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EVALUATION AND KINETICS OF TOFU WASTEWATER BIOREACTOR WITH ADDITION OF WATER HYACINTH

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ABSTRACT: Tofu wastewater is a nitrogen-rich wastewater type with a high concentration of organic contents. To enhance the digestion rate of tofu wastewater in an anaerobic bioreactor, water hyacinth was tested as an additional substrate. Water hyacinth is a dangerous weed with a high carbon-nitrogen ratio (C/N) of about 30-35. Substrate combination was intentionally used in this study to raise the C/N of wastewater. This study aims to evaluate the digestion rate of a bioreactor qualitatively based on experimental data to determine the biokinetic constants of the anaerobic bioreactor quantitatively based on microbial growth data, substrate degradation, and methane (CH_4) production. MATLAB was used as software to run mathematical modeling. The anaerobic bioreactor was designed and equipped with a circulation pump to maintain the homogeneity of the substrate and was completed with a biogas collector. Seeding and acclimatization were carried out before the main experiment started. Using a combination substrate of tofu wastewater and water hyacinth with a 5:3 volume ratio, the bioreactor was run at 20 days of hydraulic retention time (HRT) at room temperature. The bioreactor was able to remove 92.8% of chemical oxygen demand (COD) concentration and produced biogas with the highest CH_4 concentration of 56.9%. The obtained kinetic constants indicate that, in comparison with similar studies of anaerobic digestion of the low C/N wastewater, the addition of water hyacinth resulted in better performance of the bioreactor with the correlation of microbial growth rate (μ_m), substrate degradation ($Y_{X/CCOD}$) and CH_4 production ($Y_{CCH4/X}$) with the consecutive values of 0.65/day, 0.64 mg cells/mg COD, and 0.62 mg CH_4/mg cells. An appropriate ratio of water hyacinth as the high carbon source and nitrogen-rich tofu wastewater is recommended to obtain the optimum ratio of carbon to nitrogen and result in a higher percentage of methane formation.

ABSTRAK: Air sisa tauhu ialah air sisa yang kaya dengan nitrogen dengan kepekatan kandungan organik yang tinggi. Bagi meningkatkan kadar penghadaman air sisa tauhu dalam bioreaktor anaerobik, keladi bunting diuji sebagai substrat tambahan. Keladi bunting merupakan rumpai berbahaya dengan nisbah karbon nitrogen (C/N) yang tinggi iaitu kira-kira 30-35. Gabungan substrat sengaja digunakan dalam kajian ini bagi menaikkan C/N air sisa. Kajian ini bertujuan bagi menilai kadar pencernaan bioreaktor secara kualitatif berdasarkan data eksperimen dan menentukan pemalar biokinetik bioreaktor anaerobik secara kuantitatif berdasarkan data pertumbuhan mikrob, degradasi substrat, dan pengeluaran metana (CH_4). MATLAB digunakan sebagai perisian pemodelan matematik. Bioreaktor anaerobik direka bentuk dan dilengkapi dengan pam edaran bagi mengekalkan kehomogenan substrat dan

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dilengkapkan dengan pengumpul biogas. Penyemaian dan penyesuaiikliman telah dijalankan sebelum eksperimen utama bermula. Substrat gabungan air sisa tauhu dan keladi bunting digunakan dengan nisbah isipadu 5:3. Bioreaktor dijalankan selama 20 hari iaitu masa pengekalan hidraulik (HRT) pada suhu bilik. Bioreaktor tersebut mampu mengasingkan 92.8% kepekatan permintaan oksigen kimia (COD) dan menghasilkan biogas dengan kepekatan CH_4 tertinggi sebanyak 56.9%. Pemalar kinetik yang diperoleh menunjukkan bahawa, berbanding dengan kajian serupa tentang pencernaan anaerobik air sisa C/N rendah, penambahan keladi bunting menghasilkan prestasi bioreaktor yang lebih baik dengan korelasi kadar pertumbuhan mikrob (μ_m), degradasi substrat ($Y_{X/CCOD}$) dan penghasilan CH_4 ($Y_{CCH4/X}$) dengan nilai masing-masing 0.65/hari, 0.64 mg sel/mg COD, dan 0.62 mg CH_4/mg sel. Nisbah keladi bunting yang sesuai sebagai sumber karbon tinggi dan air sisa tauhu yang kaya dengan nitrogen disyorkan agar mendapatkan nisbah optimum karbon kepada nitrogen dan ini menghasilkan peratusan pembentukan metana yang lebih tinggi.

