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ORIGINAL PAPER

Simulation and Experimental Study on Iron Impregnated Microbial Immobilization in Zeolite for Production of Biogas

Chandra Wahyu Purnomo[1] · Melly Mellyanawaty[2] · Wiratni Budhijanto[1]

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Abstract Anaerobic digestion is a simple decomposition process of organic matter by microorganisms while producing biogas in a low (absence) of oxygen environment. Anaerobic microorganisms are slow in growth and metabolism which require large volume of reactors in an industrial scale. Immobilization of anaerobic microbia on solid media such as zeolites can increase and maintain its population in the reactor and hence may speed up the decomposition process. The addition of essential micronutrients such as Fe^{2+} into the zeolite as microbial support may further increase the affinity of microbial film to attach and grow on the zeolite surface. This study aimed to evaluate the effect of Fe-loading into zeolite packing on an anaerobic digestion system for biogas production using stillage as the substrate. During experiment, 0.033 mg Fe^{2+} was loaded into each gram of zeolite rings by wet impregnation method. Then, the rings were put inside batch anaerobic reactors filled with stillage in various organic contents represented by soluble Chemical Oxygen Demand (sCOD). The observation of sCOD reduction, Volatile Fatty Acid (VFA) concentration, and biogas production were conducted for 28 days of batch mode anaerobic processes. It is shown that the Fe-loaded support has significant effects on enhancing the organic digestion process in the reactors with high concentration of stillage. On the other hand for lower concentration reactors, the iron impregnated media have similar effect on the digestion process with the media without impregntion. A mathematical model was developed for simulation of the methane generation from sCOD with VFA as the intermediate product. The simulation supports the evidence that the presence of Fe^{2+} in the immobilization media had a noticeable impact on accelerating volatile fatty acids conversion into methane and preventing acidic condition in the reactors.

Keywords Anaerobic digestion · Stillage · Immobilized media

✉ Chandra Wahyu Purnomo
chandra.purnomo@ugm.ac.id

1 Chemical Engineering Department, Engineering Faculty, Universitas Gadjah Mada, Yogyakarta, Indonesia

2 Environmental Engineering Departement, Faculty of Engineering, Universitas Muhammadiyah Tasikmalaya, Tasikmalaya, Indonesia

Introduction

Many industries produce wastewater with high organic content that commonly measured by Chemical Oxygen Demand (COD) such as palm oil, sugar and bioethanol industries. This kind of wastewater, especially with COD above 50,000 mg/l can be a source for renewable energy generation if being treated with anaerobic digestion (AD) system [1]. Stillage is a wastewater from bioethenol industry which has high COD content in the range of 60,000–120,000 mg/l [2–4]. As reported by Beltran et al. [5], stillage is a potential feedstock for anaerobic treatment even though it contains inhibitory compounds of phenolic substances that can reduce the anaerobic microorganism activity. In the case of stillage from molasses, it contains nitrogen compounds and protein from glucose hydrolysis by yeast during ethanol production, thus, the C/N ratio in the stillage is relatively low [6]. The other challenging characteristic of stillage are viscous, odorous, brown color, high organic content, and sulfate content [7]. The sulfate content can enhance the activity of Sulfate Reduction Bacteria

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(SRB) which are the major competitor of the methanogen bacteria.

Anaerobic digestion is a simple wastewater treatment method facilitated by anaerobic microorganism in an enclosed air tight reactor. However, it has some drawbacks such as a long processing time which requires large reactor volume and a possibility of the microorganism washout due to a high substrate loading rate reducing reactor performance [5]. These drawbacks can be minimized by incorporating porous solid materials inside the AD reactor as a support for anaerobic microorganism which provides an ideal environment for enhancing bacterial growth [8]. The solid media addition can increase the reactor ability in reducing COD content of wastewater while preventing the biomass loss in a high loading rate. In terms of equipment investment, the increase of reactor efficiency can reduce the reactor volume demand for a specific treatment duty.

Immobilized bacteria has been studied by Shuler and Kargi [9] who reported a higher cell concentration inside the reactor that improve the efficiency while reduce the washout problem in a high substrate flowrate. Cell immobilization can provide an ideal environment for improving the bacterial performance in digesting the organic waste was also concluded by Borja et al. [10] from the study of zeolite addition into AD reactor which showed 59% increase of specific bacterial growth and about 20% increase in methane yield.

