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Alkaline Activated Natural Zeolite as a Microbial Immobilization Media in Anaerobic Digestion for Tapioca Wastewater Treatment

Zeolit Alam Teraktivasi Basa sebagai Media Imobilisasi Mikrobial di Peruraian Anaerobik untuk Pengolahan Limbah Cair Tapioka

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ABSTRAK

Produksi biogas dari limbah cair singkong menawarkan sumber energi yang berkelanjutan, meskipun efisiensinya masih menjadi tantangan. Penggunaan zeolit alami sebagai media untuk mengimobilisasi bakteri anaerob menunjukkan potensi, namun efisiensi proses ini dapat lebih ditingkatkan dengan aktivasi zeolit. Namun, pengetahuan tentang bagaimana zeolit yang diaktifkan secara basa (NaOH) mempengaruhi aktivitas mikroba dan hasil biogas masih terbatas. Dalam penelitian ini, kami menunjukkan bahwa zeolit alam Lampung yang diaktifkan secara basa signifikan meningkatkan produksi biogas dengan menyediakan media imobilisasi yang lebih efektif bagi bakteri anaerob. Analisis Fourier transform infrared spectroscopy (FTIR) mengungkapkan perubahan struktural, dengan pergeseran gelombang Si-O dan Al-O, yang menunjukkan interaksi kimia yang lebih baik setelah aktivasi. Scanning electron microscope-energy dispersive x-ray spectroscopy (SEM-EDX) mengonfirmasi peningkatan kandungan Al dan Na; Al meningkat secara signifikan dari 5,62% menjadi 7,48%, yang menunjukkan peningkatan kandungan Al dalam struktur zeolit setelah aktivasi. Sementara itu, analisis x-ray diffraction (XRD) mengungkapkan kristalinitas 21% pada zeolit yang diaktifasi, yang menunjukkan permukaan mendukung untuk perlekatan mikroba. Analisis Brunauer-Emmett-Teller (BET) menunjukkan struktur mesopori (34,335 Å) yang meningkatkan kontak bakteri dengan substrat. Reaktor dengan zeolit yang diaktifkan mencapai puncak produksi biogas sebesar 620 mL, melebihi kontrol karena peningkatan stabilitas mikroba dan pengurangan akumulasi asam lemak volatil (VFA). Hasil ini menunjukkan bahwa zeolit yang diaktivasi secara basa dapat secara signifikan meningkatkan efisiensi sistem produksi biogas dengan meningkatkan imobilisasi mikroba dan stabilitas proses.

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ABSTRACT

Biogas production from cassava wastewater offers a sustainable energy source, though efficiency remains challenging. Natural zeolite is a promising material for anaerobic bacteria immobilization media. However, there is limited knowledge of how alkaline-activated (NaOH) zeolite affects microbial activity and biogas yield. Here, we show that alkaline-activated Lampung natural zeolite significantly enhances biogas production by providing a more effective immobilization medium for anaerobic bacteria. Fourier transform infrared spectroscopy (FTIR) analysis reveals structural changes, with a shift in Si-O and Al-O vibrations, indicating improved chemical interaction post-activation. Scanning electron microscope-energy dispersive x-ray spectroscopy (SEM-EDX) confirms increased Al and Na content; the Al increased significantly from 5.62% to 7.48%, which may indicate an increase in Al content within the zeolite structure after activation. Meanwhile, the x-ray diffraction (XRD) analysis revealed a 21% crystallinity in the activated zeolite, suggesting a favorable surface for microbial attachment. Brunauer-Emmett-Teller (BET) analysis shows a mesoporous structure (34.335 Å) that improves bacterial contact with the substrate. The reactor with activated zeolite achieved peak biogas production of 620 mL, outperforming the control due to enhanced microbial stability and reduced volatile fatty acid (VFA) accumulation. These results sugest that alkaline-activated zeolite can significantly boost the efficiency of biogas production systems by improving microbial immobilization and process stability.

1. INTRODUCTION

Nigeria is the world's greatest producer of cassava, followed by Congo, Thailand, and Indonesia (Widowati et al., 2025). Lampung, Indonesia, a key food production region, holds significant potential with several prime commodities. Cassava (Manihot Esculenta Crantz) was the top national producer in 2023, with a total output of 6,719,088 tons. (Dinas Kominfotik Provinsi Lampung, 2023). Cassava is a commodity with high economic potential, thus encouraging the development of the tapioca industry. The sector of cassava processing is expanding quickly (Mujiyo et al., 2022). Cassava is used as the primary food ingredient and raw industrial material in future industrial advances; farmers and food producers may maximize the potential of cassava plants and create a wide range of high-quality food items by using cassava to make tapioca. However, due to this maximized tapioca production, the tapioca industry generates tons of waste.

