SINERGI Vol. 29, No. 2, June 2025: 423-436
http://publikasi.mercubuana.ac.id/index.php/sinergi
http://doi.org/10.22441/sinergi.2025.2.013

Evaluation of double-stage Anaerobic Fluidized Bed Reactor (AFBR) for digestion of leachate: correlation of kinetic parameter with operational condition and process

Elli Prastyo[1*], Wiratni Budhijanto[2], Hanifrahmawan Sudibyo[2]
[1]Department of Chemical Engineering, Institut Teknologi Petroleum Balongan, Indonesia
[2]Department of Chemical Engineering, Universitas Gadjah Mada, Indonesia

Abstract
The objective of this study is to investigate the performance of using an advanced fluidized bed reactor (AFBR) of a double column configuration in breaking down leachate into biogas. The relationship of the kinetic parameters with the operating conditions and the performance of the double-column reactor during anaerobic digestion was examined. The substrate concentration, microorganism population, hydraulic retention time value, growth rate, and death rate of microorganisms were employed as reference points for evaluating anaerobic digestion performance and assessing the operating conditions. The results demonstrated that there was no notable correlation between the formation of volatile fatty acids (VFA) in the acidogenic reactor (R1), the degradation of VFA in the methanogen reactor (R2), and the methane production rate in the methanogen reactor (R2). The simulation results for VFA formation (dCVFA1/dt) and VFA degradation (dcVFA2/dt) exhibited a tendency to overestimate when operated at low HRT and underestimate at short HRT compared to the experimental results. The steady state of the simulation results exhibited a faster rate of progression than the experimental outcomes. The fitting data for Ksx1 and Ksx2 predominantly comprise dynamically evolving values that exert an influence upon um1 and um1, as well as kd1 and kd2, when the reactor is operated in continuous mode. Furthermore, the factors of inhibitor compounds and microorganism adaptation were not observed across all HRT values in this investigation.

Keywords:
Acidogenic;
AFBR;
Biogas;
Kinetic;
Leachate;
Methanogen;

Article History:
Received: August 29, 2024
Revised: October 10, 2024
Accepted: November 16, 2024
Published: May 15, 2025

Corresponding Author:
Elli Prastyo
Department of Chemical Engineering, Institut Teknologi Petroleum Balongan, Indonesia
Email:
elli.prastyo@gmail.com

INTRODUCTION

One of the principal consequences of rapid population growth, coupled with economic and industrial development, is the production of substantial quantities of waste. Waste management presents one of the most significant challenges facing humanity today, particularly in developing countries where the infrastructure to deal with this issue may be lacking or inadequate. The most commonly employed method of solid waste disposal is landfill, which generates a considerable amount of leachate as a result of the natural process of decomposition and rainwater infiltration [1]. The leachate produced in landfills can comprise a heterogeneous mixture of dissolved complex organic compounds (including volatile fatty acids and combustible organic matter), ammonia, and heavy metals (Cd^{2+}, Cr^{3+}, Cu^{2+}, Pb^{2+}, Ni^{2+}, Hg^{2+}), inorganic salts, and other xenobiotics (aromatic hydrocarbons, phenols, and pesticides) [2][3]. The treatment of leachate is a critical process, as improper treatment could lead to significant environmental impacts [4]. The development of leachate treatment methods,

including physicochemical, biological (e.g., anaerobic or aerobic), and electrochemical methods, is ongoing with the objective of achieving the best possible environmental and energy efficiency outcomes [5][6]. Amongst these technologies, anaerobic digestion has been identified as the treatment method most likely to be effective, given its capacity to stabilize and reuse sludge [7]. However, the most prevalent issue in anaerobic digestion is bacterial washout. This can be mitigated by employing biofilm formation on the reactor. The biomass attached to the carrier biofilm has the capacity to move freely within the reactor's water volume, enabling its collection via the reactor's filtration system at the outlet.

The fluidized bed biofilm reactor (FBR) represents a common approach to biofilm treatment in wastewater treatment plants. The FBR offers a level of mixing between two packed bed reactors and the STR, which may be regarded as a more intensive approach to treatment [8]. A homogeneous system is more easily monitored and controlled, with the advantage of good mixing. Higher mass transfer and heat transfer rates are also to be expected. Furthermore, scale-up can be achieved without increasing the concentration gradient [9]. However, the effects of anaerobic digestion of leachate using two-stage reactors and the underlying mechanisms remain unclear at each stage, particularly in the processes of acidogenesis and methanogenesis. The optimal requirements for the growth of the various groups of microorganisms involved, including nutrient concentrations and substrate pH, vary significantly [10]. In the single-stage anaerobic digestion process, the three principal stages of hydrolysis, acidogenesis, and methanogenesis occur in a single reactor. As a consequence, volatile fatty acids (VFAs) may accumulate due to a faster acidogenesis process relative to the slower methanogenesis process. This phenomenon has the potential to negatively impact the methanogenesis process, ultimately inhibiting biogas production [11].

The double-stage reactor represents an innovative approach to the optimization of conditions at each stage of the anaerobic digestion process. Previous research has indicated that a two-stage reactor system can facilitate the most optimal growth of microorganisms under varying conditions [12]. The optimal process conditions, including those of the fermenter settings, as well as their effects on the rates of substrate degradation, are well described in the scientific literature. This pertains to two-stage methane and hydrogen production, which is derived from both wastewater and food and agricultural waste [13]. A different study has reported that two-stage decomposition of grass silage has been shown to result in 7% higher methane production compared to a single-stage system [14]. Research by [15] reported that the use of a two-stage anaerobic CSTR of food waste resulted in 23% higher methane production (419 mL CH_4/kg.VS) than a single-stage system (371 mL CH_4/kg.VS). However, the authors observed that there was no data on hydrogen production. The objective was therefore to optimise the acidification process and to maximise methane yield, with the generation of hydrogen not being a priority. Further research should be conducted to investigate the performance of the two-stage filtered bed anaerobic reactor in producing biogas from leachate at different stages of operation.

Process kinetics serves as a valuable tool for predicting and describing the performance of anaerobic digestion systems. Mathematical models assist in predicting kinetic parameters and clarify each stage of the anaerobic digestion process. Several mathematical equations have been developed to represent the anaerobic digestion process, including the exponential model, Monod model, Gompertz model, transfer function-based model, and cone model [16]. The results of a comprehensive literature survey indicate that the Monod kinetics model is a widely utilized framework to elucidate the kinetics of anaerobic digestion processes [17]. However, the applicability of Monod kinetics is limited when dealing with complex substrates such as leachate, where inhibitors are present. Indeed, other researchers have also considered the issue of specific growth rates under the assumption of Monod kinetics with substrate inhibition [17][18]. Obtaining kinetic data in anaerobic digestion to represent the conversion of acetate to methane represents a complex and limiting study within the Monod model [19]. In optimizing the design of a two-phase anaerobic digestion system, the Contois kinetics model is the preferred approach.

Research conducted by [20] revealed that the Contois kinetic model represents the most suitable approach for optimizing the design of a two-phase anaerobic digestion system to treat complex solid waste. The use of Contois kinetics with an inhibition of 30 g/L of VFA yielded satisfactory outcomes in the treatment of organic waste [21]. The mechanism of VFA inhibition was studied through a series of experiments on organic solid waste treatment [22]. The results demonstrated that non-ionized VFA and quantifiable levels of VFA were not inhibitory, while acidic pH was the inhibitory factor between a pH of 5 and 7.

This study has developed a kinetic model that describes the anaerobic digestion of leachate in two stages. The hydrolysis process was considered in the first reactor at the VFA formation stage, while the subsequent decomposition of VFA into methane and carbon dioxide by methanogenic bacteria was examined in the second reactor. The application of mathematical modelling and perfect mixing systems, as referenced in [23], is suitable for this study because of the small dimensions of zeolite particles, making mass transfer from liquids to solids negligible.