Zeolite is a common material to be used for the immobilization media. The physical properties of this material i.e. porosity and the cation exchange capability, are beneficial for reducing inhibitory substances in the feedstock such as ammonia [11]. The porosity also creates a large surface area for microbial attachment and growth. The bacterial film attached on the solid surface is less prone to washout compared with the suspended microorganism in the feedstock solution.

In this study, natural zeolite packing impregnated with Fe^{2+} was used as immobilization supprot of anaerobic treatment process. It is expected that the immobilized microbial consortium can digest the organic content effectively. The Fe^{2+} impregnation process was done by ion exchange mechanism to replace naturaly existed cation on the zeolite surface with iron (II) cation. Fe^{2+} has proved to be one of the essential micronutrients for bacterial growth and an agent for improving the activity of methanogenic bacteria in biogas production [12]. Then, the modified natural zeolit supports were incorporated into batch anaerobic reactors with stillage as the organic source.

Several studies related to metal addition directly into the various feedstock solution or impregnated into solid immobilization media (zeolite) have been done quite intensively to seek an optimum biogas production by AD treatment. Espinosa et al. [13] added trace element of Fe, Ni, Co and Mo into stillage from molasses and claimed as a successful treatment in reducing some organic acids while increasing the biogas production. Another report demonstrated reactor improvement ability in reducing Volatile Fatty Acids (VFA) and COD by using domestic and food industry wastewater with trace element addition (Co, Ni and Fe) [14]. Other attempt has been documented by Milan et al. [15] by addition of Mo, Co and Ni into zeolite powder as immobilized media of AD which was able to increase the methane production from pig manure from two to up to eight times. Using thin stillage, Moestedt et al. [16] added Fe and Co into the feedstock and observed the reduction in ammonia and sulfide compound inhibition to AD process. Many other studies have also reported the positive effects of trace element especially Ni, Co, Fe and Zn to anaerobic microorganism that lead into an increase of methane concentration and biogas production [17–23].

Many factors must be considered to evaluate the effect of the addition of Fe^{2+} in the zeolite as a microbial immobilization media in anaerobic decomposition process. It is because the decomposition process is a complex biosystem involving a variety of living microorganisms and interaction among each other in a strong connection called syntrophy. Syntrophic communities cannot be separated as monocultures to be studied separately. Moreover, it is not easy also to determine all the organic compounds contained in the substrate in detail. The addition of immobilization media in this study will add to the complexity that arise in the system, as it creates new micro-environment that is different from the fluid condition. Therefore, to objectively evaluate and quantify the results, a mathematical model was developed to measure the kinetic parameters of AD processes such as the specific growth rate (μ_g), kinetic constant of the substrate consumption and several reaction yields as lump parameters.

Experimental

Materials

In this experiment, diluted and undiluted stillage from a bio-ethanol industry was used as the feedstock substrate after mixing with inoculum solution. The sCOD content variation in this study were 20,000, 50,000 and 150,000 ppm. The highest COD content was stillage without dilution. Stillage was obtained from PT. Energi Agro Nusantara (Enero), Mojokerto, East Java, Indonesia. The inoculum was collected from the effluent of cow manure conventional bio-digester from Center of Agricultural Technology Innovation (PIAT-UGM). The stillage was mixed with inoculum solution in a certain ratio before

Table 1 Stillage and inoculum from active digester effluent characteristic

Properties	Stillage	Inoculum
sCOD (mg/l)	100,000–150,000	5000
pH	5	7.8
VFA (mg acetic acid/l)	57.5	200
Phenol (mg/l)	26	–

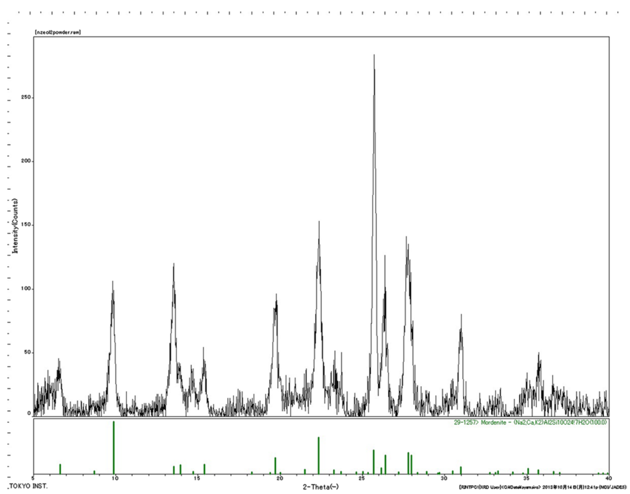


Fig. 1 Powder diffractogram of natural zeolite powder

anaerobic treatment. The characteristic of the stillage and the digester effluent is provided in Table 1.