The cassava starch industry generates solid and liquid waste throughout production, including the washing, extraction, settling, and drying stages (Adnan et al., 2020). Cassava starch waste (CSW) is mainly produced from washing and extraction processes (Prasertsung et al., 2019). Liquid waste from the cassava starch separation process contains chemical oxygen demand (COD) in the range of 7000-30,000 mg/L, total solids (TS) 4500-7645 mg/L, volatile solids (VS) 1834-3400 mg/L, and pH around 4.26 to 7.2 (Adnan et al., 2020; Ekop et al., 2019; Rajivgandhi et al., 2013; Setyawaty et al., 2011). If left untreated, liquid waste can become a severe problem because it is a significant environmental problem in several industries.

Due to its high organic matter content, tapioca starch wastewater is a valuable resource that, if properly managed, can be converted into biogas as a renewable energy source. The processing of tapioca starch wastewater into biogas is expected to produce new renewable energy sources and reduce environmental damage and greenhouse gas emissions that cause global warming. This potential utilization of tapioca starch wastewater for biogas production contributes to the attainment of the goal of achieving a renewable energy composition of 23% in 2025 (PERTAMINA, 2020), and minimizes dependence on conventional energy sources (fossil fuels) that harm the environment. Anaerobic digestion (AD) is an up-and-coming renewable energy technique as it can efficiently transform organic waste into biogas with a high methane content (Pramanik et al., 2019). Anaerobic digestion is a complex metabolic process consisting of four main biochemical phases: hydrolysis, acidogenesis, acetogenesis, and methanogenesis (Sanjaya et al., 2023). It offers several advantages. First, it eliminates the need for energy-intensive aeration.

The limitations of anaerobic processes include the possibility of microbial washout, which can result in a total biological breakdown. Washout can occur when retention times are too short, resulting in incomplete degradation and ultimately reduced biogas production (Bachmann, 2015). The addition of immobilization media was intended to facilitate the anaerobic process, particularly by preventing washout phenomena and promoting methane production (Ramadhani et al., 2020). Furthermore, this process also brings microbiological cells to the surface of porous media, where they adhere and develop into colonies. This condition will increase the surface area of contact between microbial cells and wastewater, enabling a greater degree of pollutant degradation (Handayani et al., 2018). Moreover, Kuo & Shu (2004) found that immobilized cells offer a large specific surface area for microbial proliferation and support bacteria exposed to sulfide toxicity (Suzana et al., 2013).

Various immobilization media that can be used in the anaerobic digester are charcoal, metallic components, carbon-based compounds, and zeolite (Liu et al., 2021). Natural zeolite has been widely used as a medium of immobilization in anaerobic decomposition (Montalvo et al., 2012). According to the research conducted by Silvio Montalvo and Simon Poirier, including zeolites has been demonstrated to improve anaerobic digestion, particularly by improving or maintaining the growth of microbial communities. These findings emphasize the possible application of support materials to enhance process stability in anaerobic reactors (Poirier et al., 2017) and decrease the quantity of biomass released from the reactor (Montalvo et al., 2014). Nevertheless, natural zeolites often contain impurities like iron, zinc, feldspar, and amorphous glass, which diminish the valuable content of the mineral and limit its potential applications. To address these issues, single or combined modification techniques, including thermal, chemical, surfactant, and other treatments, can be employed to remove impurities, enhance structural properties, and improve cation affinity, thereby expanding the use of natural zeolites in several applications (de Souza et al., 2018).

This research explores the potential of cassava wastewater as a raw material for biogas production and the use of activated natural zeolite as an immobilization medium. The study investigates the effect of varying hydraulic retention time (HRT) to determine the optimal retention time for maximizing biogas production. Before its use as an immobilization medium, natural zeolite is activated to enhance its properties and remove contaminants. Chemical activation with NaOH is employed, as previous studies have shown that alkali-activated zeolite improves biogas production, particularly by increasing methane production rates. This approach presents a novel advancement over prior methods by optimizing the biogas production process and using zeolite as an immobilization medium in a single integrated system.

2. METHODOLOGY

2.1 Substrate and Inoculum

The inoculum used is mesophilic sludge taken from the reactor at PT. Bumi Waras in Central Lampung, Lampung. It will be acclimatized for two weeks, and substrate feeding will be given. The reactor will be fed with tapioca wastewater from PT. Bumi Waras (PT. BW) in Central Lampung, Lampung. The characteristics data of tapioca wastewater from PT. BW is attached in Table 1.

