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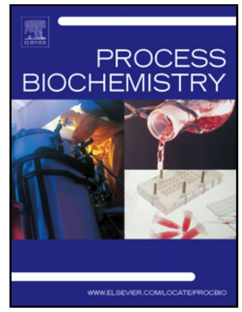

Title: Enhanced bio-methane production from ammonium-rich waste using eggshell-and lignite-modified zeolite (ELMZ) as a bio-adsorbent during anaerobic digestion

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Enhanced bio-methane production from ammonium-rich waste using eggshell-and lignite-modified zeolite (ELMZ) as a bio-adsorbent during anaerobic digestion

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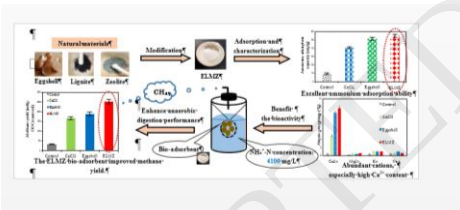
Graphical abstract



Highlights

- A bio-adsorbent, ELMZ, has been developed for ammonium-rich anaerobic digestion.
- The ammonium was adsorbed by the ELMZ adsorbent efficiently.
- The abundant cations, especially high Ca^{2+} content benefits the bioactivity.
- High bio-methane production has been achieved by addition of bio-adsorbent ELMZ.
- ELMZ is promising to recover methane from ammonium-rich substrate practically.

Abstract

In this study, a calcium-rich natural material utilized bio-adsorbent, composed of eggshell-and lignite-modified zeolite (ELMZ) was developed for enhancing methane production at high ammonia concentrations. The ELMZ showed a maximum NH_4^+-N adsorption capacity of 55 mg/g, which was 5.9 folds of the natural zeolite (9.2 mg/g) and 1.4 folds zeolite modified by calcium chloride (40 mg/g), respectively. The ammonium adsorption process well fitted the pseudo-second-order kinetic model and the Freundlich isotherm. It indicated that its process was an ion-exchange process. The high abundance of cations, especially high Ca^{2+} in ELMZ supported the ion-exchange, which stimulated the anaerobic digestion process. The anaerobic digestion with ELMZ as a fixed-bed (initial NH_4^+-N concentration: 4.1 g/L), exhibited the best performance at methane yield of 250 mL CH_4/g-$DOC_{removal}$, which is 7 folds higher than that achieved with natural zeolite (32 mL/g-$DOC_{removal}$) at the same ammonia level. As a bio-adsorbent, ELMZ contributes to enhance methane production and further benefits to a sustainable process since the eggshell waste is re-circulated.

Keywords: Eggshell and lignite modified zeolite (ELMZ); Bio-adsorbent; Ammonia inhibition; Anaerobic digestion; Bio-methane production.

1. Introduction

Along with the development of livestock industries, environmental problems such as soil contamination, eutrophication, and air pollution have increased rapidly due to the growing production of livestock wastes. Livestock waste is usually used as soil fertilizer, but repeated overload of livestock waste leads to the accumulation of nutrients and heavy metals, causing many environmental problems like soil and water contamination [1].

Anaerobic digestion is regarded as a desirable application due to its beneficial productivity with renewable energy recovery and efficient control on waste management. Furthermore, anaerobic digestion of livestock waste is also considered as one of the sustainable waste-to-energy technologies, which could convert livestock waste materials into methane as a valuable energy carrier [2].

However, the high concentration of ammonia released during the biodegradation of livestock wastes may seriously inhibit the anaerobic digestion process. Ammonia inhibition could lead to a decrease in methane production and accumulation of by-products, contributing to instability of the anaerobic digestion process [3]. Zeeman et al. [4] reported that ammonia inhibition occurs at an initial ammonium nitrogen concentration of 1700 mg/L in the thermophilic reactor, and Hobson and Shaw [5] have also reported that *Methanobacterium formicicum* could be inhibited at ammonium nitrogen concentration of 3290 mg/L.

Numerous practical solutions have been investigated for bio-methane recovery from ammonium-rich waste, such as adding ammonium-selective adsorbent, microorganism immobilization and ammoxidation [6–8]. Ammonia removal by adding ammonium-selective adsorbents has drawn a great attention owing to its non-toxicity, low cost and eco-friendly character [9]. Among numerous adsorbents, zeolite is commonly used because of its wide availability and low cost. However, the ion-exchange capacity of natural zeolite is usually quite low. Hence, modification of zeolite is considered as an attractive option, in order to increase the effective surface area and further enhance the adsorption capacity of zeolite. The adsorption behavior of the silicate-carbon modified zeolite has been reported to be effective to remove ammonium with adsorption capacity of 0.12 mg/g [10]. Meanwhile, Lei et al. [11] found that the microwave-treated zeolite could reach to adsorption capacity of 23.38 mg/g. However, due to the weak adsorption capacity of these modified zeolite, the development of more efficient adsorbent for ammonia removal is still needed.