The modified mathematical models utilized were specifically adapted to the process variables that can be analyzed using the available facilities. All input data for these models must be based on steady-state reactor performance. In these models, it is assumed that the microorganism immobilization media granules are spherical within the reactors in question, with the relative concentrations of acid-forming and methanogenic bacteria also adjusted for each reactor. The developed dynamic mathematical model provides a more quantitative description of the process operation, which facilitates the design of a better control system to improve stability, enhance the performance of the anaerobic digester, and optimize the process performance. The experimental results are compared with the theoretical data to determine their alignment.

METHOD

Material

The leachate was obtained from an integrated waste management facility (TPST) Piyungan located in Bantul Regency, Yogyakarta Province. The leachate was employed as a substrate with (soluble chemical oxygen demand) sCOD values in the range of 3000-5000 mg/L and pH 8.0-8.5. The immobilization media was composed of a mixture of natural zeolite with bentonite, with a specific surface area of 15,178 m^2/g, Total pore volume of 40,778 x 10^{-3}cc/g, and average pore radius of 53,735 Á. The zeolite was derived from Mojokerto Regency, while the other supporting raw materials were obtained from the active digester effluent of the Center for Agrotechnology Innovation (PIAT) UGM biogas plant in Berbah, Sleman Regency, Yogyakarta, and the Sleman Regency biogas plant. The inoculum was filtered prior to its introduction into the bioreactor to prevent the entry of large debris into the system. The inoculum was characterised to determine the chemical oxygen demand (COD) and volatile suspended solids (VSS) in accordance with the standard methods set out by the American Public Health Association [24]. The detailed characteristics of the inoculum utilized in this study are presented in Table 1 and Table 2.

Experiment Design

This research was conducted in two stages: batch and continuous stage. The objective of the initial batch stage is to facilitate the growth and adaptation of the microorganisms prior to transitioning to continuous operation. The batch stage was completed once the results of VFA, sCOD, and biogas volume analyses indicated stable value.

Subsequently, the reactor proceeded to operate continuously, initiating from the largest to the smallest hydraulic retention time (HRT). Modifications to the HRT values were implemented when the VFA, sCOD, and biogas volume measures remained consistent over time, indicating a state of equilibrium.

The operating conditions for each reactor are described in Table 3 and Table 4. Each reactor was operated without any input (feeding) or output until a steady state condition was achieved. The study's batch stage was carried out at leachate sCOD concentrations ranging from 3000 to 5000 mg/L in acidogenic reactors with a total volume of 15 L and methanogenic reactors of 10 L. Each reactor was dosed with a mixture of leachate water and inoculum in an 80:20 (v/v) ratio. To prepare the acidogenic reactor, an acetic acid solution was added to adjust the pH to 5.5. Conversely, a solution with a pH of 7-7.5 was added to prepare the methanogenic reactor.

Table 1. Characteristics of Inoculum KP4 PIAT UGM

Characteristic	Value
pH	7.3
TS (mg/L)	25,830.305
VS (mg/L)	16,252.500
VFA (mg acetic acid/L)	425.820
SCOD (mg/L)	1,385
Anaerobic microorganism (cells/mL)	2.0×10^7

Table 2. Characteristics of Inoculum Turgo Village

Characteristic	Value
pH	7.0
TS (mg/L)	32,102.620
VS (mg/L)	22,930.252
VFA (mg acetic acid/L)	560.820
SCOD (mg/L)	1,502
Anaerobic microorganism (cells/mL)	3.0×10^7

Table 3. Operation Condition of Acidogenic Reactor

Parameters	Values
Dimension of Reactor	iD = 19.8 cm, oD = 20 cm, V effective = 15 L
Superficial Velocity	3.8033 cm/s
Settling Velocity	7.9120 cm/s
Fluidization level	40-50%
Density of media	1.82 gr/cm^3
Mass of media	150 gr
Diameter of media	14 mesh
Temperature	32^{o}C
pH	5.5 – 6
COD influent	8500 – 9500 mg/L

Table 4. Operation Condition of Methanogenic Reactor

Parameters	Values
Dimension of Reactor	iD = 14.8 cm, oD = 15 cm, V effective = 10 L
Superficial Velocity	3.8033 cm/s
Settling Velocity	11.0580 cm/s
Fluidization level	30%-40%
Density of media	1.82 gr/cm^3
Mass of media	150 gr
Diameter of media	14 mesh
Temperature	32^{o}C
pH	7-7.5
COD influent	Effluent reactor acidogenic

The continuous phase was conducted at HRT values of 20, 15, 10, 5, 2, and 1 day with Organic Loading Rates (OLRs) of 1.2 gCOD/L.day for each reactor. The device's main components comprise a series of interconnected systems, as illustrated in Figure 1. The components of the double-stage reactor include influent tanks, effluent tanks, intermediate tanks, anaerobic reactors engaged in the acidogenesis process, anaerobic reactors engaged in the methanogenesis process, and biogas volume measuring equipment (GM).

The anaerobic reactors were constructed from transparent polymer acrylic, with an acidogenic reactor volume of 15 litres and a methanogenic reactor volume of 10 litres. Each reactor was equipped with two centrifugal pumps for circulation. The immobilization media utilised in this study was a mixture of zeolite and bentonite in the form of powder with a diameter of 0.5-0.8 mm. According to these studies, the optimal reactor circuit should allow for a smooth flow of fluid, ensure the proper functioning of all equipment, and maintain a fluidisation level (FL) within the range of 40% to 50% of the effective tube depth [25].