Table 1. The characteristics of the substrate and inoculum

Parameters	Value
a) Inoculum	
Total solid (TS) (mg/L)	26.920
Volatile solid (VS) (mg/L)	27.450
b) Substrate	
pH	4.090
COD (mg/L)	10950
sCOD (mg/L)	1170
Total solid (TS) (mg/L)	6580
Volatile solid (VS) (mg/L)	8767
Water	99.46
Ash	0.074
Protein	0.198
Lipid	0
Carbohydrate	0.269

2.2 Immobilization Medium and Activation

Natural zeolite must be activated before being applied as an immobilization medium to enhance its distinctive characteristics and remove contaminants. This study obtained Lampung natural zeolite called zeolit alam Lampung (ZAL) in Indonesian from CV. MINATAMA, Bandar Lampung, Indonesia. The chemical activation method using NaOH was selected. The process began with standardizing the zeolite size using a 5-mesh sieve. Subsequently, the zeolite was soaked in a 3M NaOH (Merck, Germany) solution for 24 hours in a beaker glass, ensuring that the container was covered to minimize excessive NaOH evaporation. After soaking, the zeolite was washed with distilled water until a neutral pH (pH ± 7) was achieved. The cleaned zeolite was then dried in an oven at 110°C for 3 hours, followed by calcination in a furnace at 400°C for 3 hours. Finally, the activated zeolite was stored in a desiccator.

2.3 Anaerobic Reactor Assembly

The equipment scheme is shown in Figure 1. The reactors used in this study are 2-litre Erlenmeyer flasks (Iwaki, Indonesia). To maintain anaerobic conditions, the tops of the flasks are sealed with tightly fitting rubber stoppers. The stoppers are pierced and fitted with glass tubes, which serve as pathways for substrate feeding and gas outlets, directing to the gas collection bags. Two reactors were operated under different conditions: The reactor with code RA was supplemented with immobilization media, while the other reactor with code RB was operated without immobilization media. 1.5 liters of inoculum and 130 grams of zeolite were packed into plain weave carbon fibers for the packed bed preparation. Six experimental trials were conducted to assess the impact of varying hydraulic retention times (HRT) on the performance of both reactors. The HRT values tested were 60, 50, 40, 30, 20, and 10 days. Each HRT variation will be evaluated to determine its impact on the anaerobic reactor's performance, particularly in biogas production and organic matter degradation. This study aims to identify the optimal HRT to achieve the highest efficiency in the anaerobic process using zeolite as the immobilization medium.

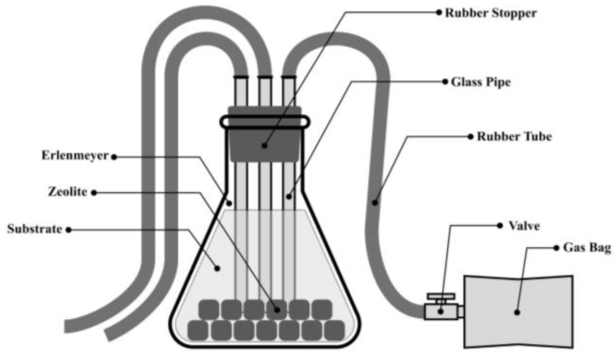

Figure 1. Apparatus of packed-bed anaerobic reactor

2.4 Analytical method

2.4.1 Sample Analysis and Zeolite Characterization

Anaerobic samples were collected thrice a week. The pH was measured using a pH meter (Si Analytics pH meter Lab 845, Germany). The concentration of volatile fatty acids (VFAs) was calculated using the distillation method (APHA 5560C), and the volume of biogas collected every day was measured using a 60 mL syringe (One Med). A gas chromatography device (Shimadzu GC-2014, Japan) was employed to analyze CH_4 concentration. The surface morphology, sizes, and composition of zeolite were studied by scanning electron microscopy with an energy-dispersive X-ray spectrometer (SEM-EDX) (JEOL JSM 6510, Japan). The fourier transform infrared (FTIR) (The Aglient CARY 630, Germany) was conducted to identify the functional groups in the zeolite with wavenumber from 4000-600 cm-1. The Brunauer-Emmett-Teller's (BET) (QUADRASORB-evo, United States) was performed to determine the size of the surface area and the characterization of its pores. The crystal structure of the zeolite was analyzed using X-ray diffraction (XRD) (Bruker D8 Advance, Germany) and validated by comparison with the crystallographic open database (COD).