KEYWORDS: *Anaerobic process, kinetic constants, C/N ratio, tofu wastewater, water hyacinth*

1. INTRODUCTION

Tofu wastewater is one of the wastes produced in tofu production with a high content of organic substances. The organic content of tofu wastewater measured as COD is fairly high, which is in the range of 10,000 to 16,000 mg/L [1, 2]. The existence of this wastewater has the potential to endanger the environment if it is directly flowed into water bodies, as is sometimes happening today. Referring to Ministerial Regulation of Indonesia No. 5 of 2014 concerning Wastewater quality Standards, the COD concentration of tofu wastewater allowed is 275 mg/L [3].

The tofu industry in Indonesia is dominated by the home-scale industry, which uses conventional waste management. An anaerobic process with the help of microorganisms is one of the applicable options due to its ability both to degrade organic compounds and to form methane. The anaerobic digestion consists of mixed culture microorganisms, such as acidogenic bacteria that are responsible for the acidogenesis process and methanogenic bacteria for the methanogenesis process. Several factors greatly influence this process. According to the Authors of [4], the ratio of carbon to nitrogen (C/N) contained in the substrate is a factor of concern that must be considered by researchers. Tofu wastewater has a low ratio of C/N, which was 5.90 [1], while the Authors of [5] suggested that the optimum ratio of C/N in the formation of methane gas is 25-30. The low ratio of C/N contained in the substrate would result in the high formation of total ammonia nitrogen (TAN) and volatile fatty acids (VFAs) [5]. The concentrations of TAN and VFA accumulation inside the bioreactor decrease pH levels in acidic conditions; this condition potentially inhibits the growth of methanogenic bacteria and their activity in producing methane. The very high ratio of C/N is also not an ideal condition for the methanogenic process and impacts the low methane yield due to the lack of nitrogen concentration. Nitrogen is needed for the bacteria's growth, especially for methanogenic bacteria.

The alternative solution to increase the C/N ratio in this study was to use a substrate combination. The combination of substrates optimizes the C/N ratio inside the bioreactor to ensure the availability of nutrients needed by anaerobic microorganisms, both for acidogenic and methanogenic bacteria. Several studies reported information on increasing biogas formation in wastewater treatment processes through anaerobic treatment using combined substrates. Combining substrates increased biogas productivity compared to single substrates only [6,7]. The researchers of [7] presented that a substrate combination of organic kitchen waste and cattle manure with a ratio of 3:1 can increase the biogas yield by 47.13%, and a substrate combination of food waste and cattle manure with a ratio of 2:1 can increase the methane yield by 41.1%. Water hyacinth is a dangerous weed that can grow very quickly and deplete the nutrients and oxygen contained in water bodies. This weed is quite harmful to the surrounding ecosystem [8]. On the other hand, water hyacinth is a plant with a high C/N ratio which is 30-35 [9]. The optimum C/N ratio in anaerobic digestion can be achieved by mixing organic materials that have a low C/N ratio with materials that have a higher C/N ratio [10].

This study aims to add water hyacinth extract to the bioreactor to increase the C/N ratio of the substrate. The effects of this treatment are evaluated qualitatively through experimental data. The kinetic constants obtained from mathematical modeling complete the quantitative evaluation of this process, both in the process of organic compound degradation and CH_4 production.

2. METHODS

2.1. Raw Materials

The raw materials used in this study were tofu wastewater, water hyacinth extract and cow rumen. The materials were collected from a tofu home industry, a fish pond, and an animal farm in the Bandung area, Indonesia, respectively. Water hyacinth extract was prepared by blending and smoothing the water hyacinth leaves and water with a 2:13 weight ratio. Tofu wastewater and water hyacinth extract were mixed with a volume ratio of 5:3. Cow rumen as the starter bacteria was diluted using water with a ratio of 1:2 [1]. The characteristics of all materials were tested before the experiment started. The analysis of total nitrogen, carbon, and phosphate contents was carried out at the Center for Material and Technical Products in Bandung, Indonesia. COD, pH, volume, and gas concentrations were analyzed in the Water and Wastewater Treatment Laboratory in Politeknik Negeri Bandung, Indonesia.