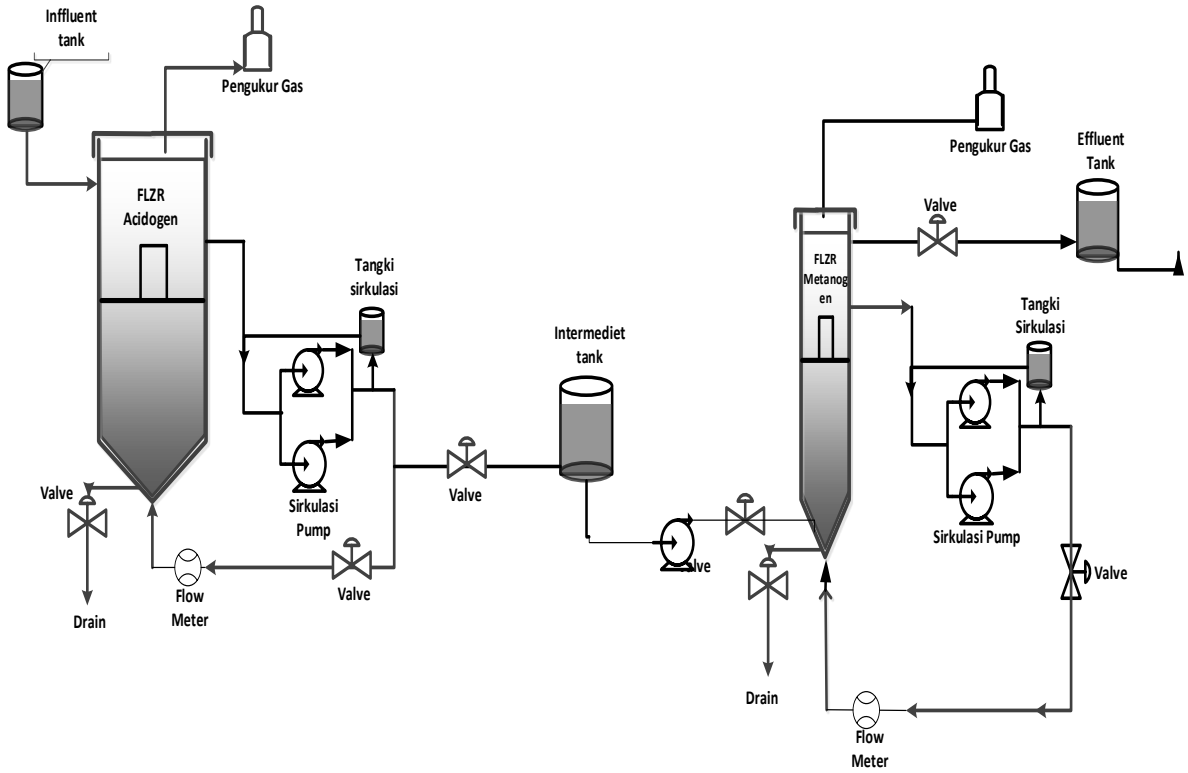

Figure 1. Scheme of AFBR Double Column

Laboratory Methods

Influent and effluent samples were taken from the acidogenic (R1) and methanogen (R2) reactors for COD, sCOD, and VFA analyses. Acidity and dissolved oxygen in the reactors were observed using Metler Toledo electrodes (Columbus, Ohio, USA). TS and VS analyses were conducted on the raw materials at the beginning of the study. The analysis method was in accordance with the APHA 2005 standard. The biogas volume was measured on a daily basis utilizing a high-gasometer apparatus. The high-gasometer is a closed cylinder or column that is partially submerged in an open container containing 75% saturated salt water with a pH of 2 as a barrier solution. To determine the volume of biogas produced, the equation proposed by [26] was employed.

Mathematical Modelling

In order to conduct a kinetic study of anaerobic digestion, kinetic data were obtained at each steady state condition in each reactor. The decomposition of soluble chemical oxygen demand (sCOD) in the acidogenic reactor (R1) was performed by acidogenic bacteria (x_1) for growth (μ_{g1}) and VFA production based on the hydraulic retention time (θ) value of the reactor. The growth of methanogen bacteria (μ_{g2}) occurs in the methanogen reactor (R2) due to the consumption of VFA and the formation of methane over time. The kinetic constant value employed in the Contois growth kinetics equation is derived from [27], where the anaerobic digestion of leachate water was conducted in batch using a single-stage immobilized anaerobic fluidized bed reactor (AFBR) with leachate as the substrate (Table 5). The batch condition was selected for the study due to its suitability for microorganisms. The data obtained from this ideal state was used as a reference point to evaluate the reactor's performance under continuous operating conditions. Based on the Contois equation, a model was created for the growth of both types of bacteria.

$$\frac{dX_1}{dt} = \mu_{nett1}.X_1 \quad (1)$$

$$\frac{dX_2}{dt} = \mu_{nett2}.X_2 \quad (2)$$

The μnett value was calculated using the following equation:

$$\mu_{nett} = \mu_g - kd \quad (3)$$

Acidogenic Reactor equation for microorganism growth, sCOD, and VFA formation

The acidogenic reactor (R1) has the primary function of degrading organic compounds with chemical oxygen demand values and generating volatile fatty acids (VFA) for the methanogen reactor (R2). The growth of methanogen bacteria is contingent upon the rate at which the influent substrate is decomposed, exhibiting a specific chemical oxygen demand (COD) value. The μ_g coefficient is derived from the Contois equation, wherein the microbial growth rate is influenced by the microbial concentration and substrate concentration, as elucidated by the following formula.

$$\mu_{g1} = \frac{\mu_{m1}.C_{sCOD}}{K_{SX1}.X_1 + C_{sCOD}} X_1 \quad (4)$$

$$\frac{dX_1}{dt} = \left(\frac{\mu_{m1}.C_{sCOD}}{K_{SX1}.X_1 + C_{sCOD}} - k_{d1} \right).X_1 \quad (5)$$

The reduction in sCOD when the growth of acidogenic bacteria is already in a steady state $\frac{dX_1}{dt}$ can be observed in the mathematical equation of sCOD, as presented in the following equation.

$$\frac{d(C_{sCOD})}{dt} = \left(\frac{(C_{sCODin} - C_{sCOD})}{\theta 1} \right) - \left[\frac{1}{Y_{X1/CsCOD}} \left(\frac{\mu_{m1}.C_{sCOD}}{K_{SX1}.X_1 + C_{sCOD}} - k_{d1} \right) X_1 \right]. \quad (6)$$

The formation of VFA, resulting from the decomposition of sCOD by acidogenic bacteria (X1), can be described by the following equation.

$$\frac{d(C_{VFA1})}{dt} = \left(\frac{(C_{VFA1} - C_{VFA\,in})}{\theta 1} \right) + \left[Y_{C_{VFA1}/X_1} \left(\frac{\mu_{m1}.C_{sCOD}}{K_{SX1}.X_1 + C_{sCOD}} - k_{d1} \right).X_1 \right] \quad (7)$$

Methanogenic Reactor Equation for microorganism growth and CH_4 formation

The methanogenic reactor serves as the principal conduit through which VFA generated by the acidogenic reactor (R1) is converted into methane (CH_4) by methanogenic bacteria. The growth and methane production of these bacteria is contingent upon the concentration of VFA derived from the acidogenic reactor.

$$\mu_{g2} = \frac{\mu_{m2}.C_{VFA2}}{K_{SX2}.X_2 + C_{VFA2}} X_2 \quad (8)$$

$$\frac{dX_2}{dt} = \left(\frac{\mu_{m2}.C_{VFA2}}{K_{SX2}.X_2 + C_{VFA2}} - k_{d2} \right).X_2 \quad (9)$$

$$\frac{d(C_{VFA2})}{dt} = \left(\frac{(C_{VFA1} - C_{VFA2})}{\theta 2} \right) - \left[\frac{1}{Y_{X_2/C_{VFA2}}} \left(\frac{\mu_{m2}.C_{VFA2}}{K_{SX2}.X_2 + C_{VFA2}} - k_{d2} \right).X_2 \right] \quad (10)$$

$$\frac{dC_{CH_4}}{dt} = \frac{C_{CH_4}}{\theta_2}.+\left[Y_{CH_4/X2}\left(\frac{\mu_{m2}.C_{VFA2}}{K_{SX2}.X_2+C_{VFA2}} - k_{d2}\right).X_2\right] \quad (11)$$

RESULT AND DISCUSSION

sCOD Degradation in Acidogenic Reactor

The decrease in the soluble chemical oxygen demand (sCOD) value of leachate water is indicative of a successful acidogenic reactor process. The simulation results utilizing equation 9, in conjunction with experimental data obtained from the acidogenic reactor, are presented in Figure 2.

The sCOD values demonstrated a notable decline when the reactor operated at a longer hydraulic retention time (HRT). An increase in the residence time was found to be correlated with an enhancement in the COD removal efficiency, suggesting a high microbial population that is capable of rapidly converting complex organic matter into useful by-products. It was reported by [28] that longer solid retention times facilitate the formation of a better system potential and result in greater COD removal due to increased contact time with microorganisms. During the course of the simulation, the sCOD value of the effluent remained constant at the beginning of the HRT operation. This phenomenon demonstrates a divergence from the results observed during the experimental phase of the process.