Generally, cations (Na^+, K^+, Ca^{2+}, and Mg^{2+}) that can exchange with ammonium are widely used in the zeolite modification processes. These cations are crucial for the cell metabolism and growth which also are trace elements important for stable process performance, e.g. by limiting the VFA accumulation [12]. Ca^{2+} has often an optimistic effect on the anaerobic digestion of organic wastes [13]. Therefore, natural materials rich in Ca^{2+} would be an eco-friendly and low-cost choice used as modifiers of adsorbents. To our best knowledge, there is no report focusing on the modification of adsorbent using calcium-rich natural material until now. Two kinds of natural material, eggshell and lignite, have been selected to modify zeolite in the present study.

Eggshell is commonly treated as waste in food industry and contains large quantities of calcium salt. In USA, 120,000 tons of waste eggshells are generated and disposed off annually [14]. The major component of eggshell is calcium carbonate. The dried eggshell contains 85-95% calcium carbonate, 1.4% magnesium carbonate, 0.8% phosphates, 4% organic matter as well as traces of sodium, potassium, zinc, manganese, iron and copper [15]. Therefore, the eggshell has an excellent potential to be used as an adsorbent material due to its high Ca^{2+} content and the presence of trace elements. On the other hand, lignite is widely allocated and abundantly available. More than 2600 billion tons of lignite accounts for about 24.4% total coal resources around the world [16]. However, because of high content of oxygen and water, low calorific value, and easy spontaneous combustion, the application of lignite has not been further investigated. Interestingly, the variety of cations in lignite, especially trace elements, may benefit for the growth of microorganisms [17]. Thus, lignite could work as a modifier to improve the ability of adsorption. The utilization of eggshell and lignite in suitable industrial application could enhance the methane production and deal with a waste problem. However, the application of both eggshell and lignite for the modification of nature zeolite has not been carried out yet.

Therefore, our aim was to develop a novel bio-adsorbent based on eggshell and lignite, with high ammonium-adsorption capacity to optimize anaerobic digestion of substrates giving rise to high concentrations ammonium. This study would provide a promising choice for a low cost and eco-friendly treatment of waste eggshell and lignite. This study was focus on (1) investigating the ammonium adsorption capacity of eggshell- and lignite-modified zeolite (ELMZ) adsorbents compared with the calcium chloride-modified zeolite and natural zeolite; (2) analyzing kinetics and thermal isotherms of the adsorption process; (3) evaluating the feasibility of modified adsorbent using ELMZ during anaerobic digestion.

2. Materials and methods

2.1 Synthesis of modified zeolite

The process of integration of calcium chloride, eggshell only, ELMZ were prepared to modify the natural zeolite, respectively. For the $CaCl_2$ modified zeolite, 8 g of natural zeolite powder, 1 g starch and 1 g $CaCl_2$ were prepared. For the eggshell only modified zeolite, 8 g of natural zeolite powder, 1 g starch and 1 g eggshell were prepared. For the ELMZ, 8 g of natural zeolite powder, 1 g starch, 0.5 g eggshell and 0.5 g lignite were prepared. Firstly, the prepared materials were mixed to homogeneity, then ultra-pure water was added to the above materials to make the raw paste, respectively. The glass tube with inner diameter of 8 mm was filled with the raw paste to obtain a uniform cube.

The cubes were shaped to balls by fingers. After that, the spherical-shaped adsorbents were oven-dried at 105 ºC for 24 hours and finally calcined in a muffle stove at 600 ºC for 1.5 hours to form the porous structure and firm the configuration of the adsorbents. All the modified adsorbents were cooled to room temperature and stored in dry environment for further studies. The natural zeolite was purchased from DAISO INDUSTRIES (Japan) and used as a control.

2.2 Inoculum and substrate

The seed sludge was digested sludge from the sewage treatment plant locating at Ibaraki prefecture, Japan. The 1g/L glucose was added to the seed sludge every other day for cultivation at 35 ºC for two weeks before the experiment. The characteristics of the digested sludge used are shown in Table 1.

