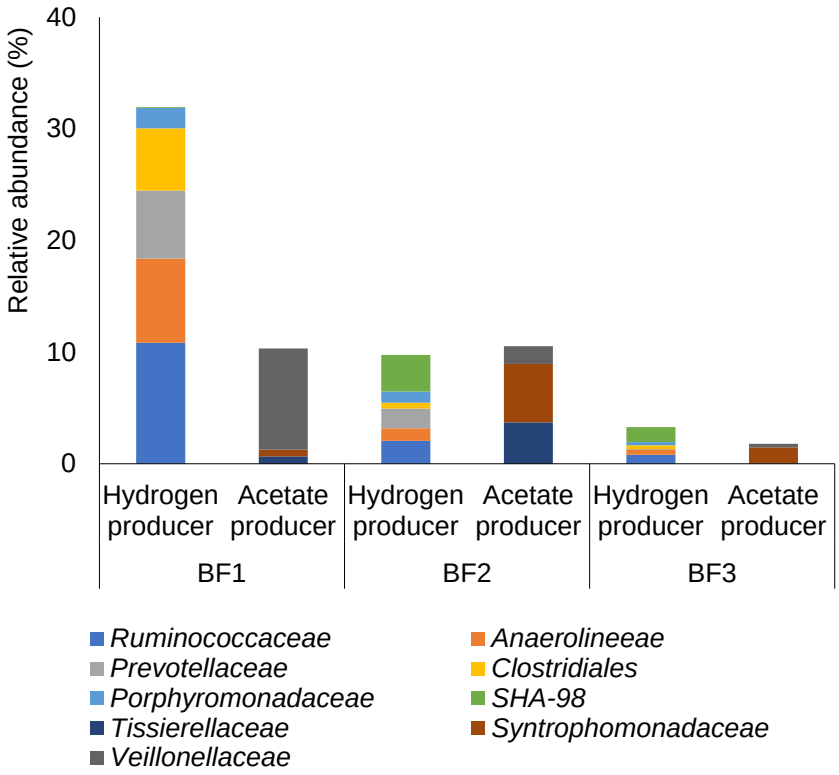


Figure 4.7 Distribution of bacteria in the bamboo carrier at family level

Overall, the hydrogen-producing bacteria (*Ruminococcaceae*, *Clostridiales*, *Prevotellaceae*, *Anaerolineae*, *Porphyromonadaceae*, *Clostridiaceae*, and *SHA-98*) were higher abundant than acetate producing bacteria (*Syntrophomonadaceae*, *Tissirelaceae*, and *Veillonellaceae*) in all reactors (at the bottom reactor and carrier biofilm) [59], [60], [61].

4.3.3. Archaeal community

According to the different metabolites, methanogens were divided into three types: (1) hydrogenotrophic methanogens that can use H_2 and CO_2 to produce methane; (2) methylotrophic methanogens, which can use methyl compounds, such as methanol, methylamine, and formic acid, and (3) the methanogen which can use of acetic acid, termed acetoclastic methanogens [85]. Fig. 4.8 and 4.9 reflects the 10 most abundant of archaeal community at the genus level. It is classified into hydrogenotrophic and acetoclastic methanogens.

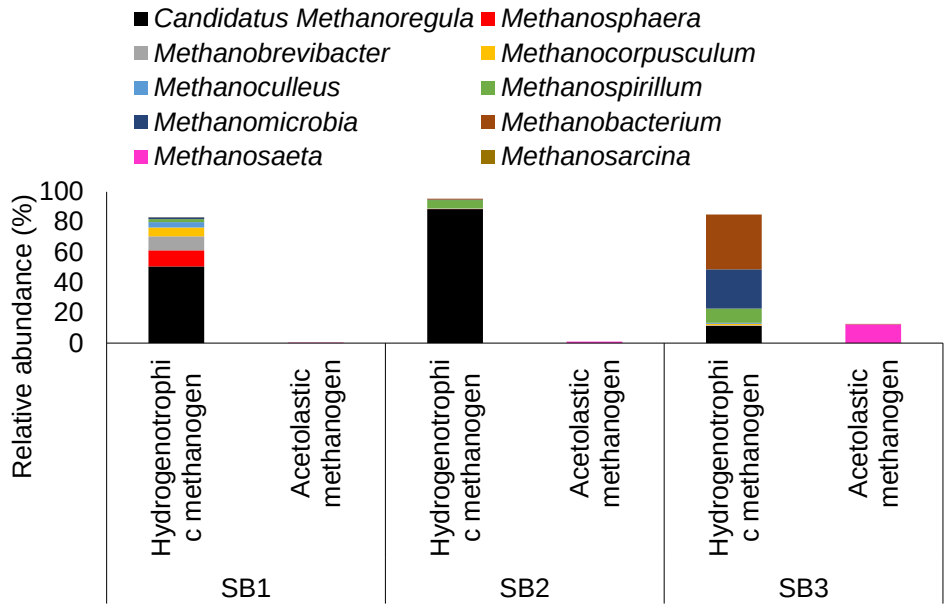


Fig. 4.8 The 10 most abundant of archaeal community in bottom reactor at genus level

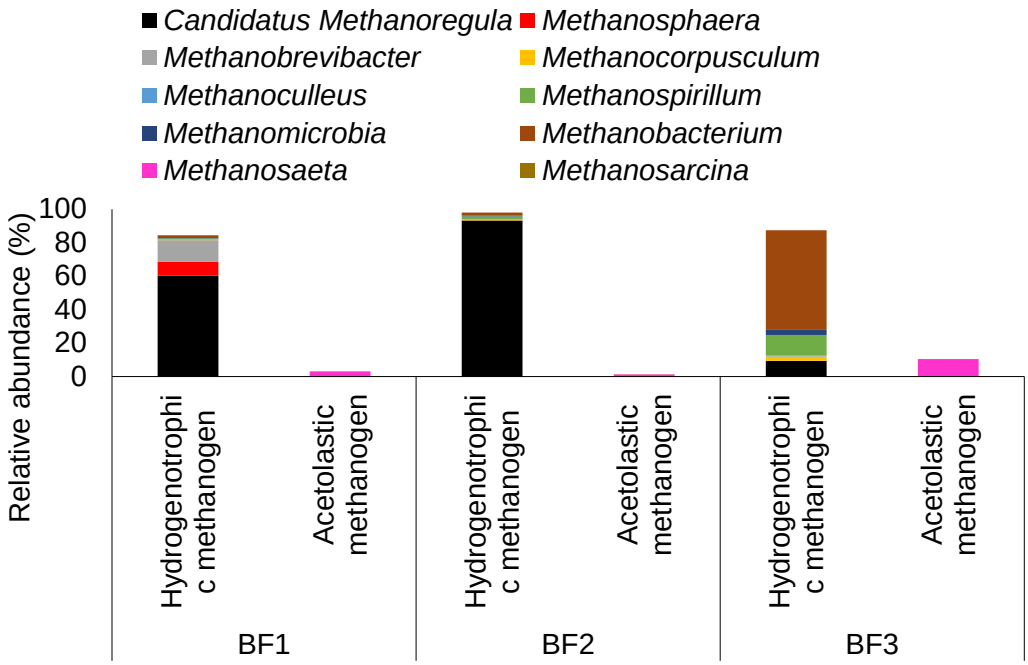


Fig. 4.9 The 10 most abundant of archaeal community in bamboo carrier at genus level

Fig. 4.8 and 4.9. show that hydrogenotrophic methanogens (*Candidatus Methanoregula*, *Methanosphaera*, *Methanobrevibacter*, *Methanocorpusculum*, *Methanoculleus*,

Methanospirillum, *Methanomicrobia*, and *Methanobacterium*) was dominant than acetoclastic methanogen (*Methanosaeta* and *Methanosarcina*) in all reactor at the bottom reactor and biofilm carrier [65] [86]. The abundance of *Methanosaeta* (acetoclastic methanogen) in R3 was higher than in R1 and R2. This might because R3 provides a higher pH level than R 1 and R2 since acetoclastic was sensitive to low pH [66].