2.2. Operational of Bioreactor

The anaerobic bioreactor used was specially designed for this research to increase biogas production through an anaerobic process, as shown in Fig. 1. The reactor was equipped with a circulation pump to maintain the homogeneity of the substrate concentration inside the bioreactor. Substrate circulation was carried out every day for 30 minutes before sampling and after feeding the wastewater. The circulation was conducted by closing valves 1 and 5 and opening valves 2, 3, and 4. The circulation pipe contained in the bioreactor was installed ¾ of the length of the feed pipe, which aims to prevent air from entering the bioreactor. The total capacity of this bioreactor (B) is 25 L with 20 L of active volume. The total capacity of the feed tank (A) is 28 L. The operating condition of this bioreactor was set at room temperature in neutral condition (pH 7), which was the optimum pH for the methanogenesis process. The bioreactor was operated at 20 days of hydraulic retention time (HRT) to prevent shock-loading problems for the microorganisms [1].

The experiment began by running the seeding stage and continued with the acclimatization process before the main process was carried out. The seeding process, as the preliminary step, was aimed to support the growth of microorganisms by adding 40 grams of glucose (2,000 mg/L) into the bioreactor. The use of an ideal substrate was proposed to shorten the lag phase and optimize the exponential phase of the microorganism's growth. The seeding process was stopped when MLVSS concentration reached more than 2,000 mg/L.

The acclimatization process was carried out by gradually replacing the ideal substrate (glucose) with tofu wastewater. One liter per day of mixing substrate was fed into the bioreactor with a concentration of 6,000 mg/L. The composition of tofu wastewater in the mixing substrate was gradually increased, starting from 5-10% per 3 days until all substrate was fully replaced by tofu wastewater. Acclimatization was ended when the fluctuation of decreasing COD was under 10% [11]. Appropriate acclimatization will result in the ability of microorganisms in the bioreactor to adapt efficiently to new substrates in the next process, namely the main process. The main research on the anaerobic bioreactor was conducted by feeding 1 L per day of tofu wastewater while keeping its concentration around 6,000 mg/L. Gas volume and pH were measured every day, while COD and MLVSS were tested every 3 days. Methane in the biogas was analyzed every 5 days to detect the methane gas formed.

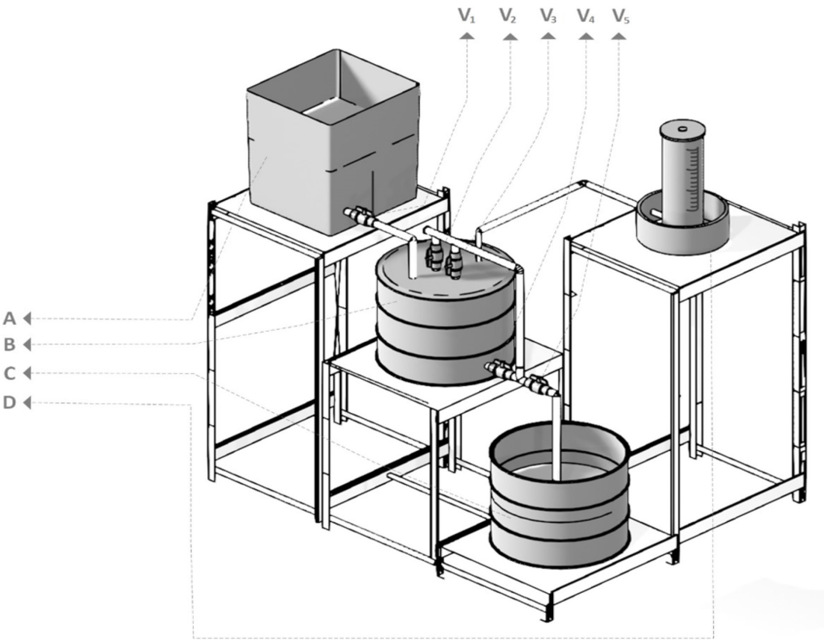

Fig. 1. Set up of bioreactor.