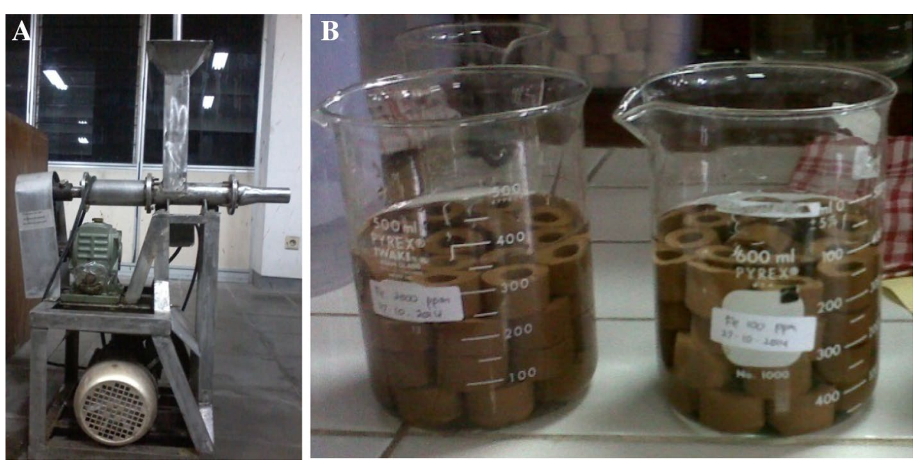
Meanwhile, natural zeolite was collected from Klaten deposit in Central Java. The zeolite mostly contains mordenite with a small fraction of clinoptillolite crystal structure which was confirmed by XRD graph (Rigaku, Multiflex) as shown in Fig. 1. The zeolite powder was mixed with commercial bentonite clay in an equal weight and water for making a pliable dough. After kneading, the dough was extruded and cut into a ring shape with outer diameter of 3.5 cm, length of 4 and 1.2 cm in wall thickness. The rings then dried inside an oven for 24 h and calcined for 2 h at 700 °C. Some of the calcined zeolite rings were immersed into $FeCl_2$ solution (200 mg/l) for 24 h, and then washed, dried and stored in a closed container. The Fe^{2+} concentration inside the solid was determined by mass balance from the iron concentration reduction of the solution before and after impregnation. The calculated iron concentration inside the solid was 0.033 mg Fe^{2+}/g solid media. Iron concentration determination was conducted using ICP (Perkin Elmer Optima 8300).

Most of the previous reports in the microbial support were using zeolite in powder form. In this study, the zeolite was shaped into rings in order to prepare the media to be used in fixed bed reactor or anaerobic filter. The performance of packed bed was tested in batch anaerobic reactors before being used in the continuous one in the near future. The extruder machine and the support media in Fe^{2+} impregnation stage are shown in Fig. 2.

Anaerobic Digestion

The 1000 ml Erlenmeyer flasks were used as AD reactors with silicone rubber cup. The cup was equipped with holes for biogas out flow and feedstock sampling port. The substrate concentration in terms of sCOD was varied from 20,000 to 150,000 mg/l by dilution. Before anaerobic treatment, the substrate was mixed with the inoculum with the substrate:inoculum volumetric ratio of 2:1. The mixture sCOD became lower than the prior substrate since the inoculum has low COD content. Before starting the digestion process, the sCOD of the feedstock mixture is always measured and recorded. The inoculum to substrate ratio was determined previously by running preliminary experiments for ensuring the minimum demand of inoculum that has to be add to enhance the digestion process without significantly altering the stillage properties due to dilution.

Fig. 2 **a** Extruder machine for shaping the zeolite ring media. **b** The zeolite support media during Fe^{2+} impregnation stage



The reactor then loaded with zeolite rings with or without Fe^{2+} loaded. The weight ratio of liquid:solid inside the reactors was 1:1. This ratio was determined in preliminary experiment by visual inspection of the reactor after being filled with the media rings. Larger number of media will provide higher surface area for microbial immobilization, however, the media has to be submerged completely inside the feedstock solution. In this case, the equal weight ratio of the liquid and solid ensures that the two constraints are met. Meanwhile, several reactors were not added any solid media as the control. For ease of identification, each of AD reactor condition was coded and listed in Table 2.