The presence of hydrogen-producing bacteria in the bacterial community had positive correlations with hydrogenotrophic methanogens. Because the influent in this study had a low pH and hydrogenotrophic methanogens are less sensitive to unfavorable pH levels than acetoclastic methanogens, hydrogenotrophic methanogens were dominant and adapted throughout the experiment [70].

4.4. Conclusions

The lab-scale treatment using three-stage FBR and cut bamboo as biofilm carrier could enhance methane recovery from acidic tofu wastewater under ambient room temperature. At OLR1.73 kg COD/m^3 day, the maximum methane yield of 0.29±0.01 NL/g COD added and 93±0.88 % of COD removal can be achieved. The hydrogenotrophic methanogenesis pathway dominated methane production in each stage of the reactor

Chapter 5

Pilot Scale application of multistage anaerobic fixed bed reactor enhanced methane recovery from acidic tofu wastewater

5.1. Introduction

The lab-scale treatment of three-stage FBR on treating acidic tofu wastewater has been assessed in the previous chapter. This system was successful in enhancing the methane recovery from acidic tofu wastewater. Further study was conducted in this chapter by applying the multistage FBR in the pilot scale. The pilot-scale of multistage was built in Sumedang regency, West Java, Indonesia.

Sumedang is well known as a tofu producer in Indonesia, with approximately 232 tofu industries, as shown in Table 5.1 [87].

Table 5.1 The distribution of tofu industries in Sumedang regency, West Java, Indonesia

No	Districts	Number of factory	Number of worker	Production capacity (kg soybean/month)
1	Cibugel	4	19	21,750
2	Cimalaka	9	16	12,000
3	Cimanggung	8	39	54,150
4	Cisitu	8	76	33,000
5	Conggeang	6	28	24,300
6	Darmaraja	8	12	10,500
7	Ganeas	1	2	200
8	Jatigede	7	32	4,750
9	Jatinangor	3	6	5,400
10	Jatinunggal	15	46	13,830
11	Pamulihan	10	50	47,250
12	Paseh	3	8	5,400
13	Situraja	17	30	28,800
14	Sumedang Selata	33	131	101,000
15	Sumedang Utara	53	148	144,760
16	Tanjungkerta	5	22	13,350
17	Tanjungsari	27	95	91,890
18	Tomo	1	2	1,500
19	Ujungjaya	4	26	6,000
20	Wado	10	24	16,800
	Total	232	812	636,630

Our study area was focuses in Sumedang Utara district, which has the largest number of

tofu industries. Since all the tofu industries still discharge their wastewater directly into the environment, a multistage FBR by applying six-stage reactors was built at Giriharja Subdistrict in Sumedang Utara. The reactor treats tofu whey wastewater from nine small-scale tofu factories. The production capacity varies from 30-400 kg-soybean per day, in a total of 2.6-3 tonnes-soybeans per day. The tofu processing still uses wood, rice husk, or sawdust as fuel. Anaerobic treatment by enhancing methane recovery expected can be used as alternative energy in tofu processing.

This work discusses the performance and the microbial population structure of pilot-scale 6-stage FBR treating acidic tofu wastewater in Indonesia.

5.2. Material and Methods

5.2.1. Reactor design and operation

The FBR was operated as a six-stage system with a total volume of 120 m^3 (Fig. 5.1) and is located in Giriharja Subdistrict, Sumedang Regency-West Java, Indonesia. The reactor was inoculated by anaerobic pond sludge obtained from the Bojongsoang Integrated Domestic WWTP in Bandung City. The reactors have been operated for ten months treating acidic tofu wastewater from 9 tofu industries at an ambient air temperature of 26 to 29.3 ˚C.

Figure 5.1 The six-stage FBR

From the factories, tofu wastewater was flowed by gravity to the six-stage FBR plant.

The FBR plant consists of a collecting tank (B), an equalizing tank (C), a feeder tank (D), six-stage anaerobic fixed-bed reactor (FBR, F), effluent ponds (M), biogas collector (E, G), biogas distribution system (H, I, L), and operator chamber (J) (Fig.5.2). The characteristics of the wastewater are shown in Table 5.2.

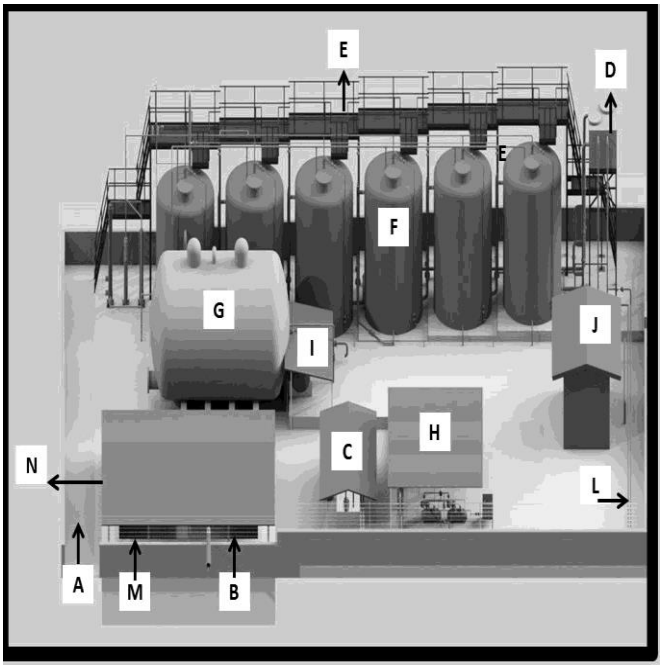


Figure 5.2 The scheme of six-stage FBR

Table 5.2 The characteristic of tofu wastewater

	Unit	Concentration
pH		5.0 ± 0.2
Total COD	g/L	13.4 ± 3.5
Soluble COD	g/L	8.5 ± 2.3
Total N	mg/L	258 ± 4
NH_3-N	mg/L	8.5 ± 0.3
Total P	mg/L	0.16 ± 0.03
Sulfate	mg/L	0.40 ± 0.04
Sulfide	mg/L	< 0.005
Natrium	mg/L	152 ± 25
Magnesium	mg/L	72 ± 2
Potassium	mg/L	46 ± 3
Calcium	mg/L	58 ± 1

TS	g/kg-sample	5.1 ± 1.3
VS	g/kg-sample	3.2 ± 1.1
TSS	g/kg-sample	1.5 ± 0.3
VSS	g/kg-sample	1.0 ± 0.4

5.2.2. Analytical method

Liquid samples were collected from the effluents port of each stage. The tofu wastewater and effluents of each stage were analyzed for total COD, total suspended solids (TSS), volatile suspended solids (VSS), and pH. COD was analyzed using the potassium dichromate method measured with a UV-VIS Spectrophotometer (Shimadzu GC14-A, Japan) at 615 nm using acetic acid standard. TSS and VSS were analyzed using the gravimetric method. Total N, P, and Sulfate were analyzed using standard methods [41].