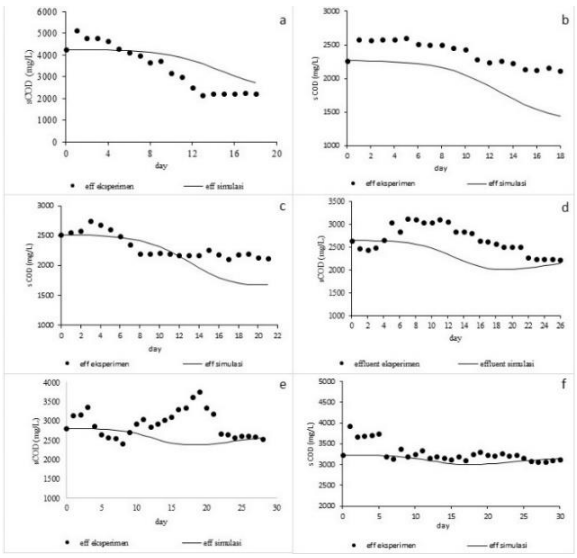

Figure 2. Profile of sCOD degradation: (a) HRT of 20 days, (b) HRT of 15 days, (c) HRT of 10 days, (d) HRT of 5 days, (e) HRT of 2 days, and (f) HRT of 1 day

During this phase, the sCOD value of the effluent exhibited an increase at the beginning of the continuous process. This indicates that the microorganisms require a certain period of time in order to recover from their previous environment and adapt to new conditions until they reach a stable equilibrium [29]. The simulation results do not take into account the microbial adaptation factor within the mathematical equation, which only measures the population level of microorganisms in terms of their specific conductivity value (sCOD) $\left(Y_{\frac{x1}{sCOD}}\right)$. Consequently, slight discrepancies occurred between the initial stages of the HRT operation and the simulation outcomes.

The simulation results suggest that a decrease in HRT value leads to an accelerated attainment of the steady state, as demonstrated by the sCOD value. This phenomenon is attributed to the assumption of a constant bacterial growth velocity (μ_{m1}) across all HRT values in the simulation. In scenarios where HRT values are reduced and incoming organic loads are augmented while μ_{m1} remains at a relatively constant low value, the dsCOD/dt value remains comparatively unchanged. The simulated sCOD value is in accordance with the mathematical model presented in (9). The experimental effluent sCOD value implies that reducing the HRT extends the adaptation period, while the value of μ_{m1} (bacterial growth velocity) increases over time.

The majority of models employed to investigate biodegradation kinetics are based upon the assumption of a maximum specific growth rate (max), which is predicated upon the existence of a short retention time, a parameter that is not feasible for complex biomasses [30]. This suggests that the steady state speed may become longer. However, at a specific point in time, the speed of bacterial growth and death will reach equilibrium, implying the achievement of a steady state [31]. The effluent sCOD value of the experimental results signifies that the optimum steady condition is reached when the reactor operates at an HRT of 10 days. At an HRT of 10 days, steady state was achieved on day 8, with a removal efficiency of 73.4% and an effluent sCOD value of 2,200 mg/L. This suggests that at 10 days of HRT, the substrate requirement is proportional for acidogenic bacteria. The proportional amount of substrate for the system ensures that the speed of bacterial growth is equal to the speed of death, thus enabling the steady state speed to be achieved [32].

The results of the acidogenic reactor simulation using the MATLAB approach demonstrated a correlation with the experimental data at a specific HRT value. This suggests that constant value data and mathematical modelling may be applicable to leachate decomposition in a dual-stage AFBR.

VFA Formation in Acidogenic Reactor

Volatile fatty acids (VFAs) result from the biotransformation of complex polymeric organic molecules into monomers. These monomers are produced by acidogenic bacteria through the hydrolysis of sCOD compounds. Figure 3 presents simulated and experimental VFA concentrations.

The value of the simulated effluent VFA shows no decline at the 20, 15, and 10 HRT, unlike the mathematical model of the ideal acidogenic reactor (R1) which indicates an increase. It can therefore be surmised that in this instance, the acidogenic bacteria population dominates in the production of VFA, while the methanogen bacteria population is suppressed to the lowest possible level. This represents the ideal condition of the simulation results.

In contrast to the mathematical model, the experimental results for HRT of 20, 15, and 10 days did not correspond with the anticipated outcomes, indicating that the reactor has not yet achieved the necessary acidogenic dominant conditions at this point in the HRT cycle.

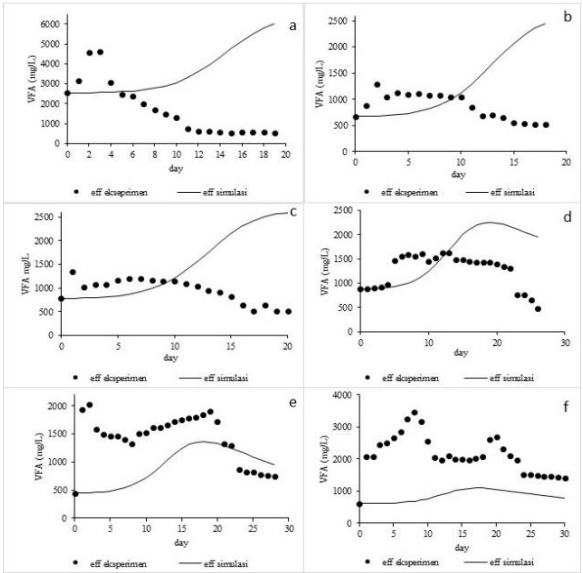

Figure 3. Profile of VFA formation: (a) HRT of 20 days, (b) HRT of 15 days, (c) HRT of 10 days, (d) HRT of 5 days, (e) HRT of 2 days, and (f) HRT of 1 day

A double-stage reactor was employed by [33] for observations, revealing a significant increase in the number of fluorescent bacteria, both methanogens and non-methanogens, in the acidogenic reactor when operated at a long HRT. It is possible that methanogenic bacteria may be able to grow well by Day 5, and therefore, when the HRT is increased, these bacteria can grow and develop in the acidogenic reactor (R1), leading to the consumption of VFA [34]. In the simulation, the optimal condition is depicted by equation 10, which illustrates the situation where methanogenic bacteria (X2) cease to grow due to the VFA consumption ($Y_{\frac{X2}{cVFA}}$).

The experiment results demonstrate a trend comparable to that of the simulation, particularly at the HRTs of 2.5 days and 1 day. The simulation showed that the lower the HRT, the less sCOD was degraded to produce VFA, contributing to a lower simulated effluent VFA value. Leachate represents a complex substrate that necessitates a protracted retention period to facilitate hydrolysis, with the subsequent production of simpler end-products in the context of anaerobic digestion. The research conducted by [35] reported that reducing the HRT facilitates VFA production when simple substrates are used, whereas longer reaction times are required to digest complex substrates. The simulated VFA values do not take into account the biofilm formation process, resulting in lower VFA formation process $\left(\frac{Y_{cVFA}}{X_1}\right)$ by acidogenic bacteria at short HRT values. The lower simulated VFA value for the effluent indicates that the population of acidogenic bacteria (X1) is considered to remain constant across all HRT values [36]. This contrasts with the experimental results, which show that the lower the HRT, the higher the VFAs in the effluent from the acidogenic reactor. The high yield value is correlated with the optimum growth rate (μ_{m1}) and the higher population of acidogenic bacteria when the reactor is operated at a short HRT. At short HRT, the increased availability of substrate for acidogenic bacteria suggests higher VFA formation by these bacteria. It can be concluded that the leachate decomposition process using the AFBR two-stage system can be optimally achieved in terms of VFA formation when the reactor is operated at short HRT. When the OLR is low, the acidogenesis and methanogenesis processes will take place simultaneously in the acidogenic reactor and the whole two-stage system will not be fully utilized [37].

By comparing the simulated and experimental results, it leads to the conclusion that mathematical modelling using single-stage AFBR constants requires adjustment to be applied in the two-stage AFBR.