Simulation Modeling

The governing equations of the model were formulated based on the work of Echiegu and Ghaly [24] with some modifications. The assumption used for all of the microbia in the process of AD was considered as pseudo-monoculture. The two types of dominant microorganism in the primary stages of AD process i.e. acidogenic (*X1*) and methanogenic bacteria (*X2*) were used as the basis for this modeling. The pseudo-mono substrate assumptions for the complex substrate was represented by the parameters of sCOD and VFA. Organic compounds in the substrate was converted into biogas with CH_4 composition at 50–70% by volume while the rest of gases were neglected for simplification, so that the complex AD process can be expressed by Eq. (1).

$$\mathrm{sCOD}\underset{\longrightarrow}{Acidogen\ (X1)}\mathrm{VFA}\underset{\longrightarrow}{Methanogen\ (X2)}\mathrm{CH_4} + \mathrm{CO_2} \tag{1}$$

The rate of substrate consumption $\left(-\frac{d[sCOD]}{dt}\right)$ by acidogenesis is the sum of substrate consumption for acidogenic bacteria growth and substrate consumption to produce VFA as shown by Eq. (1). Therefore, the rate of total sCOD consumption by acidogenic bacteria can be expressed by Eq. (2).

$$-\frac{d[SCOD]}{dt} = \left(\frac{1}{Y'_{X_1/SCOD}}\right)\frac{dX_1}{dt} \tag{2}$$

Equation (2) is a simplification to correlate the rate of overall substrate (sCOD) consumption with the rate of acidogenic bacterial growth. The analogous assumptions were taken in the development of models for methanogenic bacteria, in which the rate of methane formation $\left(\frac{d[CH_4]}{dt}\right)$ by methanogenic bacteria linearly correlated with the rate of methanogenic bacteria growth $\left(\frac{dX_2}{dt}\right)$ as expressed in Eq. (3).

$$\frac{d[CH_4]}{dt} = Y_{CH_4/X_2}\frac{dX_2}{dt} \tag{3}$$

It is also necessary to investigate the accumulated rate of the intermediate compound (VFA). This rate is important to be investigated, because VFA accumulation can be a tool for determining the performance of the AD reactor. VFA accumulation above 10,000 mg/l is generally seen as an indication of self-inhibition in an anaerobic process. The overall accumulation rate of VFA $\left(\frac{d[VFA]}{dt}\right)$ is obtained from the subtraction of the formation rate of VFA by acidogenic bacteria by the rate of VFA consumption by methanogenic bacteria, that can be written as in Eq. (4).

$$\frac{d[VFA]}{dt} = Y'_{VFA/X_1}\frac{dX_1}{dt} - \frac{1}{Y'_{X_2/VFA}}\frac{dX_2}{dt} \tag{4}$$

By using Contois [9] equation, the value of $\frac{dX_1}{dt}$ and $\frac{dX_2}{dt}$ can be evaluated by Eqs. (5) and (6).

$$\frac{dX_1}{dt} = \frac{\mu_{m_1} sCOD}{K_{SX_1}X_1 + sCOD}X_1 \tag{5}$$

$$\frac{dX_2}{dt} = \frac{\mu_{m_2} VFA}{K_{SX_2}X_2 + VFA}X_2 \tag{6}$$

The parameter of μ_m represents the maximum specific microbial growth rate, and K_{SX} is saturation constant. Then, Eqs. (1–6) will be used simultaneously for fitting the model with experimental data i.e. sCOD level, VFA concentration and CH_4 production. The method of solving the equations was using simultaneous differential equation solver in Matlab coupled with R-squared analysis for coefficient determination. The parameters values were firstly guessed to be able to calculate sCOD, VFA and CH_4 concentration for each time increment and then the calculation data were compared with the real

Table 2 AD Reactor identification

ID reactor	Substrate initial sCOD (mg/l)	Fe^{2+} loaded	Solid media added
20Z	23,250 ± 1760	No	Yes
20Z-Fe	23,250 ± 1770	Yes	Yes
20TM	23,250 ± 1770	No	No
50Z	39,430 ± 1960	No	Yes
50Z-Fe	39,430 ± 1970	Yes	Yes
50TM	39,430 ± 1950	No	No
150Z	145,050 ± 4120	No	Yes
150Z-Fe	145,050 ± 4110	Yes	Yes
150TM	145,050 ± 4100	No	No

measured data. The difference (errors) were minimized by changing the parameter values until achieving the highest possible R^2.