The gas volume and composition were analyzed by the gas meter (Ritter, Germany) and biogas analyzer (CombimassGA, Germany, and Geotech Biogas 5000, UK).

5.2.3. Microbial Community Analysis

The biomass from the reactor was collected from reactor 1 to 5 at nine-month of reactor operation to investigate the microbial community structure. DNA was extracted from the samples using a DNeasy PowerSoil Kit (Qiagen, Germany). The 16S rRNAs amplicons were amplified from the extracted DNA utilizing a polymerase chain reaction (PCR) method that implemented a 515F [42] and 806R [43] primer set that target the V3-V4 regions of bacteria. A 340F and 806Rb [44] primer set was used to target the V4-V5 regions of archaea. The PCR was carried out using a HotStarTaq DNA Polymerase (Qiagen, Hilden, Germany). The PCR conditions were as follows: denaturation at 95 °C for 15 min, annealing at 94 °C for 30 s, 55 °C for 30 s, and 72 °C for 60 s. This was performed for 25 cycles for bacteria and 37 cycles for archaea. The initial PCR products were purified using Agencourt AMPure XP (Beckman Coulter, Pasadena, USA), while following the manufacturer's standard protocols. The second

PCR was conducted to attach Illumina Adapters for DNA sequencing using the KAPA HiFi HotStart PCR Kit (Kapa Biosystems, Inc, Wilmington, USA), while following the manufacturer's protocols. The amplified DNA was purified using Agencourt AMPure XP. The 16S rRNA amplicon sequencing was performed by Illumina MiSeq platform.

Raw sequence reads were filtered from the adapter contaminant using the Trimmomatic [45]. After quality trimming, sequence reads were clustered using Usearch at 97% similarity [46]. Clustered reads were then classified into operational taxonomical units (OTU) using the UPARSE pipeline [47]. The taxonomic classification of OTUs was performed using QIIME with SILVA_128 as the reference database [48]. The microbial community abundances were generated using Microsoft Excel™.

5.3. Results and Discussions

5.3.1. Reactor performance

Fig. 5.3 shows the total COD concentration of the influent and effluent in each reactor. The results show that COD concentration gradually decreases from R1 to R5. Total COD concentration decreased from 13.4 ± 3.5 g/L in the influent to 3.6 ± 0.5 g/L in R1. On average, 73% of the total COD in the influent was removed in R1. Fluctuations mostly occurred in R2 and R3, suggesting these two stages acted as the buffer. Reactor effluent (R6) was relatively stable at 0.7 ± 0.4 g/L total COD concentration. The effluent indicates overall removal of 95%.

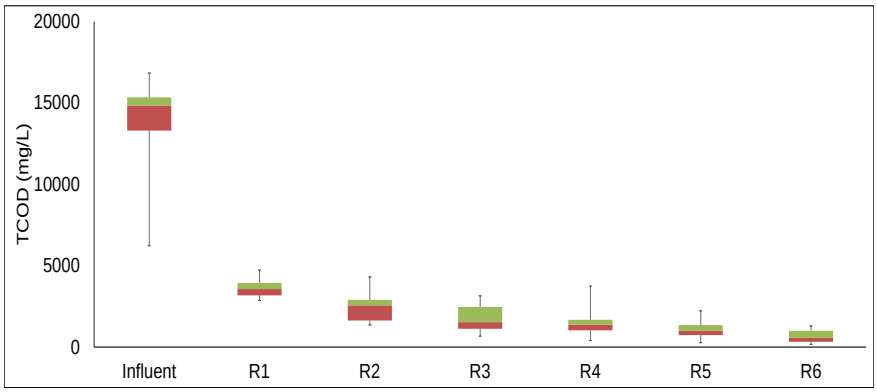


Figure 5.3 The distribution of total COD concentration in the influent and effluent of each reactor

The soluble COD concentration of influent and effluent in each reactor are shown in Fig 5.4. The soluble COD concentration shows a similar trend to total COD concentration, except the fluctuations mostly occurred in R1 and R2. The fluctuation indicates that hydrolysis could be a limiting step, particularly in R1. In R1-R4, soluble COD concentrations were significantly lower than total COD concentrations. In R5 and R6, however, there was no significant difference between total and soluble COD concentrations. This suggests that in R5 and R6, most particulate COD had been dissolved.

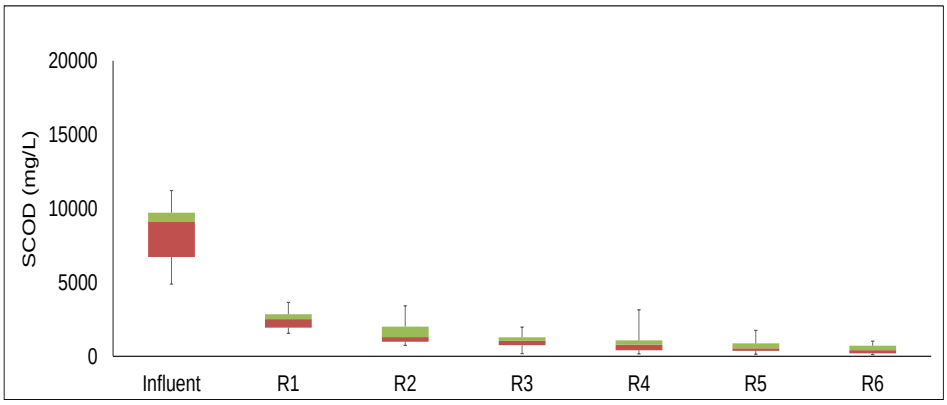


Figure 5.3 The distribution of soluble COD concentration in the influent and effluent of each reactor

The pH in each reactor is shown in Fig. 5.4. It shows that the pH increased significantly from the influent (5.0 ± 0.2) to R1 (6.6 ± 0.2), then to R2 (7.0 ± 0.2). The pH in the reactor effluent (R6) was 7.1 ± 0.2. There was no significant pH difference in R2-R6. This indicates that the six-stage AFBR was able to stabilize the pH of tofu wastewater.

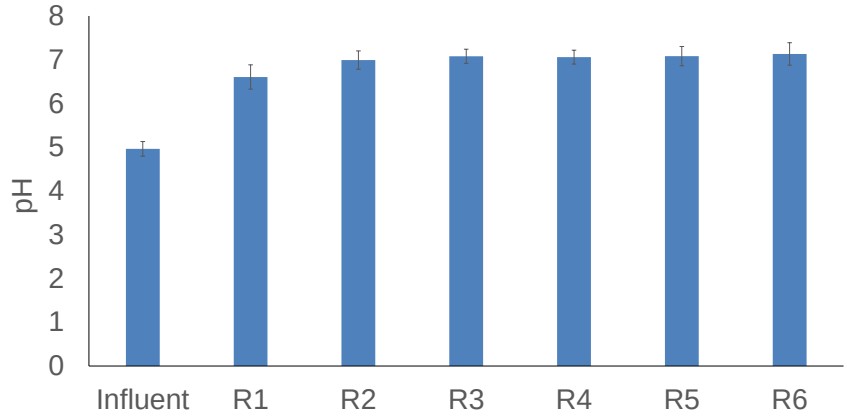


Figure 5.4 The distribution of pH in each reactor

5.3.2. Bacterial Community

Fig. 5.5 show the relative abundance of bacterial phyla in each reactor. The dominant phyla detected in all reactors were similar to lab-scale treatment in the previous chapter, *Firmicutes* and *Bateriodetes*. Members of the *Bacteroidetes* are known as acidogenic, sugar fermenting, saccharolytic, and proteolytic bacteria that produce propionate, acetate, and succinate as their primary byproducts [54]. *Firmicutes* are involved in the process of hydrolysis, acidogenesis, and acetogenesis [83]. At the phylum level, the bacterial community shows that *Bacteroidetes* and *Firmicutes* were gradually decreased from R1 to R5. This might occur because the organic matter was also progressively decreased in the reactors.