Annotation

a	: Feeding tank
b	: Anaerobic bioreactor
c	: Effluent tank
d	: Biogas collector
V_1	: Inlet valve
V_2, V_3, V_4	: Circulation valve
V_5	: Outlet valve

2.3. Analysis of COD, MLVSS, and Biogas

Chemical Oxygen Demand (COD) is a research parameter that represents the organic concentration, while Mixed Liquor Volatile Suspended Solid (MLVSS) represents the concentration of microorganisms inside the bioreactor. COD analysis was carried out using the closed reflux titrimetric method, referring to SNI 6989.73:2009, and MLVSS was tested using the gravimetric method, referring to SNI 06-6989.3-2004. All parameters were measured in duplicate. The volume of biogas formed was measured in a closed cylindrical gas device where the middle to bottom part of the cylindrical gas device containing water was immersed in an open-air container containing water (D in Fig. 1). The volume of biogas produced was

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calculated from measuring changes in water level in the cylindrical gas column. This method adopted the biogas volume measurement method outlined by [12].

2.4. Mathematical Modeling

The growth rate of anaerobic microorganisms (μ) was arranged based on cell mass balance and the specific growth rate following the Monod model. The Monod equation is defined by Eq. (1) where S is the substrate concentration (mg/L). In this study, it is measured as COD concentration (C_{COD}).

$$\mu = \frac{\mu_m . S}{K_{SX} + S} \quad (1)$$

The Monod equation for the growth rate of anaerobic bacteria is described in equation (2).

$$\frac{dX}{dt} = \left(\frac{\mu_m . C_{COD}}{K_{SX} + C_{COD}}\right) . X \quad (2)$$

The mathematical model for an anaerobic bioreactor for tofu wastewater for the batch system was defined from the mass balance on the substrate (COD) and product (CH_4). This mathematical model is adapted to the model used by [11] and combined with the model developed by [13].

The substrate (COD) mass balance is written as follows:

[rate of accumulation] = [rate of mass input] - [rate of mass output] + [rate of formation] - [rate of consumption]

$$V \frac{d(C_{COD})}{dt} = 0 - 0 + 0 - V . \frac{1}{Y_{X/CCOD}} \frac{dX}{dt} \quad (3)$$

Divided by the volume (V) and substituting the equation (2), the equation obtained is as follows:

$$\frac{d(C_{COD})}{dt} = -\frac{1}{Y_{X/CCOD}} \left(\frac{\mu_m . C_{COD}}{K_{SX} + C_{COD}}\right) . X \quad (4)$$

The product (CH_4) mass balance is written as:

[rate of accumulation] = [rate of mass input] - [rate of mass output] + [rate of formation] - [rate of consumption]

$$V \frac{d(C_{CH4})}{dt} = 0 - 0 + V . \frac{1}{Y_{X/CCH4}} \frac{dX}{dt} - 0 \quad (5)$$

Divided by the volume (V) and substituting the equation (2), the equation obtained is as follows:

$$\frac{d(C_{CH4})}{dt} = Y_{CCH4/X} \left(\frac{\mu_m . C_{COD}}{K_{SX} + C_{COD}}\right) . X \quad (6)$$

Nomenclature

C_{COD}	Chemical oxygen demand	mg/L
C_{CH4}	Methane concentration	mg/L
X	Cell concentration	mg/L
μ_m	Maximum specific growth rate	1/day
K_{SX}	Half-saturation constant	mg COD/mg cell
$Y_{X/CCOD}$	Yield of cell formation per mg COD reduction	mg cell/mg COD
$Y_{CCH4/X}$	Yield of CH_4 formation per mg cell	mg CH_4/mg cell

The simultaneous ordinary differential equations were solved by fitting the experimental data using MATLAB software with the use of ODE15S and FMINCON solvers resulting in the kinetic constants of the process. The effect of water hyacinth addition to the anaerobic bioreactor of tofu wastewater was shown on the experimental data and on the kinetic constants obtained from the model.

3. RESULTS AND DISCUSSION

The initial characteristics of all materials used in this study are shown in Table 1. The C/N ratio of tofu wastewater was very low, at 5.90, far below the optimum C/N ratio range for anaerobic processes (25-30) [5,7]. In addition, the optimum C/N ratio depends on the substrate characteristics and process operating conditions.