Results and Discussion

In the batch anaerobic reactors, the reduction of sCOD over time has been observed (Fig. 3). It is shown that in low concentration of sCOD, the Fe^{2+} loaded media has no difference with non-impregnated ones in terms of sCOD reduction profile. The reduction of sCOD in reactor with the lower and medium initial concentration (20Z and 50Z) using neither impregnated nor non-impregnated support have not shown any significant differences. Figure 3a, b show that the reduction of sCOD occur rapidly during sCOD concentrations below 20,000 mg/l. In Fig. 3a, it appears that initial sCOD concentration of approximately 14,000 mg/l decreased dramatically over 50% in the first week. Similarly in the reactor with medium sCOD concentration (50Z and 50Z-Fe), the sCOD concentration decline rapidly in the second week when sCOD in the reactor has reached below 20,000 mg/l. Meanwhile, for the highest initial sCOD concentration reactor i.e. 150TM, 150Z and 150Z-Fe, the sCOD reduction profiles were varied, even though the general pattern suggests that the decrease of sCOD is quite slow (Fig. 3c). Until the 4th week all reactors could only remove sCOD to about one-third or even less than the initial concentration. The improvement of sCOD removal in the reactor with zeolites media was clearly observed. Nevertheless, the zeolite impregnation by iron (II) did not show significant improvement compared with non-loaded one in term of sCOD reduction.

These COD reduction results in different initial concentration are relevant to the previous research [25]. This study reported that the COD degradation ability in an AD reactor increased from initial COD of 15,000–25,000 mg/l, and decreased from 25,000 to 30,000 mg/l. The reactor with the initial COD concentration of 25,000 mg/l gave the best performance for the COD degradation of the stillage of 33.6%. Based on the review from Satyawali and Balakrishnan [26], one of the reasons of the slow reduction rate in a high concentrated substrate is due to the long adaptation time which can be reduced by the addition of micronutrient such as iron, boron and molybdenum. The reduction of adaptation time in this study can be observed on the highest concentration reactor (Fig. 3c) which has a significant improvement of COD reduction by iron loading media after 2 weeks of treatment time.

From the VFA measurement during the anaerobic digestion, in Fig. 4., it is shown that the reactors with media (Z and Z-Fe) have more stable VFA concentration throughout the process compared with fluctuating VFA concentration in the reactor without support media (TM). The fluctuation of VFA concentration could reach an excessive level which can be a trigger for self-inhibition mechanism due to increasing acidity. Acidic environment may decrease the microbial population and create instability of reactor performance. In general, VFA over 10,000 mg/l will hamper the methane production.

The addition of Fe impregnated zeolite and non-impregnated media did not exhibit any significant difference unless for the highest initial sCOD reactor. The iron impregnated media reactor (150Z-Fe) has the lowest VFA content almost in all the observation time as shown in Fig. 4c. Addition of iron has also been reported previously

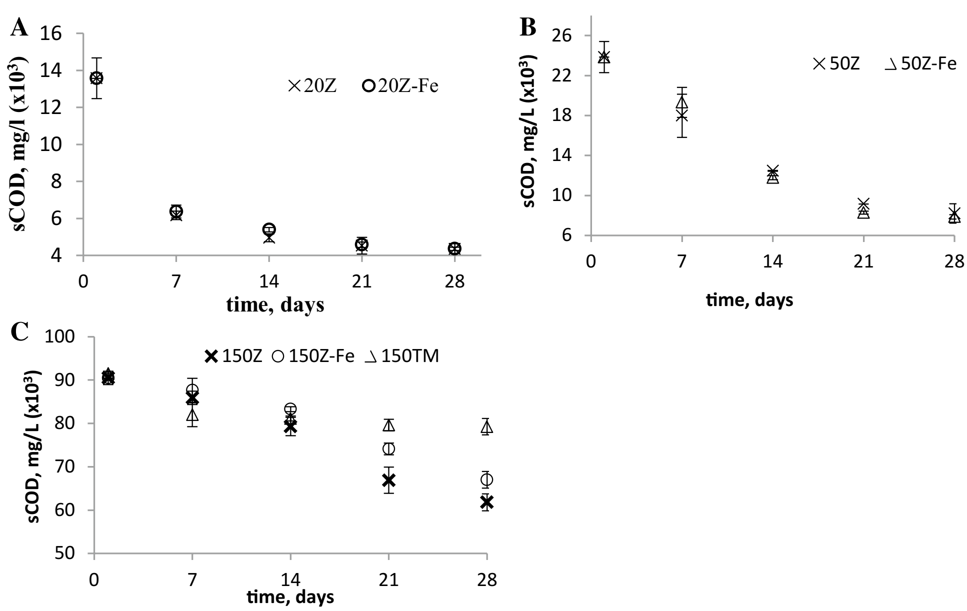


Fig. 3 sCOD reduction profile with different initial concentration of stillage: **a** 20,000 mg/l; **b** 50,000 mg/l; **c** 150,000 mg/l