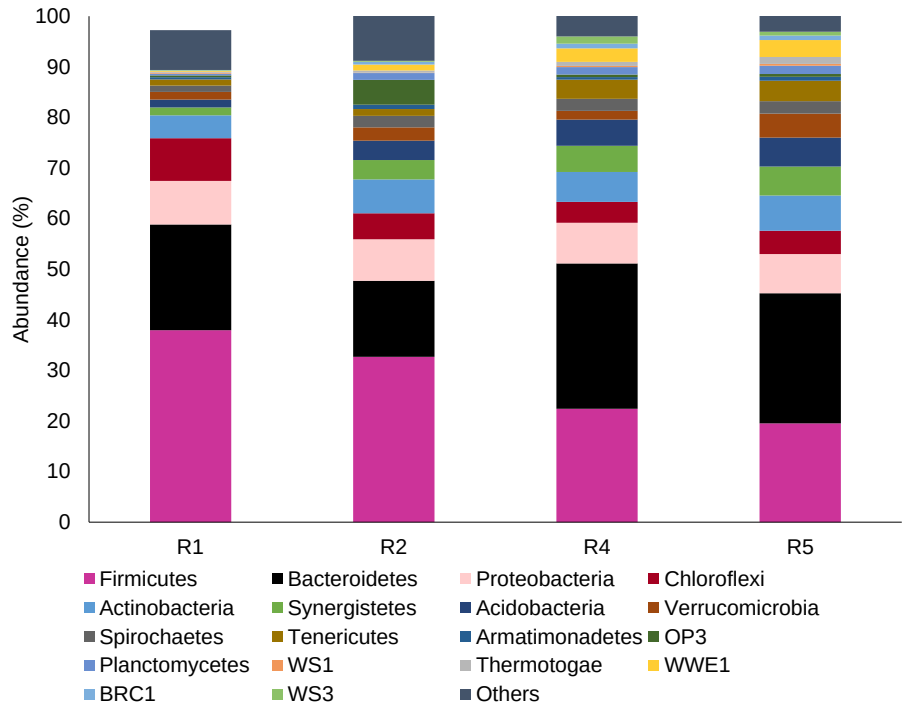


Figure 5.5 Distribution of bacteria in each reactor at phylum level

By specified the bacterial community into two groups, hydrogen-producing bacteria and acetate producer bacteria (Fig 5.6), the results show that hydrogen-producing bacteria (*Prevotellaceae*, *Clostridales*, *Ruminococcaceae*, *Porphyromonadaceae*, and *Anaerolineae*) and acetate-producing bacteria (*Syntrophomonadaceae* and *Veillonellaceae*) existed in all reactors) [59], [60], [61]. The acetate producing bacteria has higher abundant in R1 and R2 than hydrogen-producing bacteria. Then, acetate producing bacteria has lower abundant than hydrogen-producing bacteria in R3-R5.

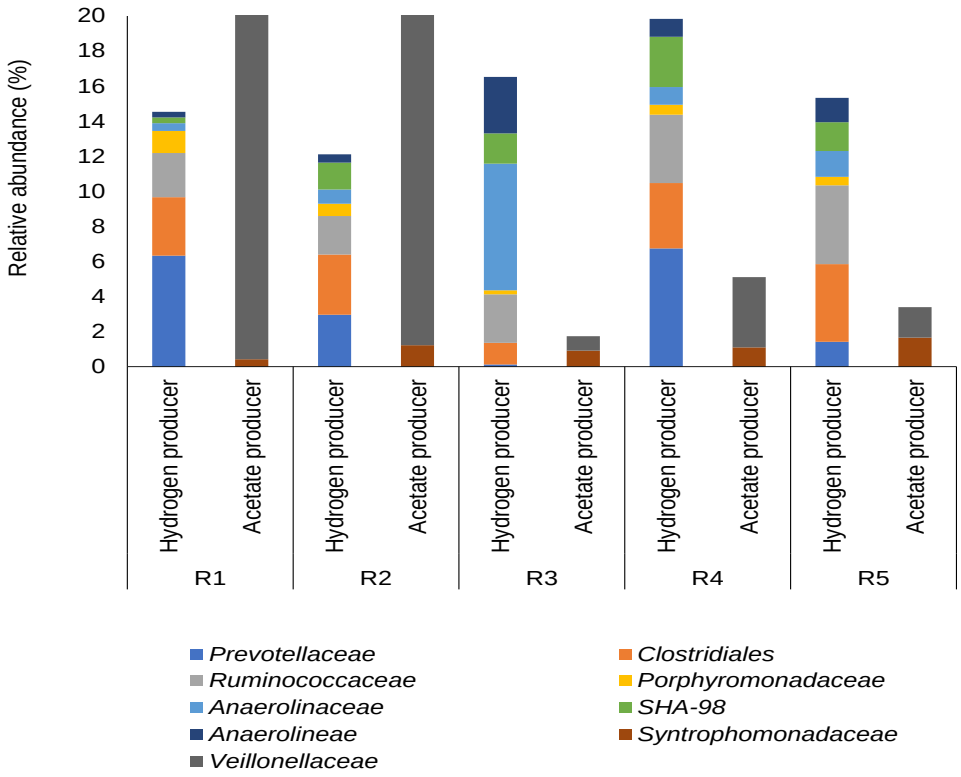


Figure 5.6 Distribution of bacteria in each reactor at family level

5.3.3. Archaeal Community

The top 13 most abundant archaeal community in each reactor at genus level is shown in Fig. 5.6. The result indicates that hydrogenotrophic methanogen (*Candidatus Methanoregula*, *Methanoregulaceae*, *Methanobrevibacter*, *Methanobacterium*, *Methanomicrobiales*, *Methanospirilum*, *Methanocorpusculum*, *Methanosphaera*, *Methanobacteriales*, *Methanocella*, *Methanomicrobia*, and *Methanoculleus*) was dominant than acetoclastic methanogen (*Methanosaeta*) in all reactors [65] [86]. Acetoclastic methanogen has a higher abundance in R3-R5 than R1-R2. This might because R3 provides a higher pH level than R1 and R2 since acetoclastic was sensitive to low pH [66].

As the predominant methanogen was found to be hydrogen-utilizing methanogen in this study, the oxidation of acetic acid into hydrogen and carbon dioxide ($\Delta G^0 = +104$ kJ/mol) also has to be considered [88]. We assumed that although in R1-R2 acetate producing bacteria is higher abundant than hydrogen-producing bacteria, hydrogenotrophic is dominant in R1-R2.

These results indicate that hydrogen-producing bacteria consume acetate produced by acetate producing bacteria to produce hydrogen. Therefore the accumulated hydrogen in R1-R2 can be utilized by hydrogenotrophic methanogen to produce methane.