VFA Degradation in Methanogenic Reactor

The acidogenic reactor effluent provides the substrate for the methanogenic reactor in the form of VFA. Figure 4 shows the influent VFA value of the simulation results and experimental data for the methanogenic reactor.

The experimental and simulated effluent VFA values revealed different trends across all HRT values. When the methanogenic reactor was operated at a short HRT, the effluent VFA value of the simulation results did not exhibit any increase at first. This is due to the fact that the simulation results did not take into account the adaptation process of the micro-organisms, which influences their growth rate. If the growth rate (µg2) decreases due to the adaptation process, the yield value produced will also decrease. [38] showed that the methanogenic microbial growth tends to decline until day six and then rise until the final day, when the bacterial concentration drops from 6x107 to 0.6x107 to 1x107/mL, and the activity is no longer convertible. The experimental effluent VFA value, which increased after day 20 at each HRT, was attributed to changes in HRT within the acidogenic reactor.

These changes cause the concentration of substrate flowing into the methanogenic reactor to increase; thus, when the system becomes unbalanced at a short HRT, it can only metabolize the substrate into organic acids (measured as VFA) without sufficient time to convert them into CH_4 [39].

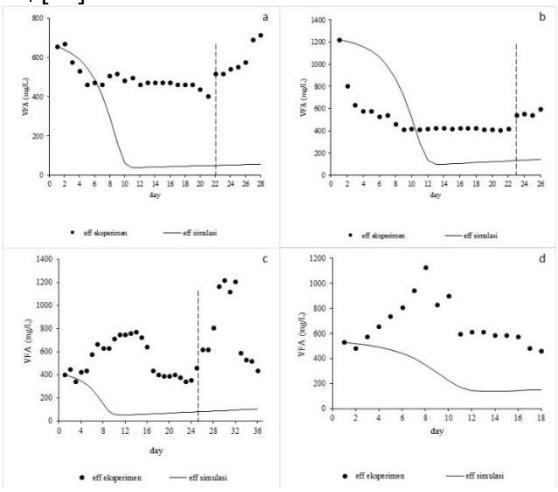

Figure 4. Profile of VFA degradation: (a) HRT of 20 days, (b) HRT of 10 days, (c) HRT of 5 days, (d) HRT of 2 days.

Additionally, [40] stated that the hydrodynamics associated with anaerobic digestion can influence cell physiology, resulting in a shift from further production to the generation of unwelcome secondary metabolites accumulated within the system. Furthermore, inhibition of fermentative bacteria populations by their primary product, volatile fatty acids (VFAs), has been observed when glucose serves as the primary substrate [41]. These observations suggest that the bacteria require a period of time to adapt to changes in substrate concentration or influent discharge. This adaptation process impacts the growth rate constant and the yield value in the methanogenic reactor. These results cannot be replicated by simulation, where the constant value remains unchanging regardless of the microorganism recovery process. The constant value that remains throughout the process contributes to the discrepancy between simulation and experimental results. The constant velocity value is correlated with the steady state observed in the simulation results.

The simulated effluent VFA value signifies that the steady state can be achieved with a similar temporal pattern between the 10th and 11th day. In contrast, the effluent VFA values of experimental results demonstrate that each HRT exhibits a distinct temporal profile in reaching the steady state. This is attributed to the varying growth speeds at different HRTs, which consequently influence the duration of reaching the steady state. The simulated effluent VFA value reached a steady state of 100 mg/L at each HRT. The experimental effluent VFA value reached a steady state of 400 mg/L at each HRT. This indicates that methanogenic bacteria require a certain minimum VFA concentration to survive [42]. In order to prevent a reduction in effluent VFA levels below 400 mg/L, it was necessary to determine the optimal substrate concentration for X2 production. The results of the reactor simulation suggest that the minimum substrate requirement for X2 bacteria is only 100 mg/L, a finding that is consistent with the experimental observations. The differing minimum substrate requirements are specified due to the influence that biofilm formation has on the microbial load in a bioreactor [43]. Research conducted by [44] explained that an increase in biofilm thickness is anticipated with extended operation times within a reactor, which may influence the simulation outcome.

CH_4 Formation in Methanogenic Reactor

The formation of methane (CH_4) is an indicator of the success of the methanogenic reactor. Methane production results from the

consumption of VFA by methanogenic bacteria. The value of VFA produced is proportional to the value of sCOD decomposed. Figure 5 and Figure 6 exhibits the methane production data of experimental and simulation results in the methanogenic reactor.

In comparison to the experimental results observed in the continuous phase of the methanogenic reactor, the simulated results tend to underestimate the CH_4 production values.

This phenomenon appears to be correlated with the findings presented in Figure 5, where at shorter residence times, there was a notable increase in CH_4 production, which consequently affected the accumulation value observed in Figure 6.

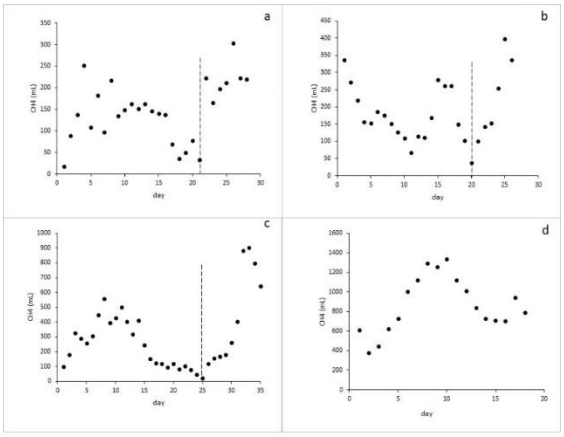

Figure 5. Profile of Production CH_4: (a) HRT of 20 days, (b) HRT of 10 days, (c) HRT of 5 days, and (d) HRT of 2 days

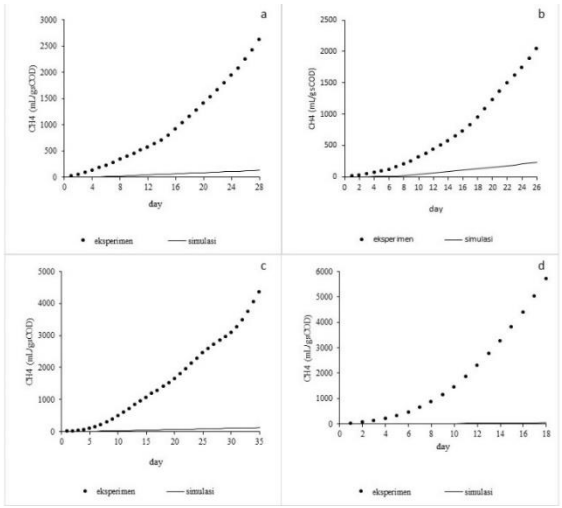

Figure 6. Profile of Accumulation Production CH_4: (a) HRT of 20 days, (b) HRT of 10 days, (c) HRT of 5 days, and (d) HRT of 2 days

Gradual increase in OLR and HRT has been demonstrated to enhance the efficacy of the anaerobic digestion process, with a concomitant impact on methane production [45]. The $YCH_4/X2$ constant demonstrated an inherent low value, which can be attributed to the characteristics observed during the batch phase. During this phase, there was a decline in substrate demand over time, reducing the yield of methane, a product of methanogenic bacteria. The kinetic constants observed in different reactor types exhibited distinct values and characteristics [46], and thus, adaptations are imperative when adopting batch reactor kinetic constants for continuous reactor operation.