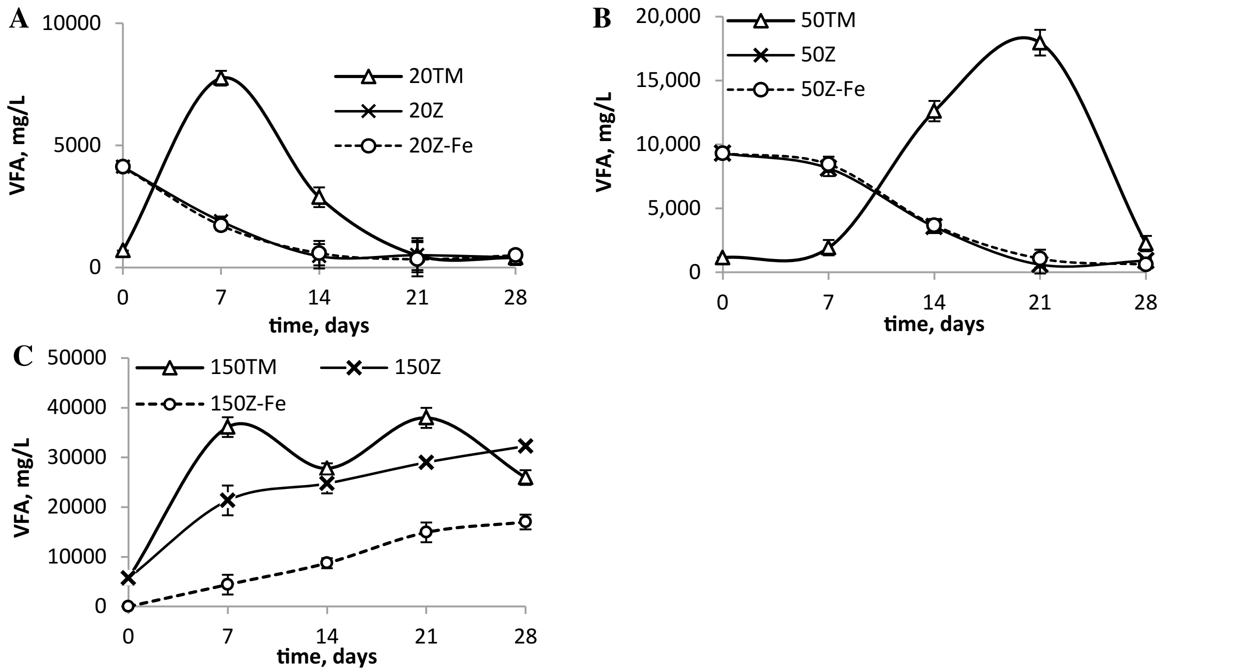


Fig. 4 VFA reduction profile of reactor with immobilized media (Z and Z-Fe) and without media (TM) in different sCOD initial concentration

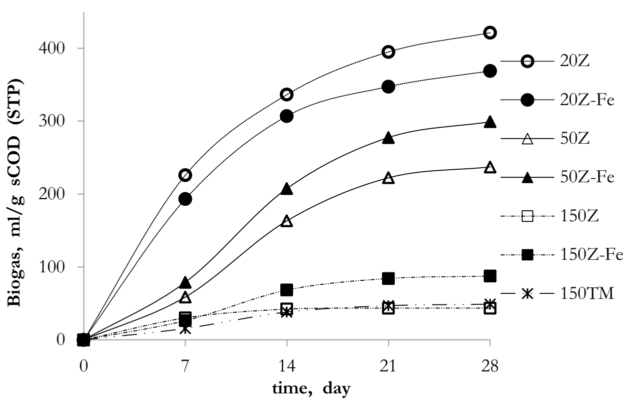


Fig. 5 Accumulated Biogas Production over sCOD content of each reactor type

Table 3 Phenolic compound reduction

Reactor ID	Phenol (%)		Reduction (%)
	Initial	Final	
20Z	0.0976	0.0336	65.57
20Z-Fe	0.0976	0.0422	56.76
50Z	0.1952	0.0855	56.20
50Z-Fe	0.1952	0.0819	58.09
150Z	0.4860	0.3170	34.77
150Z-Fe	0.4860	0.1421	70.76

to enhance the capacity for degradation of VFA in biogas processes [14].