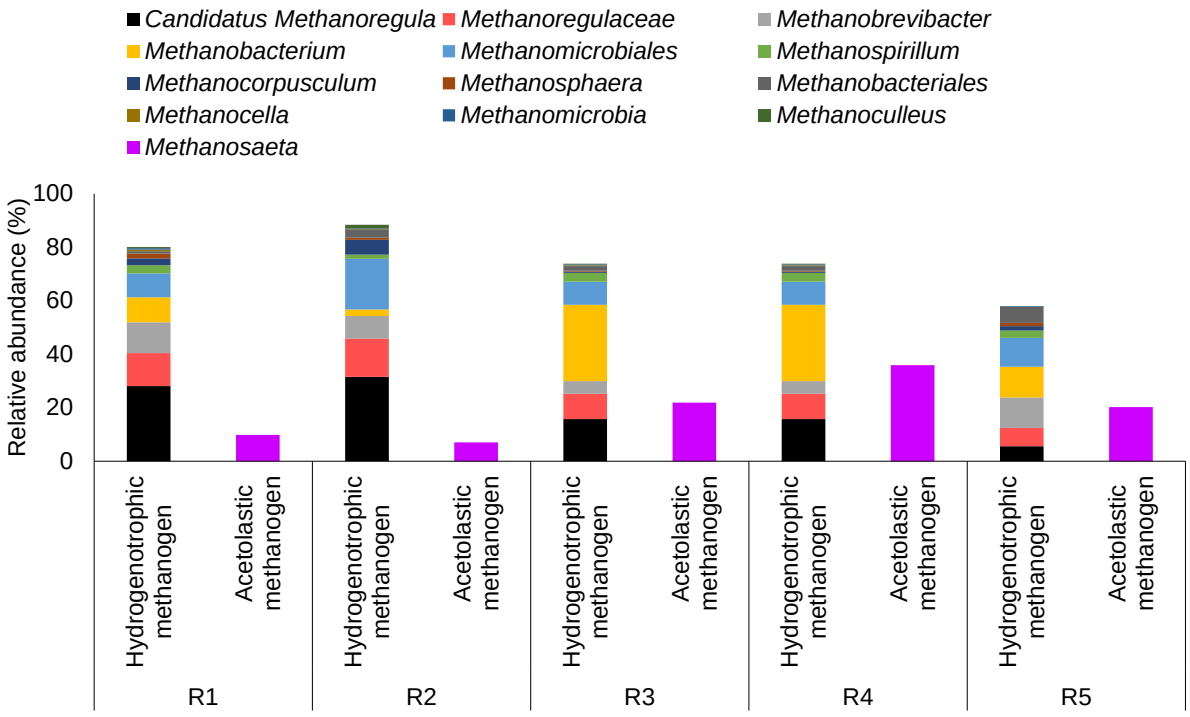


Figure 5.5 The top 13 most abundant of archaeal community in each reactor at genus level

5.4. Conclusions

The six-stage FBR could operate for treating acidic tofu wastewater on a pilot-scale. COD removal of 95% can be achieved, and neutral pH can be maintained in the system. The separation of acidogenesis and methanogenesis in a multistage system has benefits in maintaining the stability of the reactor performance. Hydrogenotrophic methanogenesis was the dominant pathway in all digesters.

Chapter 6
Conclusions

Overall, this study highlights the low-cost anaerobic treatment for treating acidic and sulfate-containing tofu wastewater. FBR using cut bamboo as biofilm carrier exhibited better performance than UASB reactor. The FBR treatment of one-stage and multistage could maintain the stability of reactor performance and applicable to the treatment at low temperature and low pH. Methane recovery can be achieved from acidic and sulfate-containing wastewater treatment without any pH adjustment methods.

The bamboo carrier FBR process is useful for wastewater treatment in small-scale tofu processing industries in Asian countries due to its low cost. Approximately 10 L of wastewater is generated per 1 kg tofu produced. In this study, 30 L of methane gas (2460 kJ) could be recovered per 1 kg tofu produced. This methane can be used as a renewable energy source in such tofu industries. In Indonesia, the energy source used in tofu processing is mainly the direct burning of wood in small scale industries located in rural areas, while wood boilers are used in middle scale industries in larger towns. Smoke generated from wood-burning may affect worker health. The use of methane gas may improve the overall working environment. In addition, since bamboo is widespread in Asian counties, its effective utilization as a wastewater treatment carrier may be useful for the management of bamboo groves.

The overall methane generation was predominated by hydrogenotrophic methanogen, work syntrophy with hydrogen-producing bacteria.

The use of FBR treatment for methane recovery from tofu wastewater provides benefits to the environment, such as produce fuel that can be used onsite, improves water quality, sanitation and public health.

However, the treatment of sulfate-containing tofu wastewater needs to improve since

the sulfide appears as a limiting factor that prevents methanogenesis, and sulfate reduction still low.

References

[1] F. Belén, J. Sánchez, E. Hernández, J.M. Auleda, M. Raventós, One option for the management of wastewater from tofu production: Freeze concentration in a falling-film system, J. Food Eng. 110 (2012) 364–373. https://doi.org/10.1016/j.jfoodeng.2011.12.036.

[2] T. McHugh, How Tofu is processed, Food Technol. 70 (2016) 1–4.

[3] Widyarani, E.S. Butar Butar, F. Dara, U. Hamidah, L. Sriwuryandari, H.R. Hariyadi, N. Sintawardani, Distribution of protein fractions in tofu whey wastewater and its potential influence on anaerobic digestion, IOP Conf. Ser. Earth Environ. Sci. 277 (2019). https://doi.org/10.1088/1755-1315/277/1/012012.

[4] K. Saio, E. Koyama, T. Watanabe, Protein-calcium-phytic acid relationships in soybean: Part I. Effects of calcium and phosphorus on solubility characteristics of soybean meal protein, Agric. Biol. Chem. 31 (1967) 1195–1200. https://doi.org/10.1080/00021369.1967.10858947.

[5] REEEP, Tofu production: a massive opportunity for RE biogas in Indonesia, (2014). https://www.reeep.org/news/tofu-production-massive-opportunity-re-biogas-indonesia (accessed June 17, 2019).

[6] W. Shurtleff, A. Aoyagi, Tofu & soymilk production : a craft and technical manual, Soyfoods Center, Lafayette, 2000.

[7] S. Anggarini, N. Hidayat, N.M.S. Sunyoto, P.S. Wulandari, Optimization of Hydraulic Retention Time (HRT) and Inoculums Addition in Wastewater Treatment Using Anaerobic Digestion System, Agric. Agric. Sci. Procedia. 3 (2015) 95–101. https://doi.org/10.1016/j.aaspro.2015.01.020.

[8] M. Faisal, F. Mulana, A. Gani, H. Daimon, Physical and chemical properties of wastewater discharged from Tofu industries in Banda Aceh city, Indonesia, Res. J. Pharm. Biol. Chem. Sci. 6 (2015) 1053–1058.

[9] C. Lay, B. Sen, S. Huang, C. Chen, C. Lin, Sustainable bioenergy production from tofu-processing wastewater by anaerobic hydrogen fermentation for onsite energy recovery, Renew. Energy. 58 (2013) 60–67. https://doi.org/10.1016/j.renene.2013.03.011.

[10] G. Lettinga, Anaerobic digestion and wastewater treatment systems, Antonie Van Leeuwenhoek. 67 (1995) 3–28. https://doi.org/10.1007/BF00872193.