The VFA substrate obtained from the methanogenic reactor in the continuous phase transferred from the acidogenic reactor had a consistently high level of availability and stimulated the growth rate of methanogenic bacteria (μ_{m2}). It is evident that the high substrate value influences the CH_4 yield generated by methanogenic bacteria and their accelerated growth speed (µm2), which consequently affects the elevated RS value (substrate consumption) [47]. The growth rate of X2 (methanogenic) bacteria will impact the $YCH_4/X2$ value produced in the methanogenic reactor. Furthermore, it is possible that the number of methanogenic bacteria will be lower than that of acidogenic bacteria that grow at a faster rate [48] at the acidogen reactor (R1) when operating at short residence times. The accumulation of acid at this stage is associated with the formation of significant quantities of methane. This observation is in accordance with prior studies which reported that conditions with high OLR can result in methane concentrations exceeding 60% [49]. The constant value employed in the batch phase proved to be unsuitable when the reactor operates continuously. This inadequate constant value resulted in simulation data that is significantly divergent from the experimental data.

Kinetic Constant Evaluation

The objective of evaluating kinetics through mathematical modelling is to identify any discrepancies that may arise as a result of the kinetics data and mathematical equations employed. A comparison of the experimental data from the AFBR double-column reactor with the simulation results of the methanogen reactor (R2) revealed significant discrepancies in the decomposition of volatile fatty acids (VFA) and methane (CH_4) production.

Table 5. Summary of kinetic parameters

Constant	Value
μ_{m1}	0.6739 day^{-1}
μ_{m2}	0.6041 day^{-1}
K_{sX1}	0.6054 mg COD/mg acidogenic
K_{sX2}	0.7110 mg VFA/mg methanogen
$Y_{X1/COD}$	2.3018 mg acidogenic/mg COD
$Y_{X2/VFA}$	0.0892 mg methanogen/ mg VFA
$Y_{CH4/X2}$	580.95 (mg CH4/L) / (mg methanogen cell/L)
$Y_{VFA/X1}$	0.9332 mg VFA/mg acidogenic
kd_1	0.2427 day^{-1}
kd_2	0.2248 day^{-1}

The simulation data indicates an underestimation of CH_4 production, while the VFA decomposition simulation data tends to overestimate compared to the experimental results. The underestimation in CH_4 production suggests that the constant Ks value used in the continuous reactor may not be accurate. As the microorganism adaptation process increases, so does the tendency for the Ks value to decrease, particularly at low HRT or high OLR. The values of Ks that tend to decrease have an impact on the affinity between microorganisms and the substrate they are growing on, as well as the growth rate of these microorganisms. The Ks value based on the Monod equation implies that when good affinity between bacteria and substrate is observed, the growth rate of bacteria will approach the maximum growth rate. This is less relevant for reactors that operate continuously. In contrast, low affinity between bacteria and substrate will impact the growth rate of methanogenic bacteria during the process [50]. The Monod equation's Ks value, as well as the Contois kinetics model, are more pertinent for ideals substrate scenarios and are therefore not applicable for complex ones, such as leachate. The Contois model incorporates a microbial concentration term, which enables a description of both the microbes and the finite conditions of the substrate [51]. In his explanation regarding the differing conduct of the Ks values observed in continuous anaerobic digesters and batch reactors, [52] highlighted the importance of recognizing these variations.

Table 5 demonstrates the unchanging growth value of methanogen microorganisms (μ2), which is inconsistent with previous findings, indicating that the growth rate of microorganisms (μmax) tends to rise as the Ks value increases [36]. An increase in growth could potentially be explained by the utilization of microbial immobilization media for biofilm formation. Research carried out by [53] reported that the addition of immobilization media can affect the efficiency of biogas formation. A subsequent study suggests a positive correlation between biogas formation and the use of zeolite as an immobilization medium, with an impact on the specific growth rate of 59% and a methane yield of 320 mL-CH_4/g-COD [54]. The formation of biofilm on immobilized media is not accommodated within the constraints of conventional kinetics, [55] posits that biofilm growth is driven by diffusion, interactions, and competition for bacterial growth at elevated VFA concentrations.

Furthermore, the deviation of CH_4 production values ($Y\frac{CH_4}{X_2}$) in the methanogen reactor is due to the constant. The increase in VFA concentration transferred from R1 to R2 resulted in an increase in CH_4 production by X2. The increase in VFA concentration at low HRT should have resulted in a higher Y value of $Y\frac{X_2}{XH_4}$, thus increasing the value of $Y\frac{CH_4}{X_2}$. This conclusion is consistent with findings reported by [55, 56, 57], which demonstrated that methane production tends to increase in concert with elevated concentrations of VFA and is associated with the proliferation of methanogenic microorganisms (μ2). It is evident that there is a discrepancy between the simulated and experimental results with regards to the degradation of VFA. This discrepancy can be attributed to the fact that the kinetic constants associated with the acidogenic reactor were not included. Consequently, the value of $Y\frac{X_2}{VFA}$ was not taken into consideration. This has led to the assumption that all the substrate is utilised by X1 to μm1, resulting in the production of VFA $\left(Y\frac{VFA}{X_1}\right)$.

CONCLUSION

The verification results between the calculated simulation and experimental data lead to the conclusion that the mathematical modification of the Contois and Monod kinetics constants is representative of the double-stage AFBR reactor at short HRT operation in the decomposition of leachate water complex substrate. The mathematical model considers the specific characteristics of each acidogenic and methanogen reactor under ideal conditions. Despite the simplifications made in this mathematical model, it still encompasses the phenomena observed in biological processes. However, the batch phase kinetic constant value is not suitable for application to the double-stages AFBR reactor when the reactor operates continuously with a long HRT. Furthermore, the proposed model also accounts for OLR changes, accurately predicting the trends observed.

ACKNOWLEDGMENT

Authors would like to show gratitude to Bioprocess Engineering Group of the Department of Chemical Engineering at Universitas Gadjah Mada for facilitating and funding this research.

REFERENCES

[1] I. D. Barbosa Segundo, F. C. Moreira, T. F. C. V Silva, A. D. Webler, R. A. R. Boaventura, and V. J. P. Vilar, "Development of a treatment train for the remediation of a hazardous industrial waste landfill leachate: A big challenge," *Science of The Total Environment*, vol. 741, p. 140165, 2020, doi: 10.1016/j.scitotenv.2020.140165.

[2] M. H. Saghi *et al.*, "Characteristics and pollution indices of leachates from municipal solid waste landfills in Iranian metropolises and their implications for MSW management," *Scientific Reports*, vol. 14, no. 1, p. 27285, Dec. 2024, doi: 10.1038/s41598-024-78630-w.

[3] J. Wang and Z. Qiao, "A comprehensive review of landfill leachate treatment technologies," *Frontiers in Environmental Science*, vol. 12, Sep. 2024, doi: 10.3389/fenvs.2024.1439128.

[4] M. Hussein, K. Yoneda, Z. Mohd. Zaki, N. Othman, and A. Amir, "Leachate characterizations and pollution indices of active and closed unlined landfills in Malaysia," *Environmental Nanotechnology, Monitoring & Management*, vol. 12, p. 100232, 2019, doi: 10.1016/j.enmm.2019.10023.

[5] R. Xiang *et al.*, "A Review on Landfill Leachate Treatment Technologies: Comparative Analysis of Methods and Process Innovation," Apr. 01, 2025, *Multidisciplinary Digital Publishing Institute (MDPI),* doi: 10.3390/app15073878.

[6] R. de Almeida, R. F. Porto, B. R. Quintaes, D. M. Bila, M. C. Lavagnolo, and J. C. Campos, "A review on membrane concentrate management from landfill leachate treatment plants: The relevance of resource recovery to close the leachate treatment loop," *Waste Management & Research*, vol. 41, no. 2, pp. 264–284, Aug. 2022, doi: 10.1177/0734242X221116212.