2.4.2 Biogas Potential

The maximum biogas yield from the derived empirical molecular formula is then calculated using the stoichiometry in Equation (1) (Achinas & Euverink, 2016; Shuler & Kargi, 1992).

$$C_nH_aO_bN_c + \left[n - \frac{a}{4} - \frac{b}{2} + 3\left(\frac{c}{8}\right)\right] H_2O \rightarrow \left[n - \frac{a}{4} - \frac{b}{2} + 3\left(\frac{c}{8}\right)\right] CO_2 + \left[n - \frac{a}{4} - \frac{b}{2} + 3\left(\frac{c}{8}\right)\right] CH_4 + cNH_3 \quad \text{Eq. 1}$$

Based on Equation (1), the maximum potential yield of CH_4 gas from 1 mole of $C_nH_aO_bN_c$ is $\left[n + \frac{a}{8} - \frac{b}{4} - 3\left(\frac{c}{8}\right)\right]$Mole. Using this value as a reference, the success rate of anaerobic decomposition can be quantitatively assessed as a percentage of the maximum biogas potential. This method of assessment allows for evaluating different variations to determine the best results in biogas production.

3. RESULTS AND DISCUSSION

3.1 Carbon Characteristics of Activated Natural Zeolite

Fourier transform infrared (FTIR) analysis was employed in this research to identify the functional groups in zeolite compounds and explore the molecular interactions between them (Mohamed et al., 2017). Figure 2a and Table 2 illustrate the results of the FTIR analysis conducted on zeolite. FTIR characterization reveals wavenumbers in the 1600-3700 cm^{-1} range, indicating the presence of water contained within zeolite. At approximately 1625 cm^{-1}, the presence of H_2O bending is observed (Favvas et al., 2016), which shifts to 1640 cm^{-1} after activation (Maache et al., 1995). The functional group at 3384 cm^{-1} is observed in inactivated zeolite, indicating the presence of hydrogen-bonded Si-OH groups and loosely held water molecules, which disappear after activation due to desilication. The isolated OH functional group is observed at 3623 cm^{-1} and their intensities remain unchanged with NaOH treatment except when treated at high NaOH concentrations (Ates, 2018). The siloxane functional group Si-O-Si is observed in both zeolites. In inactivated zeolite, this group is detected at a wavenumber of 1013.8 cm^{-1}, which shifts to 998.9 cm^{-1} after activation with NaOH. This shift is attributed to the dealumination and desilication processes in the natural zeolite. Similar observations were noted by Ayten Ates, where activation of zeolite with NaOH resulted in a vibrational peak shift from 1020 cm^{-1} to 1010 cm^{-1} (Ates, 2018).

To determine its composition, natural zeolite was analyzed using energy-dispersive X-ray spectroscopy (EDS). The SEM-EDX results for inactivated zeolite and activated zeolite are shown in Table 2, with the composition of natural zeolite summarized. In this study, the zeolite was activated with an alkaline treatment, resulting in a Si content of 28.89, an increase from 28.61, and an aluminum content of 7.48, a rise from 5.62. The primary components of natural zeolites, such as Na, Mg, K, Ca, and Fe (Alshameri et al., 2014), also increased. These ions and minerals are essential nutrients for bacterial growth (Purnomo et al., 2019). Similar to the findings of Milán et al, magnesium plays a key role as a cofactor in several enzyme carriers during anaerobic digestion (Milán et al., 2003). The Si and Al contents increase because the alkaline activation of zeolite causes desilication, thereby decreasing the Si/Al ratio (Marin Senila, 2024). Conversely, zeolite activated through acidic treatment shows a decrease in Al content, as this activation can cause dealumination of the zeolite, thus increasing the Si/Al ratio (Wang et al., 2019).

The predominant types of natural zeolites in Indonesia are clinoptilolite [$(Na,K,Ca)2\text{-}3Al_3(Al,Si)_2Si_{13}O_{36}\cdot 12H_2O$] and mordenite [$(Ca,Na_2,K_2)Al_2Si_{10}O_{24}\cdot 7H_2O$], with mordenite being commonly found in Java deposits, such as those in Bayah, Banten Province. The provided XRD diffractogram of ZAL in Figure 2b demonstrates the crystallographic changes induced by the activation process. The intensity peaks observed in the graph indicate the degree of crystallinity and the presence of several phases within the sample. Alkaline activation using NaOH alters the crystallinity degree of the natural zeolite elements. Sodium hydroxide reacts with the natural zeolite, acting as an activator that dissolves Si and Al into silicate and aluminate salts, thereby changing the Si/Al ratio (Ngapa et al., 2016). This process effectively removes impurities without damaging the zeolite material structure, making it a valuable immobilization agent with ample space for microorganisms and an optimal surface structure. Particularly for the activated Lampung Natural Zeolite (ZAL), the Match-Score analysis using High Score Plus Software indicates that clinoptilolite with monoclinic crystal system crystalline phases exhibits the highest intensity. These results confirm previous findings by Kurniawan and Aziz (2019), which identified the natural zeolites from Lampung as clinoptilolite (Aziz et al., 2019; Kurniawan, 2019).