[11] A.P. Picanço, M.V.G. Vallero, E.P. Gianotti, M. Zaiat, C.E. Blundi, Influence of porosity and composition of supports on the methanogenic biofilm characteristics developed in a fixed bed anaerobic reactor, Water Sci. Technol. 44 (2001) 197–204.

https://doi.org/10.2166/wst.2001.0220.

[12] L.W. Hulshoff Pol, W.J. de Zeeuw, C.T.M. Velzeboer, G. Lettinga, Granulation in UASB-Reactors, Water Sci. Technol. 15 (1983) 291–304. https://doi.org/10.2166/wst.1983.0172.

[13] Y. Liu, H. Lou Xu, S.F. Yang, J.H. Tay, Mechanisms and models for anaerobic granulation in upflow anaerobic sludge blanket reactor, Water Res. 37 (2003) 661–673. https://doi.org/10.1016/S0043-1354(02)00351-2.

[14] M. Evren, H. Ozgun, R. Kaan, I. Ozturk, Anaerobic Treatment of Industrial Effluents: An Overview of Applications, Waste Water - Treat. Reutil. (2011) 2–28. https://doi.org/10.5772/16032.

[15] W.P. Tritt, H. Kang, Slaughterhouse wastewater treatment in a bamboo ring anaerobic fixed-bed reactor, Environ. Eng. Res. 23 (2018) 70–75. https://doi.org/10.4491/eer.2017.040.

[16] D.G.B. Torres, S.D.M. Lucas, C.L. Andreani, K.Q. de Carvalho, S.R.M. Coelho, S.D. Gomes, Hydrogen Production and Performance of Anaerobic Fixed-Bed Reactors Using Three Support Arrangements From Cassava Starch Wastewater, Eng. Agrícola. 37 (2017) 160–172. https://doi.org/10.1590/1809-4430-eng.agric.v37n1p160-172/2017.

[17] G. Lettinga, Anaerobic digestion and wastewater treatment systems, Antonie Van Leeuwenhoek. 67 (1995) 3–28. https://doi.org/10.1007/BF00872193.

[18] J.B. van Lier F P van der Zee C T M J Frijters M E Ersahin, Celebrating 40 years anaerobic sludge bed reactors for industrial wastewater treatment, (2015). https://doi.org/10.1007/s11157-015-9375-5.

[19] M.S. Switzenbaum, Anaerobic fixed film wastewater treatment, Enzyme Microb. Technol. 5 (1983) 242–250. https://doi.org/10.1016/0141-0229(83)90072-8.

[20] A. Lettinga, G., van Velsen, A. F. M., Hobma, S. W., de Zeeuw, W. J., Klapwijk, Use of the Upflow Sludge Blanket (USB) reactor concept for biological wastewater treatment, especially for anaerobic treatment, Biotechnol. Bioeng. 22 (1980) 699–734.

[21] M. Alvarez, J., Ruiz, I., Gomez, M., Presas, J., Soto, Start-up alternatives and performance of an UASB pilot plant treating diluted municipal wastewater at low temperature, Bioresour. Technol. (2006) 1640–1649.

[22] J.-H. Liu, Y., Xu, H.-L., Yang, S.-F.,Tay, Mechanisms and models for anaerobic granulation in upflow anaerobic sludge blanket reactor, Water Res. 37 (2003) 661–673.

[23] C.A. de Lemos Chernicharo, Anaerobic Reactors, IWA Publishing, London, New York, 2007. https://doi.org/10.2166/9781780402116.

[24] R.C. Weast, CRC Handbook of Chemistry and Physics, 58th ed., Press, Inc., West Palm Beach, FA,USA, 1978.

[25] Emmerich. Wilhelm, R. Battino, R.J. Wilcock, Low-pressure solubility of gases in liquid water, Chem. Rev. 77 (1977) 219.

[26] R.K. Thauer, K. Jungermann, K. Decker, Energy conservation in chemotrophic anaerobic

bacteria., Bacteriol. Rev. 41 (1977) 100–180. https://doi.org/10.1073/pnas.0803850105.

[27] A. Boek, A. Prieger-kraft, P. Sehnheit, Pyruvate - a novel substrate for growth and methane formation in Methanosarcina barkeri, Arch. Microbiol. 161 (1994) 33–46.

[28] E. Colleran, S. Finnegan, P. Lens, Anaerobic treatment of sulphate-containing waste streams, Antonie Van Leeuwenhoek. 67 (1995) 29–46. https://doi.org/10.1007/BF00872194.

[29] G.R. Gibson, Physiology and ecology of the sulphate-reducing bacteria, J. Appl. Bacteriol. 69 (1990) 769–797. https://doi.org/10.1111/j.1365-2672.1990.tb01575.x.

[30] P. Hu, J. Liu, L. Wu, L. Zou, Y.-Y. Li, Z.P. Xu, Simultaneous release of polyphosphate and iron-phosphate from waste activated sludge by anaerobic fermentation combined with sulfate reduction, Bioresour. Technol. 271 (2019) 182–189.

[31] F. Widdel, Microbiology and ecology of sulfate- and sulfur-reducing bacteria, Biol. Anaerob. Microorg. (1988) 469–585.

[32] C. Zeynep, . Dolfing Jan, T. Jessica, K. J Purdy, Ö. Kevin, COD/sulfate ratio does not affect the methane yield and microbial diversity in anaerobic digesters, Water Res. 155 (n.d.) 444–454.

[33] G.F. Parkin, N.A. Lynch, W.-C. Kuo, E.L. Van Keuren, S.K. Bhattacharya, Interaction between sulfate reducers and methanogens fed acetate and propionate, Res. J. Water Pollut. Control Fed. 62 (1990) 780–788.

[34] H. Kroiss, F. Wabnegg, Method to Characterize Anaerobic Sludge and Anerobic Removal of Substrates, in: Poster Present. 3rd Int. Conf. Anaerob. Dig., Boston, Mass, 1983.

[35] J. Teknik, K. Fakultas, U.D. Semarang, TEKNOLOGI PENANGANAN LIMBAH CAIR TAHU Oleh : Sriharti , Takiyah Salim dan Sukirno Pencucian kedelai, (2004) 1–6.

[36] C. Liu, X. Yuan, G. Zeng, W. Li, J. Li, Prediction of methane yield at optimum pH for anaerobic digestion of organic fraction of municipal solid waste, Bioresour. Technol. 99 (2008) 882–888. https://doi.org/10.1016/j.biortech.2007.01.013.

[37] A.E. Ghaly, A comparative study of anaerobic digestion of acid cheese whey and dairy manure in a two-stage reactor, Bioresour. Technol. 58 (1996) 61–72. https://doi.org/10.1016/S0960-8524(96)00105-8.

[38] J. De Haast, T.J. Britz, J.C. Novello, Effect of different neutralizing treatments on the efficiency of an anaerobic digester fed with deproteinated cheese whey, J. Dairy Res. 53 (1986) 467–476. https://doi.org/10.1017/S0022029900025085.

[39] E.J. Fox, C.J. Clanton, P.R. Goodrich, B.D. Backus, H.A. Morris, Liming an anaerobic cheese whey digester, Trans. Am. Soc. Agric. Eng. 35 (1992) 269–274. https://doi.org/10.13031/2013.28599.

[40] H.Q. Yu, Z.H. Hu, T.Q. Hong, G.W. Gu, Performance of an anaerobic filter treating soybean processing wastewater with and without effluent recycle, Process Biochem. 38 (2002) 507–513. https://doi.org/10.1016/S0032-9592(02)00175-9.