Table 1. Characteristics of raw materials

Parameters	(1)	(2)	(3)	(4)
T (ºC)	27	35	25	25
pH	6.80	5.45	6.25	5.75
COD (mg/L)	8,538.3	10,267.6	8,106.4	8,646.4
C/N	2.40	5.90	13.27	10.49

Annotation:
(1): Cow rumen, (2): Tofu wastewater, (3): Water hyacinth extract, (4): The mixture of tofu wastewater and water hyacinth extract

The mixture of tofu wastewater and water hyacinth extract was proposed to increase the C/N ratio of the bioreactor substrates. Tofu wastewater and water hyacinth extract were mixed with a volume ratio of 5:3, according to the research outlined in [1]. However, based on Table 1, the actual C/N ratio of the mixed substrate was 10.49. This might be caused by the water hyacinth extract used in this study, which contained a low ratio of C/N, which was only 13.27.

A low C/N ratio increases the pH value inside of the bioreactor. This is caused by the release of ammonia due to the large amount of nitrogen contained in the substrate. Apart from that, alkaline conditions higher than 8.5 have the potential to poison anaerobic bacteria, especially acidogenic bacteria. The pH value should be in the range of 7.0 to 7.5 to increase methane formation. Low pH values (<7.0) inhibit methane yield because alkalinity is not sufficient to buffer the production of volatile fatty acids that inhibit methanogenic activity [14].

3.1. Seeding Process

The seeding process, the first stage of this study, was carried out for 26 days. The main focus of this process was to cultivate anaerobic bacteria in the bioreactor. This stage is very important to determine the optimum process for the entire anaerobic treatment. Glucose as an ideal substrate was used because it has a high carbon content and is easily degraded by anaerobic bacteria. Organic matter measured as COD in the anaerobic bioreactor was maintained in the range of 6,000-8,500 mg/L. Very low concentrations of organic matter were avoided to ensure the availability of nutrients required for the growth of anaerobic bacteria. The COD concentration was set to a moderate value to prevent organic shock loads of the bacteria. In addition to the ideal media, operating conditions were also set to ideal conditions at this stage. During the seeding process, the pH conditions of the bioreactor were maintained in the range of 6.28-7.03 by adding sodium bicarbonate (Na_2CO_3) as a buffer solution. The growth of anaerobic bacteria is represented by the concentration of Mixed Liquor Volatile Suspended Solid (MLVSS), as shown in Fig.2.

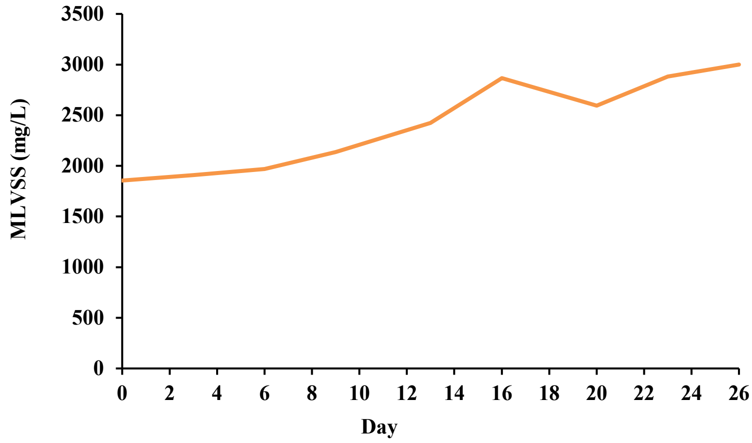

Fig. 2. MLVSS concentration versus time during the seeding process.

As seen in Fig. 2, the MLVSS concentration rose from 1,855 mg/L at the beginning of the seeding process to 3,000 mg/L on day 26. This means that the concentration of anaerobic bacteria met the minimum concentration required in the anaerobic process for wastewater treatment. The seeding process was stopped on the 26th day with a COD value of 6,755 mg/L and the MLVSS at 3,000 mg/L.

3.2. Acclimatization Process

The acclimatization process is referred to as the adaptation phase of microorganisms to process real organic matter contained in wastewater [15]. The addition of glucose prevented the risk of organic shock loads for microorganisms due to changes in the ideal substrate to the wastewater mixture [1]. Mixed substrates are more complex and difficult to degrade than glucose. After gradually replacing glucose with tofu wastewater, starting on day 16, the acclimatization process only used a mixture of tofu wastewater and water hyacinth until the end of the process on day 20, as shown in Fig. 3. Overall observations were made on the parameters of COD, MLVSS, and the volume of biogas formed.