Figure 5 shows the cumulative volume of biogas and methane over initial sCOD in each reactor. For the reactors with low sCOD the presence of Fe^{2+} in the zeolite rings did not give any improvement for methane generation. Different case for reactor with medium initial sCOD (50Z and 50Z-Fe), reactor with impregnated zeolite media produced higher total volume of biogas than 50Z. The addition of Fe^{2+} loaded zeolite media at medium sCOD concentrated reactor can increase methane production by 24.9%. The improvement of cumulative biogas production at the highest reactor concentration by Fe^{2+} loaded media (150Z-Fe) is also observable. The reactor with unmodified zeolite rings has comparable biogas production with reactor without support media 150^{TM}. These results are in agreement with the report from Espinosa [27] that described about the addition of iron for increasing the activity and growth of the hydrogen oxidizing methanogens and the bacteria converting acetic acid to methane.

By observing the biogas production data, the Fe-loaded media has a significant effect in higher substrate concentration reactor. In a higher substrate concentration, the possibility of inhibitor substance accumulation inside a reactor is much higher. The results suggest that the main contribution of iron loading media is stabilizing the reactor in term of controlling the inhibition processes. As shown in Table 3, the loaded Fe media has significant role in reducing phenol in high substrate concentrated reactors. It is known that phenol is an unwanted compound for anaerobic digestion system. Phenol in a high concentration may inhibit methanogenic bacteria in producing methane and degrading carbonaceous substrate [28]. The analysis of phenol was performed only at the beginning and the end of the digestion process.

Table 4 Simulation results of parameter and R-squared values in several reactors

Parameter	20Z	20ZFe	50Z	50ZFe
$\mu_{m1} Y'_{X_1/SCOD}$	21.2083	21.2623	11.9772	16.2967
K_{SX1}	1801.04	1800.04	972.14	1106.94
$Y'_{X_1/SCOD}$	0.0495	0.0461	0.0532	0.2916
μ_{m2}	0.6131	0.4735	0.7056	0.8070
K_{SX2}	22.4038	16.9322	5.9076	9.6837
Y_{CH_4/X_2}	0.9324	0.9247	0.1367	0.3960
$Y'_{VFA/X1}$	15.1232	20.3492	0.0346	0.1313
$Y'_{X2/VFA}$	0.0134	0.0077	0.0099	0.0399
R-squared				
sCOD	0.99	0.97	1.00	0.99
VFA	0.98	0.97	0.99	0.91
CH_4	0.70	0.49	0.85	0.68

As a cross-check of the aforementioned interpretation of the plotted data, the mathematical models' parameters were also evaluated to compare various treatments tested in this study. For the model fitting to determine the constants, only data with high biogas yield were used. The kinetics parameters determined for each treatment are listed in Table 4. While for the curve data and simulation fittings from selected reactors are represented in Fig. 6.

Figure 6 shows the simulation results of sCOD reduction, VFA concentration as well as CH_4 production in the medium COD loading reactors. In general the model can fit the data quite well especially for sCOD and VFA evolution during experiment. Meanwhile for CH_4 production, the model shows a significant deviation especially at the end time period. In the batch system, it is predicted that after several days of high production rate the biogas (CH_4) production will decrease since the organic matter (sCOD) has been mostly consumed. However, the CH_4 production data at the last section still show a significant activity of the methanogenic microbia in producing methane in particular for the reactor with iron loading media (50Z-Fe). It