The XRD analysis revealed that the peak intensities of Lampung natural zeolites were relatively low, indicating a low crystallinity phase, which suggests a high amorphous silica content. Additionally, the XRD analysis in this study showed a crystallinity value of 21%. The 21% crystallinity indicates that most of the sample (79%) consists of an amorphous structure where the atoms or molecules are randomly arranged without a regular pattern. The stability of the zeolite structure was consistently maintained throughout each activation process, confirming that chemical activation can enhance both surface area and pore size while preserving the crystalline structure. Notably, the activation process effectively removed impurities without compromising the integrity of the zeolite material. These findings are significant as they demonstrate the potential to create an immobilization agent with ample space for microorganisms and a well-structured surface.

Based on the shape of the adsorption isotherm presented in Figure 2c and following the classification by Thommes et al. in the IUPAC (International Union for Pure and Applied Chemistry) Technical Report (Thommes et al., 2015), the observed isotherm is identified as a Type IV (a) isotherm. For materials with pore widths greater than 4 nm, a hysteresis loop is observed in the desorption branch of the isotherm. Conversely, materials with less than 4 nm pore widths tend to show an almost perfect overlap between the adsorption and desorption branches. This type of isotherm is a hallmark of mesoporous materials, frequently displaying a pronounced hysteresis loop (Cychosz & Thommes, 2018; Islamoglu et al., 2022). According to a study by (Cahyono et al., 2018), the activation method significantly influences the surface area of zeolite. The surface area and the pore size distribution as an effect of the activation method were evaluated and shown in Figure 2d. The graph indicates that the majority of pores are sized at 20Å. This result confirmed the surface area analysis that the chemical activation produced activated zeolite as an immobilization agent more effectively from the viewpoint of available space for microorganisms. In addition, the pore size of Lampung zeolite was categorized as mesopore region, which was more than 2 nm (1 Å ≈ 0.1 nm).

Figure 3 illustrates the results of the SEM analysis at various magnifications, revealing the morphological structure of the zeolite. Images of the SEM show the presence of clinoptilolite zeolite, which has been observed in previous studies using SEM analysis for zeolites from Sardinia, Italy (Brundu & Cerri, 2015). However, the zeolite activated by NaOH displays irregular crystalline shapes, including trapezoidal flakes, as depicted in Figure 3 (b). These flaking structures are likely a result of NaOH treatment, leading to the deagglomeration of the zeolite crystals (Ates, 2018). Additionally, the analysis highlights the surface morphology of the zeolite. Figure 3(b) shows that the activated zeolite's surface is more structured than without activation. This structural change is significant for its role as an immobilization medium in anaerobic processes, as surface area is a critical parameter for effective anaerobic reactions (Cahyono et al., 2018). The activation of alkaline also impacts the pore structure of the zeolite. Figure 3 illustrates the difference in pore sizes, indicating that the activated zeolite has larger pores than the natural zeolite. This increase in pore size and surface area is consistent with the study of Y. Zhang et al., who reported that NaOH activation remarkably enhanced the specific surface area, pore size, and total pore volume of zeolite (Zhang et al., 2019). Such modifications are beneficial for zeolite being used as immobilization media in that a larger quantity of microorganisms can attach to the surface of zeolite, facilitating more efficient contact with substrate particles and more effective degradation processes (Cahyono et al., 2018).