[7] C. T. Mary, K. Swarnalatha, and S. J. Harishma, "Experimental analysis on the effects of trace metals as micronutrients in enhancing biomethane production," *Sustainable Energy Research*, vol. 11, no. 1, Jan. 2024, doi: 10.1186/s40807-023-00093-w.

[8] U. E. Senadheera *et al.*, "Up-Flow Anaerobic Sludge Bed Reactors for Sustainable Wastewater Management: Challenges, Innovations, and Future Directions," Feb. 01, 2025, *Multidisciplinary Digital Publishing Institute (MDPI)*. doi: 10.3390/w17040476.

[9] Y. Ding *et al.*, "Research on biological fluidized bed system treatment performance and nitrogen removal process for seafood processing wastewater with different operation conditions," *Water (Switzerland)*, vol. 13, no. 19, Oct. 2021, doi: 10.3390/w13192630.

[10] H. Tong, B. Zhou, C. Liu, A. C. Wachemo, X. Li, and X. Zuo, "Improving biomethane yield by strengthening acidification of maize stover in two-phase anaerobic digestion," *International Journal of Agricultural and Biological Engineering*, vol. 13, no. 4, pp. 226–231, 2020, doi: 10.25165/j.ijabe.20201304.4654.

[11] E. Prasetyo, H. Sudibyo, and W. Budhijanto, "Determination of the optimum hydraulic retention time in two-stage anaerobic fluidized bed bioreactor for landfill leachate treatment," *Journal of Engineering and Technological Sciences*, vol. 49, no. 3, pp. 388–399, 2017, doi: 10.5614/j.eng.technol.sci.2017.49.3.7.

[12] B. Singh *et al.*, "Enhancing efficiency of anaerobic digestion by optimization of mixing regimes using helical ribbon impeller," *Fermentation*, vol. 7, no. 4, Dec. 2021, doi: 10.3390/fermentation7040251.

[13] Y. Hu, Z. Zhou, and C. Shen, "Microbial community acclimation during anaerobic digestion of high-oil food waste," *Sci Rep*, vol. 14, no. 1, Dec. 2024, doi: 10.1038/s41598-024-77136-9.

[14] X. Zheng and R. Li, "Critical Review on Two-Stage Anaerobic Digestion with H2 and CH4 Production from Various Wastes," Jun. 01, 2024, *Multidisciplinary Digital Publishing Institute (MDPI)*. doi: 10.3390/w16111608.

[15] M. M. Montes, S. E. Vigueras-Carmona, S. Esteban, V. Carmona, and J. P. Vargas, "Two-stage anaerobic digestion of fruit and vegetable wastes," *Journal of Biotech Research*, vol. 10, pp. 29-37, 2019.

[16] B. Velázquez-Martí, O. Meneses Quelal, J. Gaibor, and Z. Niño, *Review of Mathematical Models for the Anaerobic Digestion Process*, 2018, doi: 10.5772/intechopen.80815.

[17] J. Wang, B. Liu, M. Sun, F. Chen, M. Terashima, and H. Yasui, "A Kinetic Model for Anaerobic Digestion and Biogas Production of Plant Biomass under High Salinity," *International Journal of Environmental Research and Public Health*, vol. 19, p. 6943, Jun. 2022, doi: 10.3390/ijerph19116943.

[18] S. Kumar Pramanik, F. Suja, M. Porhemmat, and B. Pramanik, "Performance and Kinetic Model of a Single-Stage Anaerobic Digestion System Operated at Different Successive Operating Stages for the Treatment of Food Waste," *Processes*, vol. 7, p. 600, Sep. 2019, doi: 10.3390/pr7090600.

[19] M. Mihi *et al.*, "Modeling and forecasting biogas production from anaerobic digestion process for sustainable resource energy recovery," *Heliyon*, vol. 10, no. 19, p. e38472, 2024, doi: 10.1016/j.heliyon.2024.e38472.

[20] M. Hanaki, J. Harmand, Z. Mghazli, A. Rapaport, T. Sari, and P. Ugalde, "Mathematical study of a two-stage anaerobic model when the hydrolysis is the limiting step," *Processes*, vol. 9, no. 11, Nov. 2021, doi: 10.3390/pr9112050.

[21] M. Atasoy and Z. Cetecioglu, "The effects of pH on the production of volatile fatty acids and microbial dynamics in long-term reactor operation," *Journal of Environmental Management*, vol. 319, p. 115700, 2022, doi: 10.1016/j.jenvman.2022.115700.

[22] X. Zhou, Y. Lu, L. Huang, Q. Zhang, X. Wang, and J. Zhu, "Effect of pH on volatile fatty acid production and the microbial community during anaerobic digestion of Chinese cabbage waste," *Bioresource Technology*, vol. 336, p. 125338, 2021, doi: 10.1016/j.biortech.2021.125338.

[23] E. Echiegu and A. Ghaly, "Kinetic Modelling of Continuous-Mix Anaerobic Reactors Operating Under Diurnally Cyclic Temperature Environment," *American Journal of Biochemistry and Biotechnology*, vol. 10, pp. 130–142, Jan. 2014, doi: 10.3844/ajbbsp.2014.130.142.

[24] N. Abbasi *et al.*, "The Effect of Mixed Liquor Volatile Suspended Solids and Hydraulic Retention Time on the Removal Efficiency of Chemical Oxygen Demand through Aerobic Activated Sludge Process of Textile Wastewater," *International Journal on Emerging Technologies*, vol. 12, no. 2, pp. 54–59, 2021

[25] N. Fernández *et al.*, "Performance evaluation of an anaerobic fluidized bed reactor with natural zeolite as support material when treating high-strength distillery wastewater," *Renewable Energy*, vol. 33, no. 11, pp. 2458–2466, 2008, doi: 10.1016/j.renene.2008.02.00.

[26] M. Walker, Y. Zhang, S. Heaven, and C. Banks, "Potential errors in the quantitative evaluation of biogas production in anaerobic digestion processes," *Bioresource Technology*, vol. 100, no. 24, pp. 6339–6346, 2009, doi: 10.1016/j.biortech.2009.07.018.

[27] H. Sudibyo, D. Guntama, and W. Budhijanto, "Assessment of start-up mechanisms for anaerobic fluidized bed reactor in series based on mathematical simulation," *AIP Conference Proceedings*, vol. 1840, no. 1, p. 110007, May 2017, doi: 10.1063/1.4982337.

[28] F. Sakaveli, M. Petala, V. Tsiridis, and E. Darakas, "Enhanced mesophilic anaerobic digestion of primary sewage sludge," *Water (Switzerland)*, vol. 13, no. 3, Feb. 2021, doi: 10.3390/w13030348.

[29] O. A. Olafadehan and A. T. Alabi, "Modelling and Simulation of Methanogenic Phase of an Anaerobic Digester," *Materials for Renewable and Sustainable Energy*, vol. 12, pp. 105–116, 2023, doi: 10.1007/s40243-023-00233-8.

[30] Y. Momoh and I. Nwaogazie, "The effect of waste paper on the kinetics of biogas yield from the co-digestion of cow manure and water hyacinth," *Biomass Bioenergy*, vol. 35, pp. 1345–1351, Sep. 2013.

[31] S. Attar and F. A. Haugen, "Dynamic model adaptation to an anaerobic digestion reactor of a water resource recovery facility," *Modeling, Identification and Control*, vol. 40, no. 3, pp. 143–160, 2019, doi: 10.4173/mic.2019.3.2.

[32] B. Kumar Biswal, H. Huang, J. Dai, G.-H. Chen, and D. Wu, "Impact of low-thermal pretreatment on physicochemical properties of saline waste activated sludge, hydrolysis of organics and methane yield in anaerobic digestion," *Bioresour Technol*, vol. 297, p. 122423, 2020, doi: 10.1016/j.biortech.2019.122423.