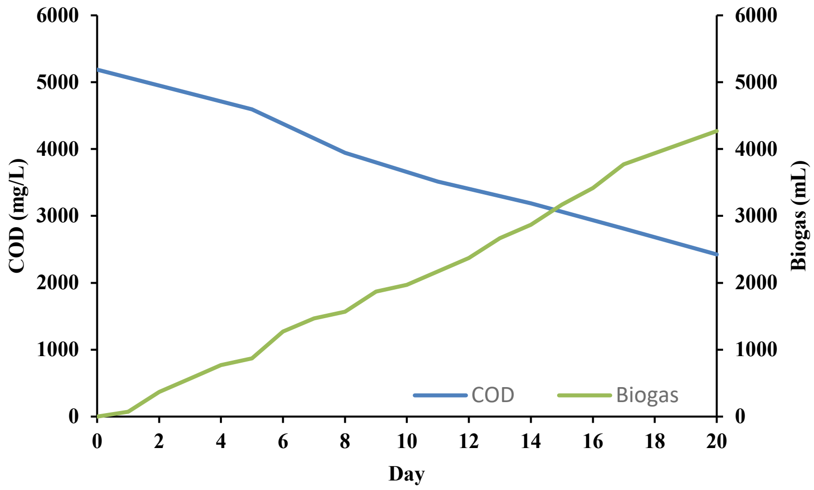

Fig. 3. COD concentration and biogas production during the acclimatization process

Fig. 3 shows that the COD concentration decreased progressively since the beginning of the acclimatization process from 5.187 mg/L to 2.424 mg/L at the end of the process. This shows that the anaerobic bacteria that were cultured in the previous stage were able to properly degrade complex organic compounds contained in the liquid waste. Reducing COD concentration is one of the determining factors for the success of the acclimatization process as evidenced by the presence of biogas formed, which was measured starting on day 1 and continued to increase until day 20, namely 4,270 mL of cumulative biogas (Fig. 3). The decrease in COD concentration and the formation of biogas indicate that all stages of the anaerobic process with the main stages of acidogenesis and methanogenesis processes were successfully conducted [16].

3.3. Main Anaerobic Process

The tofu wastewater anaerobic bioreactor was operated at an HRT of 20 days with an organic loading rate of 6,000 mg COD/L/day of fresh tofu wastewater. This tofu wastewater was fed into the bioreactor every day. At this stage, several research parameters were measured to be observed qualitatively, including pH (Fig. 4), MLVSS (Fig. 5), COD (Fig. 6), and methane formation (Fig. 7). These parameter data were used to solve the mathematical model presented in Eq. (2), Eq. (4) and Eq. (6) using MATLAB software and to generate calculation data based on model simulations. In addition, the kinetic constants obtained from the mathematical model were evaluated quantitatively.

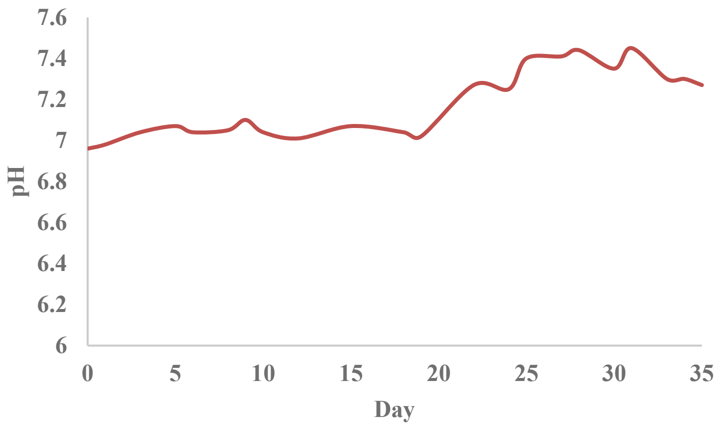

Fig. 4. pH measurements during anaerobic treatment

Fig. 4 provides bioreactor pH data during the main process. The bioreactor was run with an initial pH of 6.8, which is in the optimum pH range for anaerobic degradation. This stable condition was maintained until day 20. On day 31, the pH gradually increased to 7.5, indicating that the methanogenic process was more dominant than the acidogenic process.