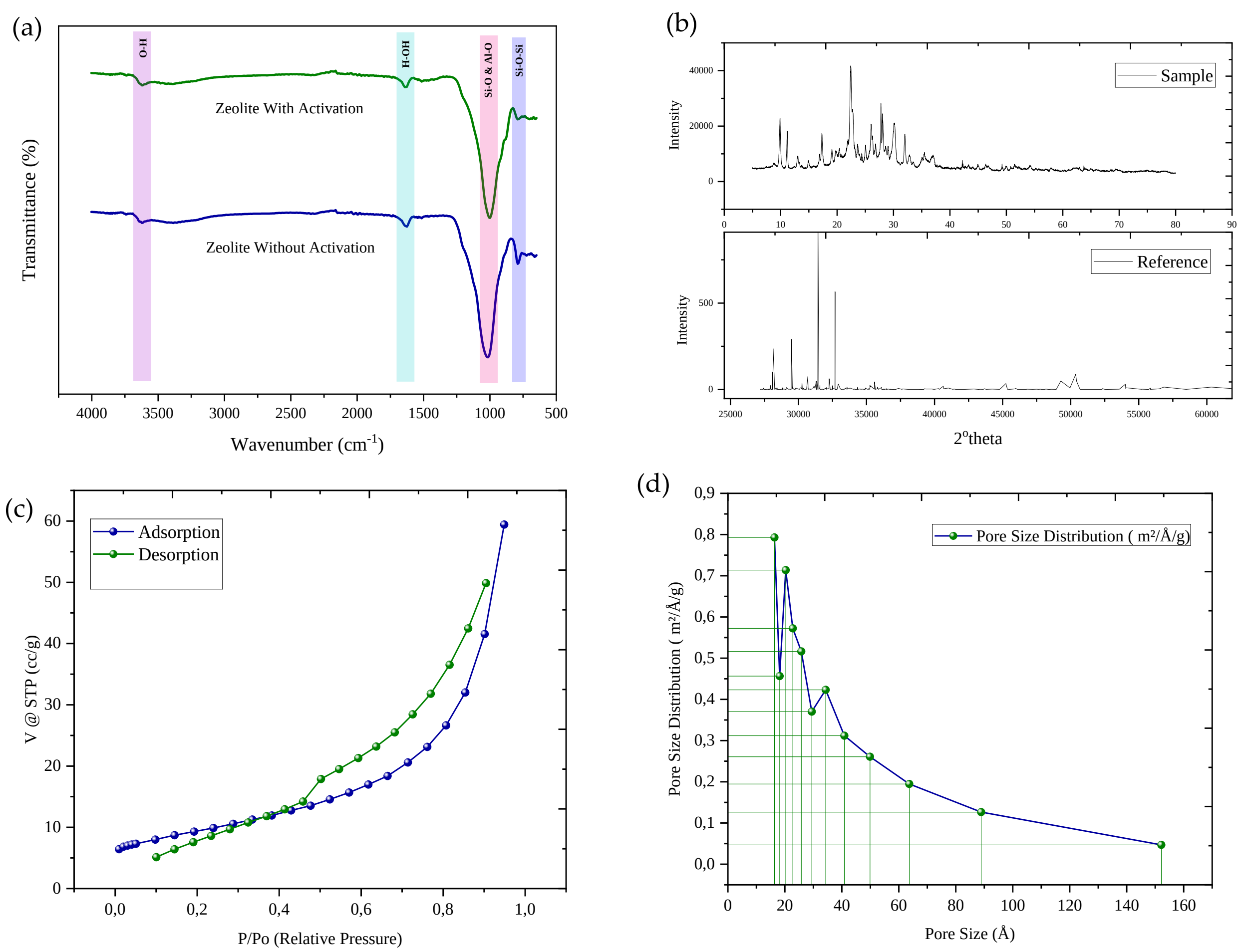

Figure 2. Characteristic immobilization media: (a) fourier transform infrared (FTIR), (b) X-ray diffraction (XRD) analysis was compared with a crystallographic open database (COD), (c) nitrogen adsorption-desorption isotherms of ZAL, (d) pore size distribution of ZAL