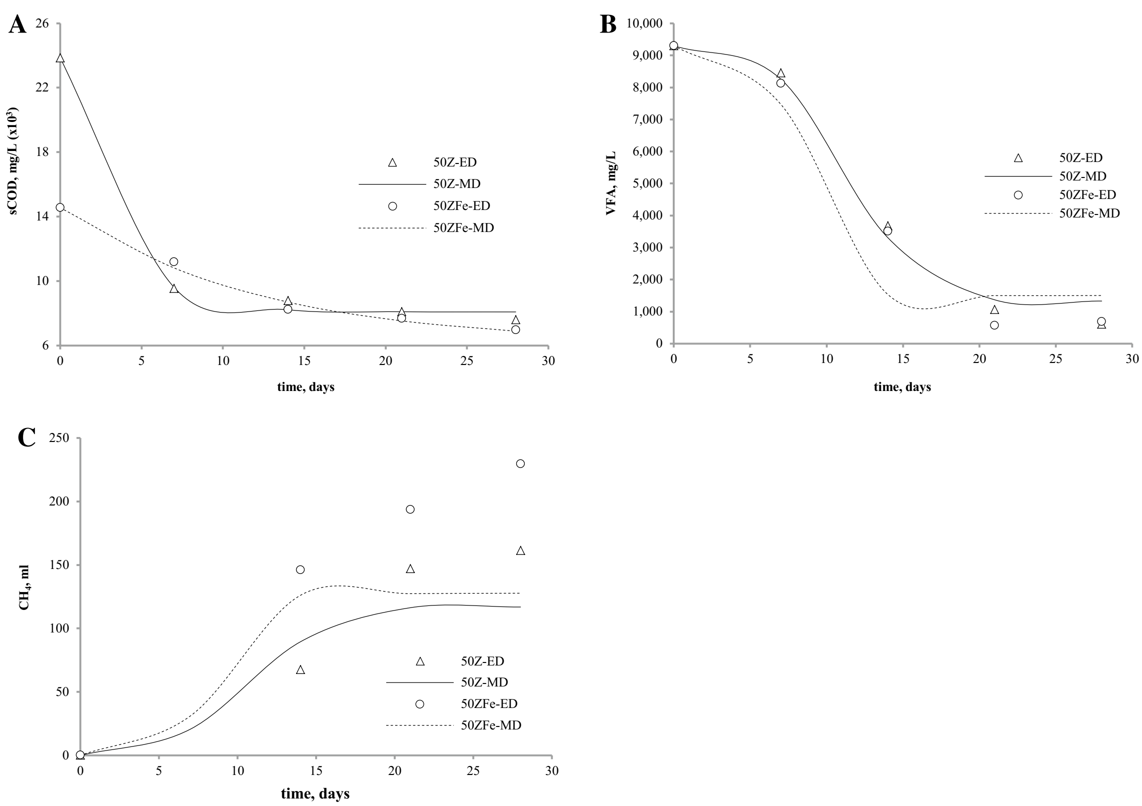


Fig. 6 Experiment data (ED) and simulation (MD) plot for the medium COD reactors (50Z and 50Z-Fe) of sCOD (**a**), VFA (**b**) and CH_4 (**c**) profiles

indicates that the iron addition can maintain the biogas production rate even at the limited substrate availability.

Table 4 provides calculated parameters obtained from mathematical simulation using R squared method. R^2 closer to 1 means better model accuracy with the experimental data. As indicated in Fig. 6 as well, for sCOD and VFA the model errors are minimum, while for CH_4 the R^2 value are quite low especially for reactor with iron loaded media. The model predicts that CH_4 production will be terminated when the substrate sCOD and the intermediate product (VFA) are in a low level. The effect of iron loading is obvious, even though cannot be anticipated by the simple simulation model.

The value of maximum specific bacterial growth rate for acidogenesis (μ_{m1}) and methanogenesis (μ_{m2}) for higher concentrated reactor is much lower. This could be an indicator that higher concentrations of stillage contain larger inhibition substances that reduce the growth rate of bacteria. This also can be a hint of the lower biogas production potential because the microorganisms have to spend most of their effort to survive in unfavorable condition rather than producing the gas. The other parameter in the growth kinetics, which is the saturation constants (K_{SX1} for acidogenic bacteria and K_{SX2} for the methanogenic bacteria), are shown to be affected more by the concentration of the stillage but did not change much with the Fe impregnated zeolite. With respect to yields (assumed to be constant), the value of Y'_{VFA/X_1}, can be used as productivity indicator of acidogenic bacteria. The greater the yield means that the same amount of acidogenic bacteria able to produce more intermediate VFA product while reproduce themselves. Table 3 shows that the yield of VFA increases by using Fe-loaded media. Y_{CH_4/X_2} shows the productivity of methanogenic bacteria in producing biogas. Incorporation of Fe^{2+} in the zeolite media shows an enhancement of the gas production yield especially in high concentration reactor.

Conclusion

The loading of Fe^{2+} into zeolite rings as microbial immobilized media had shown a significant impact on the anaerobic digestion process. The improvement was noticeable in high organic content of wastewater with inhibitor substances such as phenol. The most important role of microbial support were reducing the VFA fluctuation and reducing the inhibitory substance i.e. phenol. On the other hand, at low substrate concentration, the modification of microbial support did not significantly change the reactor performance.

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