Fig. 5 shows the measured microorganism concentrations obtained from research data (MLVSSdata) and also the predicted concentrations from mathematical calculations (MLVSScal). It can be seen that the concentration of microorganisms swiftly rose from 1,067 mg/L to 2,649 mg/L. This shows that the addition of water hyacinth to the mixed substrate played a positive role in supplying additional nutrients needed by microorganisms in the anaerobic digestion process, especially in processing waste with low C/N content. This is reinforced by the growth rate constant (μ_m) obtained, which was equal to 0.65/day, as shown in Table 2. This growth rate constant was much higher and comparable to the growth rate constant for several anaerobic processes in general, namely 0.17 – 0.25/day [17] and 0.21 – 1.54/day [18].

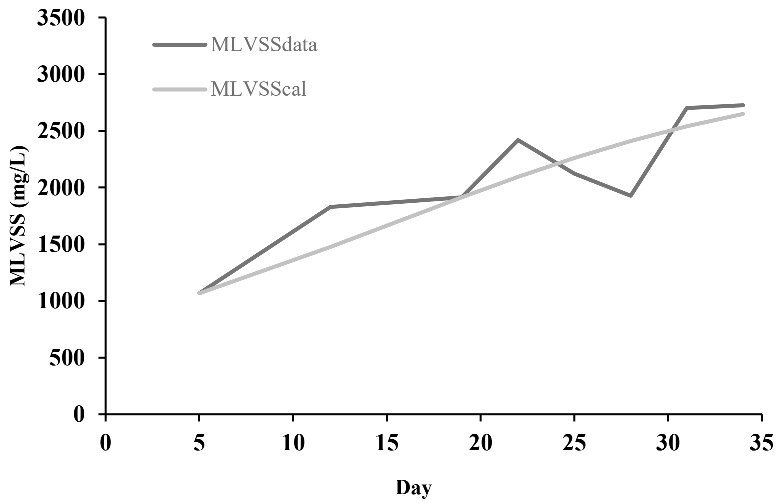

Fig. 5. Comparison of data and calculation of MLVSS concentrations

Table 2. Kinetic constants of anaerobic bioreactor

Constants	Value	Unit
μ_m	0.65	1/day
K_{SX}	9.62	mg COD/mg cells
$Y_{X/CCOD}$	0.64	mg cells/mg COD
$Y_{CCH4/X}$	0.62	mg CH_4/mg cells

An increase in MLVSS concentration was followed by a significant decrease in pollutant content in wastewater (Fig. 6). By maintaining the substrate concentration at 6,000 mg/L, anaerobic digestion with the addition of water hyacinth was able to reduce the COD concentration by 92.82% on day 35 with a final concentration of 431 mg/L. The kinetic constant associated with this decrease is $Y_{X/CCOD}$ which is equivalent to 0.64 mg cells/mg COD (Table 2).