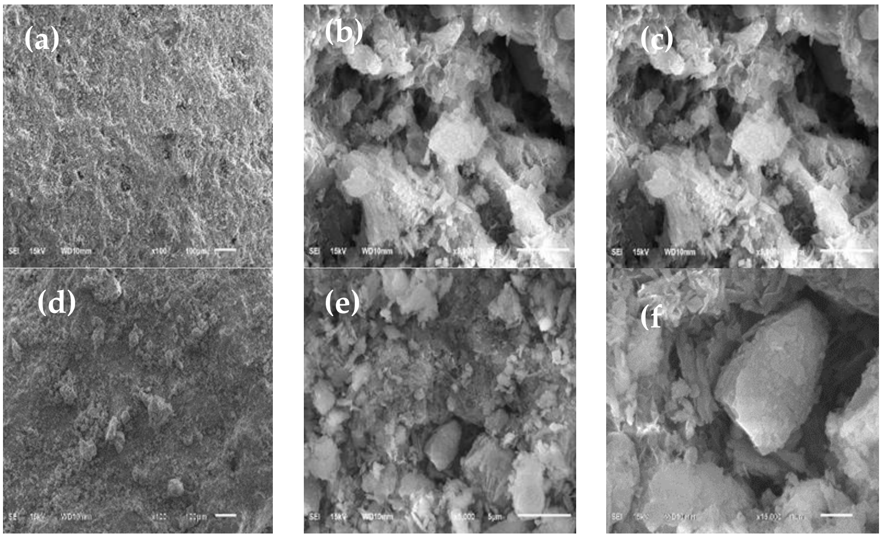

Figure 3. SEM analysis result of activated zeolite observed at: (a) magnifications of 100x, (b) 5000x, (c) 15000x; and in inactivated zeolite: (d) 100x, (e) 5000x, and (f) 15000x magnifications

Table 2. Summary of characteristics of natural zeolite as immobilization media

No	Parameter	Natural Zeolite without Activation	Natural Zeolite with Activation	Assignment
1.	Wavelength (cm^{-1})	790.2	790.2	Symmetric Si-O-Si
		1013.8	998.9	Asymmetric Si–O and Al–O
		1625.1	1640	H_2O Banding
2.	Constituent Compounds	3623	3623	Nonbonded hydroxyl group, OH stretch
		12.06	8.06	C
		48.8	45.25	O
		0.15	2.51	Na
		0.32	0.43	Mg
		5.62	7.48	Al
		28.61	28.89	Si
		2.02	2.53	K
		0.97	2.31	Ca
		1.45	2.54	Fe
3.	Surface Area (m^2/g)	27.255	33.208	N/A
4.	Pore Volume (cc/g)	0.132	0.088	N/A
5.	Pore Size (Å)	48.514	34.335	N/A

*N/A: data not available

3.2 Anaerobic Digestion Performance

Potential biogas production can be accurately forecasted using theoretical calculations based on empirical molecular formula methodology, as proposed by Shuler and Kargi. This approach yielded a predicted biogas volume of 403,442 L/D. This study used different hydraulic retention times (HRT) to evaluate their effects on biogas production. The data show that at an HRT of 20 days, the reactor with immobilized media produced the highest amount of Methane, approximately 61.97 mL/g. In contrast, the non-immobilized media process produced a significantly lower volume of biogas, only 4.15 mL/g. This compelling evidence confirms the significant increase in biogas yield achieved by incorporating immobilized media, highlighting its far superior efficiency to the non-immobilized method.

Figure 4a illustrates the actual biogas production results using both immobilization and non-immobilization media, highlighting the effects of Hydraulic Retention Time (HRT) on biogas production. Biogas was generated over various organic loading rate (OLR) and HRT conditions, extending to 168 days for both reactors. During the initial HRT stages, biogas production in Reactor A (RA) and Reactor B (RB) exhibited fluctuations, significantly increasing and decreasing biogas volume. This variability can be attributed to the anaerobic bacteria's adaptation to the new environment and initial reactor instability (Luo et al., 2022). As the operational period advanced and OLR increased, biogas production also rose, eventually reaching a steady-state phase at an OLR of 37.5 mL/D for 34 days, continuing to an OLR of 75 mL/D for 32 days. This observation aligns with previous studies indicating that increasing OLR can enhance biogas volume production (Sun et al., 2017). The peak biogas production was achieved at

620 mL on the 150th day, with an OLR variation of 150 mL/D for ten days. This peak performance was followed by a decline, signifying that the system equilibrium had been disrupted (X. Liu et al., 2018). This disruption was caused by the excessively short HRT coupled with a significant increase in OLR, which did not allow the bacteria sufficient time to effectively digest the substrate, leading to the accumulation of volatile fatty acids (VFA) that disrupted the biochemical balance within the anaerobic digester (Parajuli et al., 2022). In the presence of immobilization media, most acidogenic and methanogenic microorganisms will stick and grow on the zeolite surface to form a biofilm layer for promoting the fast of organic content to methane (Damayanti et al., 2020). This biofilm formation mechanism demonstrates that adding immobilization media can significantly enhance biogas production (M. Liu et al., 2021) and help prevent system washout (Safak et al., 2022).