[33] E. Holl *et al.*, "Two-stage anaerobic digestion: State of technology and perspective roles in future energy systems," *Bioresource Technology,* vol. 360, p. 127633, 2022, doi: 10.1016/j.biortech.2022.127633.

[34] M. Atasoy and Z. Cetecioglu, "Butyric acid dominant volatile fatty acids production: Bio-augmentation of mixed culture fermentation by Clostridium butyricum," *Journal of Environmental Chemical Engineering*, vol. 8, no. 6, pp. 104496, 2020, doi: 10.1016/j.jece.2020.104496.

[35] C. Zhang, H. Su, Z. Wang, T. Tan, and P. Qin, "Biogas by Semi-Continuous Anaerobic Digestion of Food Waste," *Applied Biochemistry and Biotechnology*, vol. 175, Mar. 2015, doi: 10.1007/s12010-015-1559-5.

[36] S. Emebu, J. Pecha, and D. Janáčová, "Review on anaerobic digestion models: Model classification & elaboration of process phenomena," *Renewable and Sustainable Energy Reviews*, vol. 160, p. 112288, 2022, doi: 10.1016/j.rser.2022.112288.

[37] P. A. Cremonez, J. G. Teleken, T. R. Weiser Meier, and H. J. Alves, "Two-Stage anaerobic digestion in agroindustrial waste treatment: A review," *Journal of Environmental Management*, vol. 281, p. 111854, 2021, doi: 10.1016/j.jenvman.2020.111854.

[38] Y. Wang, Y. Zhang, J. Wang, and L. Meng, "Effects of volatile fatty acid concentrations on methane yield and methanogenic bacteria," *Biomass Bioenergy*, vol. 33, pp. 848–853, May 2009, doi: 10.1016/j.biombioe.2009.01.007.

[39] S. Harirchi *et al.*, "Microbiological insights into anaerobic digestion for biogas, hydrogen or volatile fatty acids (VFAs): a review," 2022, *Taylor and Francis Ltd.* doi: 10.1080/21655979.2022.2035986.

[40] Y. Hu *et al.*, "Investigation of hydrodynamics in high solid anaerobic digestion by particle image velocimetry and computational fluid dynamics: Role of mixing on flow field and dead zone reduction," *Bioresource Technology*, vol. 319, p. 124130, 2021, doi: 10.1016/j.biortech.2020.124130.

[41] Y. Jiang, E. McAdam, Y. Zhang, S. Heaven, C. Banks, and P. Longhurst, "Ammonia inhibition and toxicity in anaerobic digestion: A critical review," *Journal of Water Process Engineering*, vol. 32, p. 100899, 2019, doi: 10.1016/j.jwpe.2019.100899.

[42] Q. Zhang, J. Hu, D.-J. Lee, Y. Chang, and Y.-J. Lee, "Sludge treatment: Current research trends," *Bioresource Technology*, vol. 243, pp. 1159–1172, 2017, doi: 10.1016/j.biortech.2017.07.070.

[43] R. D. A. Cayetano *et al.*, "Biofilm formation as a method of improved treatment during anaerobic digestion of organic matter for biogas recovery," *Bioresource Technology*, vol. 344, p. 126309, 2022, doi: 10.1016/j.biortech.2021.126309.

[44] G. B. Abera *et al.*, "Biofilm application for anaerobic digestion: a systematic review and an industrial scale case," Dec. 01, 2024, *BioMed Central Ltd.*, doi: 10.1186/s13068-024-02592-4.

[45] L. Zhang, K.-C. Loh, and J. Zhang, "Enhanced biogas production from anaerobic digestion of solid organic wastes: Current status and prospects," *Bioresource Technology Reports*, vol. 5, pp. 280–296, 2019, doi: 10.1016/j.biteb.2018.07.005.

[46] M. Shuler and F. Kargi, *Bioprocess Engineering: Basic Concepts*, 3rd Ed., Pearson, 2017, UK.

[47] S. Wainaina, L. Lukitawesa, M. K. Awasthi, and M. Taherzadeh, "Bioengineering of anaerobic digestion for volatile fatty acids, hydrogen or methane production: A critical review," *Bioengineered*, Jan. 2019, doi: 10.1080/21655979.2019.1673937.

[48] N. Kontogianni, E. M. Barampouti, S. Mai, D. Malamis, and M. Loizidou, "Effect of alkaline pretreatments on the enzymatic hydrolysis of wheat straw," *Environmental Science and Pollution Research*, vol. 26, no. 35, pp. 35648–35656, Dec. 2019, doi: 10.1007/s11356-019-06822-3.

[49] T. A. Mendiola-Rodriguez and L. A. Ricardez-Sandoval, "Robust control for anaerobic digestion systems of Tequila vinasses under uncertainty: A Deep Deterministic Policy Gradient Algorithm," *Digital Chemical Engineering*, vol. 3, p. 100023, 2022, doi: 10.1016/j.dche.2022.100023.

[50] A. I. Bayu *et al.*, "Kinetic study of thermophilic anaerobic digestion of sugarcane vinasse in a single-stage continuous stirred tank reactor," *Results in Engineering*, vol. 14, p. 100432, 2022, doi: 10.1016/j.rineng.2022.100432.

[51] K. Waszkielis, I. Białobrzewski, and K. Bułkowska, "Application of anaerobic digestion model No. 1 for simulating fermentation of maize silage, pig manure, cattle manure and digestate in the full-scale biogas plant," *Fuel*, vol. 317, p. 123491, 2022, doi: 10.1016/j.fuel.2022.123491.

[52] E. O. Otieno, R. Kiplimo, and U. Mutwiwa, "Optimization of anaerobic digestion parameters for biogas production from pineapple wastes co-digested with livestock wastes," *Heliyon*, vol. 9, no. 3, Mar. 2023, doi: 10.1016/j.heliyon.2023.e14041.

[53] A. Rabii, S. Aldin, Y. Dahman, and E. Elbeshbishy, "A review on anaerobic co-digestion with a focus on the microbial populations and the effect of multi-stage digester configuration," *Energies (Basel)*, vol. 12, no. 6, 2019, doi: 10.3390/en12061106.

[54] P. Kongjan and S. O-Thong, “Biogas Production from Anaerobic Co-digestion of Palm Oil Mill Effluent and Empty Fruit Bunches,” *Energy Procedia*, vol. 138, pp. 717–722, Oct. 2017, doi: 10.1016/j.egypro.2017.10.206.
[55] N. El Khattabi, I. Ahlamine, and abdellah Alla, “Mathematical modelling of the anaerobic digestion process with acidogenic and methanogenic biomasses,” *Authorea*, Apr. 14, 2024. doi: 10.22541/au.171309199. 90952619/v1.
[56] A. Martin, R. Hartono, and R. Asrian, “Utilizing waste heat gasoline engine in the design and fabrication of a fin and tube evaporator for the Organic Rankine Cycle (ORC),” *SINERGI*, vol. 27, no. 2, pp. 171-178, 2023, doi: 10.22441/sinergi.2023.2.004
[57] C. Charnier *et al.*, “Fast characterization of solid organic waste content with near infrared spectroscopy in anaerobic digestion,” *Waste Management*, vol. 59, pp. 140–148, 2017, doi: 10.1016/j.wasman.2016.10.029.
[58] T.-S. Le *et al.*, “Bacterial community composition in a two-stage anaerobic membrane bioreactor for co-digestion of food waste and food court wastewater,” *Bioresource Technology*, vol. 391, p. 129925, 2024, doi: 10.1016/j.biortech.2023.129925.