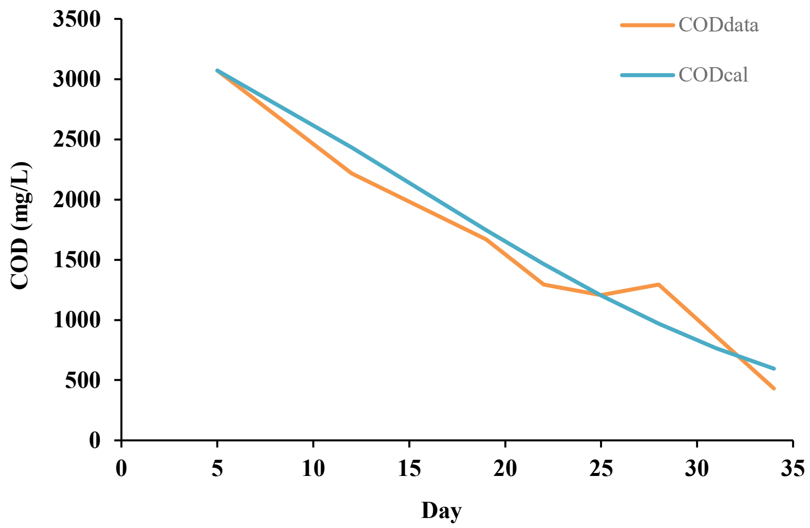

Fig. 6. Comparison of data and calculation of COD concentrations

This constant ($Y_{X/CCOD}$) represents the percentage of COD consumed for cell formation. Based on Eq. (4), a decrease in $Y_{X/CCOD}$ resulted in a higher percentage reduction in COD. A study on the anaerobic process to treat wastewater with low C/N (Piyungan sanitary landfill leachate) was completed by [10] and resulted in a COD reduction of 62% with $Y_{X/CCOD}$ of 0.783-0.91 mg cells/mg COD. The total COD removal resulting from this research is higher than the result obtained by [10]. This shows that water hyacinth augmentation has contributed to providing a favorable atmosphere for the growth of microorganisms by providing the essential nutrients needed. Conducive anaerobic conditions were also supported by the pH of the bioreactor, which can be maintained in the range of 6.8-7.5. These factors have an impact on the high conversion of COD into fatty acids resulting from the acidogenic process, compared to the use of organic materials for the survival of microorganisms. Therefore, an aerobic system can be one of the advanced processing recommendations for completing anaerobic processing when the main concern is to meet waste standards before being discharged into the environment [1].

A comparison of the methane gas formed based on research data and prediction data is shown in Fig. 7. This figure presents the methane gas formed as the main product of the methanogenic process. The model predicts a constant increase in methane formation. These results are in accordance with experimental data up to day 27 of the experiment. Methane rose rapidly to 1,431 mL/g COD on day 30.

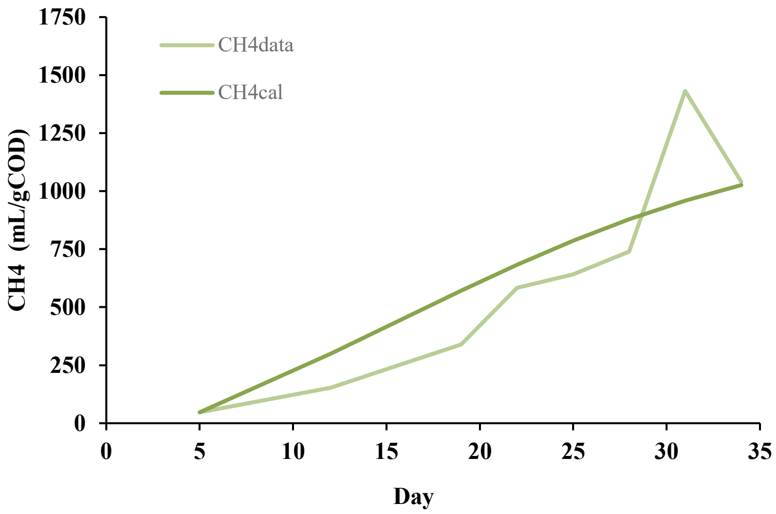

Fig. 7. Comparison of data and calculations of methane formation

The kinetic constant associated with this formation is $Y_{CCH4/X}$ (mg CH_4/mg cell), which is 0.62 mg CH4/mg cells, as illustrated in Table 2. This constant controls the amount of CH_4 produced in the bioreactor [10]. From Eq. (6), it can be concluded that increasing $Y_{CCH4/X}$ has an impact on increasing the percentage of methane formation. The highest methane concentration achieved was 56.9% on day 21. The $Y_{CCH4/X}$ resulting from this research was much higher than the $Y_{CCH4/X}$ for several anaerobic processes with the carbon-poor wastewater, namely 0.14 mg CH_4/mg cells [17], 0.27 mg CH_4/mg cells [19] and 0.35 mg CH_4/mg cells [20]. The appropriate ratio of water hyacinth as a high carbon source and tofu wastewater, which is rich in nitrogen, is recommended in subsequent research to obtain an optimal carbon-to-nitrogen ratio, resulting in a higher percentage of methane formation.

4. CONCLUSION

The use of water hyacinth as an additional substrate has great potential in improving bioreactor performance through qualitative evaluation based on research data with a COD reduction percentage of 92.8% and a methane concentration percentage of 56.9%. These results are supported by the kinetic constants resulting from this research which show better performance compared to previous similar anaerobic digestion using low C/N wastewater, namely microbial growth rate (μ_m), substrate degradation ($Y_{X/CCOD}$) and CH_4 production ($Y_{CCH4/X}$) with respective values of 0.65/day, 0.64 mg cells/mg COD and 0.62 mg CH_4/mg cells. These results complete the evaluation quantitatively.

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