The changes in VFA patterns and their impact on methane production in the anaerobic fermentation system are illustrated in Figure 4b. Both VFA graphs exhibit significant fluctuations in VFA concentrations. There are notable peaks around days 60 and 150, where VFA concentrations reach their highest levels. In the initial phase (0-40 days), VFA levels fluctuate due to the adaptation of microorganisms to the substrate, although methane yield remains relatively stable. The observed reduction in VFA concentrations during the initial period (up to day 3) suggests that the microorganisms were in the adaptation phase, during which they were actively utilizing the substrate (Ramadhani et al., 2018). In the middle phase (40-120 days), there is an increase in VFA concentrations, marked by several high peaks, indicating very active fermentation. As a result, methane yield also increases, but there are some points where methane production decreases due to VFA accumulation. In the final phase (120-157 days), VFA levels continue to fluctuate, but methane yield in reactors A and B shows significant differences. The RA reactor, benefiting from the immobilization media, demonstrates a more pronounced increase in methane production due to enhanced microbial stability. The addition of immobilization media caused the reactor to exhibit less VFA accumulation than the control. This phenomenon was possibly caused by the media's simultaneous effects of VFA adsorption onto the media and enhanced methanogenic performance in converting VFA into biogas (Budhijanto et al., 2024).

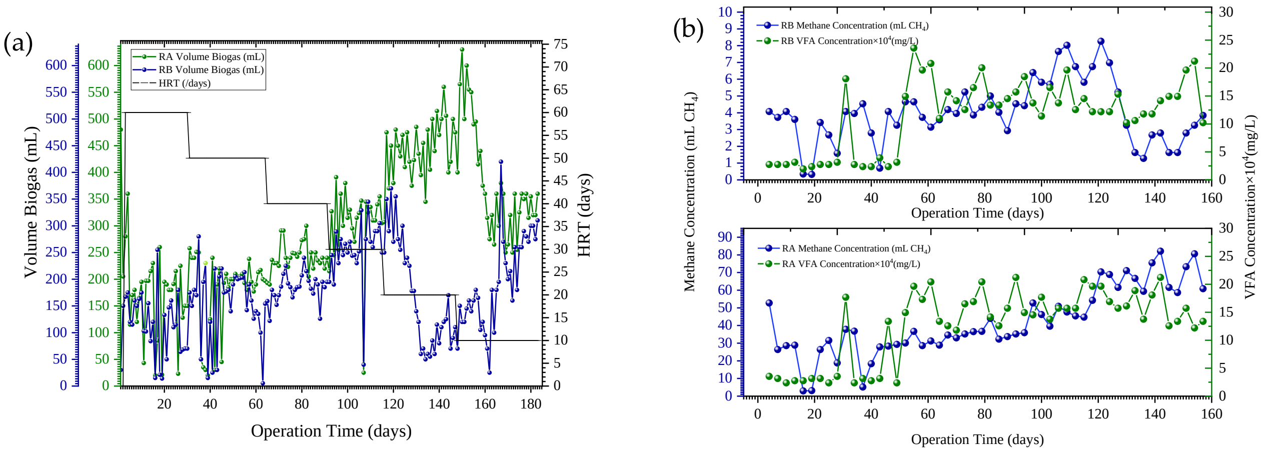

Figure 4. Anaerobic digestion performance (a) comparison of biogas production in reactors with immobilization (RA) and reactors without immobilization (RB) under varying hydraulic retention time (b) influence of volatile fatty acids (VFAs) on methane productivity

4. CONCLUSION

The FTIR analysis revealed structural changes in the zeolite post-activation, particularly shifts in Si-O-Si and Al-O peaks, indicating a successful modification of its chemical structure. These changes were confirmed by SEM-EDX analysis, which showed a significant drop in the amount of carbon and oxygen in the sample. At the same time, sodium and aluminum levels rose because they were absorbed during the activation process. The alkaline activation with NaOH changed the zeolite's elemental composition and made it more crystallized, as shown by the XRD results showed a crystallinity value of 21%. However, most of the structure stayed amorphous. Activated Lampung Natural Zeolite has large pores that microorganisms can penetrate, thus facilitating increased biogas production. This pore enlargement and improved surface accessibility allow bacteria to remain on the surface of the zeolite, thereby increasing their contact with the substrate. As a result, better interaction between bacteria and the substrate promotes a more efficient degradation process, resulting in increased biogas yield. The enhancement in microbial stability and contact area resulted in maximum biogas production of 620 mL on the 150th day at HRT 20, validating that immobilization media allowed the anaerobic process to survive even at low hydraulic retention times (HRT). Zeolite keeps bacteria stable and active, which lowers the buildup of volatile fatty acids (VFA) and keeps methane production steady, even in situations that are hard for microbial communities.

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