[41] W. APHA, AWWA, Standard Methods for examination of water and wastewater. 22nd Ed,

Washington, DC, USA, 2012.

[42] J.G. Caporaso, C.L. Lauber, W.A. Walters, D. Berg-Lyons, J. Huntley, N. Fierer, S.M. Owens, J. Betley, L. Fraser, M. Bauer, N. Gormley, J.A. Gilbert, G. Smith, R. Knight, Ultra-high-throughput microbial community analysis on the Illumina HiSeq and MiSeq platforms, ISME J. 6 (2012) 1621–1624. https://doi.org/10.1038/ismej.2012.8.

[43] D.P.R. Herlemann, M. Labrenz, K. Jürgens, S. Bertilsson, J.J. Waniek, A.F. Andersson, Transitions in bacterial communities along the 2000 km salinity gradient of the Baltic Sea, ISME J. 5 (2011) 1571–1579. https://doi.org/10.1038/ismej.2011.41.

[44] M. Bahram, F. Hildebrand, S.K. Forslund, J.L. Anderson, N.A. Soudzilovskaia, P.M. Bodegom, J. Bengtsson-Palme, S. Anslan, L.P. Coelho, H. Harend, J. Huerta-Cepas, M.H. Medema, M.R. Maltz, S. Mundra, P.A. Olsson, M. Pent, S. Põlme, S. Sunagawa, M. Ryberg, L. Tedersoo, P. Bork, Structure and function of the global topsoil microbiome, Nature. 560 (2018) 233–237. https://doi.org/10.1038/s41586-018-0386-6.

[45] A.M. Bolger, M. Lohse, B. Usadel, Trimmomatic: A flexible trimmer for Illumina sequence data, Bioinformatics. 30 (2014) 2114–2120. https://doi.org/10.1093/bioinformatics/btu170.

[46] R.C. Edgar, Search and clustering orders of magnitude faster than BLAST, Bioinformatics. 26 (2010) 2460–2461. https://doi.org/10.1093/bioinformatics/btq461.

[47] R.C. Edgar, UPARSE: highly accurate OTU sequences from microbial amplicon reads, Nat. Methods. 10 (2013) 996–998. https://doi.org/10.1038/nmeth.2604.

[48] J.G. Caporaso, J. Kuczynski, J. Stombaugh, K. Bittinger, F.D. Bushman, E.K. Costello, N. Fierer, A.G. Peña, J.K. Goodrich, J.I. Gordon, G.A. Huttley, S.T. Kelley, D. Knights, J.E. Koenig, R.E. Ley, C.A. Lozupone, D. McDonald, B.D. Muegge, M. Pirrung, J. Reeder, J.R. Sevinsky, P.J. Turnbaugh, W.A. Walters, J. Widmann, T. Yatsunenko, J. Zaneveld, R. Knight, QIIME allows analysis of high-throughput community sequencing data., Nat. Methods. 7 (2010) 335–6. https://doi.org/10.1038/nmeth.f.303.

[49] M. PL, Anaerobic waste treatment fundamentals, Public works, 1964.

[50] E. Ten Brummeler, L.W. Hulshoff Pol, J. Dolfing, Methanogenesis in an upflow anaerobic sludge blanket reactor at pH 6 on an acetate-propionate mixture, Appl. Environ. Microbiol. 49 (1985) 1472–1477.

[51] D. Karadag, O.E. Köroğlu, B. Ozkaya, M. Cakmakci, A review on anaerobic biofilm reactors for the treatment of dairy industry wastewater, Process Biochem. 50 (2015) 262–271. https://doi.org/10.1016/j.procbio.2014.11.005.

[52] H. Gannoun, E. Khelifi, H. Bouallagui, Y. Touhami, M. Hamdi, Ecological clarification of cheese whey prior to anaerobic digestion in upflow anaerobic filter, Bioresour. Technol. 99 (2008) 6105–6111. https://doi.org/10.1016/j.biortech.2007.12.037.

[53] M. Alves, A. Pereira, M. Mota, J.M. Novais, E. Colleran, Staged and non-staged anaerobic filters: Microbial activity segregation, hydrodynamic behaviour and performance, J. Chem.

Technol. Biotechnol. 73 (1998) 99–108. https://doi.org/10.1002/(SICI)1097-4660(1998100)73:2<99::AID-JCTB934>3.0.CO;2-O.

[54] J. Cardinali-Rezende, R.B. Debarry, L.F.D.B. Colturato, E. V. Carneiro, E. Chartone-Souza, A.M.A. Nascimento, Molecular identification and dynamics of microbial communities in reactor treating organic household waste, Appl. Microbiol. Biotechnol. 84 (2009) 777–789. https://doi.org/10.1007/s00253-009-2071-z.

[55] Y.L. Qiu, X.Z. Kuang, X.S. Shi, X.Z. Yuan, R.B. Guo, Paludibacter jiangxiensis sp. nov., a strictly anaerobic, propionate-producing bacterium isolated from rice paddy field, Arch. Microbiol. 196 (2014) 149–155. https://doi.org/10.1007/s00203-013-0951-1.

[56] F. Abram, A.M. Enright, J. O'Reilly, C.H. Botting, G. Collins, V. O'Flaherty, A metaproteomic approach gives functional insights into anaerobic digestion, J. Appl. Microbiol. 110 (2011) 1550–1560. https://doi.org/10.1111/j.1365-2672.2011.05011.x.

[57] J.X. Lim, Y. Zhou, V.M. Vadivelu, Enhanced volatile fatty acid production and microbial population analysis in anaerobic treatment of high strength wastewater, J. Water Process Eng. 33 (2020). https://doi.org/10.1016/j.jwpe.2019.101058.

[58] K.G. Cibis, A. Gneipel, H. König, Isolation of acetic, propionic and butyric acid-forming bacteria from biogas plants, J. Biotechnol. 220 (2016) 51–63. https://doi.org/10.1016/J.JBIOTEC.2016.01.008.

[59] Y. Jiang, C. Dennehy, P.G. Lawlor, Z. Hu, M. McCabe, P. Cormican, X. Zhan, G.E. Gardiner, Exploring the roles of and interactions among microbes in dry co-digestion of food waste and pig manure using high-throughput 16S rRNA gene amplicon sequencing, Biotechnol. Biofuels. 12 (2019) 1–16. https://doi.org/10.1186/s13068-018-1344-0.

[60] Y.F. Li, J. Shi, M.C. Nelson, P.H. Chen, J. Graf, Y. Li, Z. Yu, Impact of different ratios of feedstock to liquid anaerobic digestion effluent on the performance and microbiome of solid-state anaerobic digesters digesting corn stover, Bioresour. Technol. 200 (2016) 744–752. https://doi.org/10.1016/j.biortech.2015.10.078.

[61] F. Lü, A. Bize, A. Guillot, V. Monnet, C. Madigou, O. Chapleur, L. Mazéas, P. He, T. Bouchez, Metaproteomics of cellulose methanisation under thermophilic conditions reveals a surprisingly high proteolytic activity, ISME J. 8 (2014) 88–102. https://doi.org/10.1038/ismej.2013.120.

[62] K.C.Y. Lee, X.C. Morgan, P.F. Dunfield, I. Tamas, I.R. McDonald, M.B. Stott, Genomic analysis of Chthonomonas calidirosea, the first sequenced isolate of the phylum Armatimonadetes, ISME J. 8 (2014) 1522–1533. https://doi.org/10.1038/ismej.2013.251.

[63] H. Zhang, J.E. Banaszak, P. Parameswaran, J. Alder, R. Krajmalnik-Brown, B.E. Rittmann, Focused-Pulsed sludge pre-treatment increases the bacterial diversity and relative abundance of acetoclastic methanogens in a full-scale anaerobic digester, Water Res. 43 (2009) 4517–4526. https://doi.org/10.1016/j.watres.2009.07.034.

[64] A.J.M. Stams, D.Z. Sousa, R. Kleerebezem, C.M. Plugge, Role of syntrophic microbial

communities in high-rate methanogenic bioreactors, Water Sci. Technol. 66 (2012) 352–362. https://doi.org/10.2166/wst.2012.192.

[65] W.E. Balch, G.E. Fox, L.J. Magrum, C.R. Woese, R.S. Wolfe, Methanogens: reevaluation of a unique biological group, Microbiol. Rev. 43 (1979) 260–296. https://doi.org/10.1128/mmbr.43.2.260-296.1979.

[66] J. De Vrieze, T. Hennebel, N. Boon, W. Verstraete, Methanosarcina: The rediscovered methanogen for heavy duty biomethanation, Bioresour. Technol. 112 (2012) 1–9. https://doi.org/10.1016/j.biortech.2012.02.079.

[67] G. Borrel, P.W. O'Toole, H.M.B. Harris, P. Peyret, J.F. Brugère, S. Gribaldo, Phylogenomic data support a seventh order of methylotrophic methanogens and provide insights into the evolution of methanogenesis, Genome Biol. Evol. 5 (2013) 1769–1780. https://doi.org/10.1093/gbe/evt128.

[68] B. Dridi, M.L. Fardeau, B. Ollivier, D. Raoult, M. Drancourt, Methanomassiliicoccus luminyensis gen. nov., sp. nov., a methanogenic archaeon isolated from human faeces, Int. J. Syst. Evol. Microbiol. 62 (2012) 1902–1907. https://doi.org/10.1099/ijs.0.033712-0.

[69] M. Poulsen, C. Schwab, B. Borg Jensen, R.M. Engberg, A. Spang, N. Canibe, O. Højberg, G. Milinovich, L. Fragner, C. Schleper, W. Weckwerth, P. Lund, A. Schramm, T. Urich, Methylotrophic methanogenic Thermoplasmata implicated in reduced methane emissions from bovine rumen, Nat. Commun. 4 (2013). https://doi.org/10.1038/ncomms2432.

[70] L.-P. Hao, F. Lü, L. Li, L.-M. Shao, P.-J. He, Shift of pathways during initiation of thermophilic methanogenesis at different initial pH, Bioresour. Technol. 126 (2012) 418–424. https://doi.org/10.1016/J.BIORTECH.2011.12.072.

[71] G. Lettinga, L.W. Hulshoff Pol, UASB-Process Design for Various Types of Wastewaters, Water Sci. Technol. 24 (1991) 87–107.

[72] P. Weiland, Development of anaerobic filters for treatment of high strength agro-industrial wastewaters, Bioprocess Eng. 4 (1989) 261–264.

[73] P.N.L. Lens, A. Visser, A.J.H. Janssen, L.W. Hulshoff Pol, G. Lettinga, Biotechnological treatment of sulfate-rich wastewaters, Crit. Rev. Environ. Sci. Technol. 28 (1998) 41–88. https://doi.org/10.1080/10643389891254160.

[74] V. O'Flaherty, P. Lens, B. Leahy, E. Colleran, Long-term competition between sulphate-reducing and methane-producing bacteria during full-scale anaerobic treatment of citric acid production wastewater, Water Res. 32 (1998) 815–825.

[75] A.I. Qatibi, A. Boris, J.. Garcia, Effect of sulfate on lactate and C2, C3 volatile fatty acids anaerobic degradation by mixed microbial culture, Antonie Van Leeuwenhoek. 53 (1990) 241–248.

[76] C. O'Reilly, E. Colleran, Effect of influent COD/SO42- ratios on mesophilic anaerobic reactor biomass populations: Physico-chemical and microbiological properties, FEMS Microbiol. Ecol.

56 (2006) 141–153. https://doi.org/10.1111/j.1574-6941.2006.00066.x.

[77] Z. Isa, S. Grusenmeyer, W. Vestraete, Sulfate reduction relative to methane production in high-rate anaerobic digestion: Technical aspects, Appl. Environ. Microbiol. 51 (1986) 572–579. https://doi.org/10.1128/aem.51.3.572-579.1986.

[78] Z. Isa, S. Grusenmeyer, W. Verstraete, Sulfate Reduction Relative to Methane Production in High-Rate Anaerobic Digestion: Technical Aspects, Appl. Environ. Microbiol. 51 (1986) 572–579.

[79] G. Zeeman, G. Lettinga, The role of anaerobic digestion of domestic sewage in closing the water and nutrient cycle at community level, Water Sci. Technol. 39 (1999) 187–194.

[80] K.G. Anderson, B. Kasapgil, O. Ince, Microbiological study of two-stage anaerobic digestion during start-up, Water Res. 28 (1994) 2383-2392.

[81] S. Ghaniyari-Benis, R. Borja, S.A. Monemian, V. Goodarzi, Anaerobic treatment of synthetic medium-strength wastewater using a multistage biofilm reactor, Bioresour. Technol. 100 (2009) 1740–1745. https://doi.org/10.1016/j.biortech.2008.09.046.

[82] Y. Qin, L. Li, J. Wu, B. Xiao, T. Hojo, K. Kubota, Y.. Li, Co-production of biohydrogen and biomethane from food waste and paper waste via recirculated two-phase anaerobic digestion process: bioenergy yields and metabolic distribution, Bioresour. Technol. 276 (2019) 325–334.

[83] R. Wirth, E. Kovács, G. Maráti, Z. Bagi, G. Rákhely, K.L. Kovács, Characterization of a biogas-producing microbial community by short-read next generation DNA sequencing, Biotechnol. Biofuels. 5 (2012) 1–16. https://doi.org/10.1186/1754-6834-5-41.

[84] X. Su, W. Zhao, D. Xia, The diversity of hydrogen-producing bacteria and methanogens within an in situ coal seam, Biotechnol. Biofuels. 11 (2018) 1–18. https://doi.org/10.1186/s13068-018-1237-2.

[85] C. KC, L. JA, Metabolic versatility in methanogens, Curr. Opin. Biotechnol. 29 (2014) 70.

[86] D. Karakashev, D.J. Batstone, E. Trably, I. Angelidaki, Acetate oxidation is the dominant methanogenic pathway from acetate in the absence of Methanosaetaceae, Appl. Environ. Microbiol. 72 (2006) 5138–5141.

[87] D. AD, E. S, N. A, R. L, Y. A, Pemanfaatan teknologi tepat guna dalam penerapan cleaner production di industri kecil pengolahan tahu di Subang dan Sumedang, LIPI press, Jakarta, 2015.

[88] Y. Maspolim, Y. Zhou, C. Guo, K. Xiao, W.J. Ng, Determination of the archaeal and bacterial communities in two-phase and single-stage anaerobic systems by 454 pyrosequencing, J. Environ. Sci. (China). 36 (2015) 121–129. https://doi.org/10.1016/j.jes.2015.02.017.