In this study, a synthetic medium containing the substrate which was made according to the description in the previous study, Acetate (2.5 g/L), glucose (2.5 g/L), NH_4Cl (200 mg/L), KH_2PO_4 (16 mg/L), yeast extract (300 mg/L) and a trace mineral solution (200 mL/L), have been added to the bioreactor at first [18]. The chemical composition of trace mineral solution as follows: $FeSO_4 \cdot 7H_2O$ (0.5 g/L), $MgSO_4 \cdot 7H_2O$ (62.5 mg/L), $MgCl_2 \cdot 6H_2O$ (62.5 mg/L), $CoCl_2 \cdot 6H_2O$ (12.5 mg/L), $MnSO_4$ (12.5 mg/L), $ZnSO_4 \cdot 7H_2O$ (12.5 mg/L), $NiCl_2 \cdot 6H_2O$ (12.5 mg/L), $CuSO_4 \cdot 5H_2O$ (12.5 mg/L), $Na_2MoO_4 \cdot 2H_2O$ (12.5 mg/L) and H_3BO_3 (12.5 mg/L) [18]. have been added to the bioreactor at first. To create the ammonium-rich condition, NH_4Cl was added to each bioreactor. The initial pH of the substrate was adjusted to 7.0 using 1M NaOH and 1M HCl.

2.3 Ammonium adsorption experiments

The adsorption ability of different formulations was confirmed by dissolving NH_4Cl in deionized water at an initial ammonium nitrogen concentration of 4.1 g/L at ambient temperature (25±3 ºC). The adsorption ability of ELMZ was calculated using the following equation [19]:

$$Q = (C_0 - C_{eq})\ V/m \qquad (1)$$

where Q is the adsorption ability of adsorbent (mg/g), C_0 and C_{eq} are the initial and equilibrium concentration of NH_4^+-N (mg/L), *V* is the solution volume (L), and *m* is the adsorbent dosage (g). The experiment was conducted in triplicate.

2.4 Kinetic models and isotherms

The adsorption isotherm was studied by the determination of ammonium adsorption capacity of adsorbent with different initial ammonium nitrogen concentration from 1 g/L to 6 g/L. The dosage of adsorbent was 10 g/L, and the contact time was 48 hours. The kinetics of ammonium adsorption was analyzed by the determination of ammonia uptake mass at different time intervals.

To understand the mechanism on exchange reactions of the ELMZ adsorbent, a study on adsorption kinetics was performed by applying 3 typical models. Thus, the Lagergren`s pseudo-first-order, Ho`s pseudo-second-order, and intraparticle diffusion kinetic models were employed to investigate ammonia adsorption process by ELMZ. The integration of the Lagergren`s pseudo-first-order, Ho`s pseudo-second-order kinetic equations and intraparticle diffusion kinetic models followed in the previous study [20].

The linear form of the Langmuir and the Freundlich isotherm equation is characterized to explore the adsorption process according to description by Wang et al. [19].

2.5 Anaerobic digestion experiment

The anaerobic digestion experiment was set up at 35 ºC. Serum bottles (300 mL, SIBATA) with working volume of 200 mL were used as fixed-bed bio-reactor. Each bioreactor contained 80% substrate and 20% (w/w) digested sludge. Novel eggshell-and lignite-modified zeolite (ELMZ), eggshell-modified zeolite, calcium chloride-modified zeolite were added to different bioreactors as adsorbent, respectively. The bioreactors with natural zeolite only acted as controls. 2 g adsorbent was fixed in the bedding materials and suspended in the bioreactor. Along with a previous study, CPE (chlorinated polyethylene; DAISO INDUSTRIES, Japan) was selected as the bedding material which was low-cost, easily obtained and already proved to be efficient as a bedding material applied in ammonium-rich anaerobic digestion [21]. Before use, the CPE was washed and immersed in distilled water for 24 hours, and then dried in oven at 105 ºC for 12 h. The initial concentration of NH_4^+-N was 4.1 g/L and the pH was 7.0 adjusted by 1 M NaOH and 1 M HCl. Each bioreactor was conducted in duplicate for 3 repetitions.

2.6 Analytical methods

For characterization of developed adsorbent, the specific surface area was determined using Brunauer-Emmett-Teller (BET) specific surface analysis device (Coulter SA-3100, US). The content of ion-exchange cations and morphology of modified zeolite were analyzed by scanning electron microscopy with energy dispersive X-ray detection (SEM-EDS, Hitachi FESEM S-4800 EDX). The ammonium nitrogen concentration was determined using the standard Nessler's reagent spectrophotometry method at 420 nm as standard method according to China State Environmental Protection Administration (HJ 535-2009).

For the anaerobic digestion experiments, total solid content (TS), volatile solid content (VS), total nitrogen (TN) determinations were conducted along with the standard methods (APHA) [22]. The ammonium nitrogen concentration was determined using the standard Nessler's reagent spectrophotometry method at 420 nm as standard method according to China State Environmental Protection Administration (HJ 535-2009). The pH, biogas production and concentration were detected every day. The pH was measured everyday by a pH meter (TES 1380). The biogas was gathered by the plastic syringes and the production of biogas was determined by the scale on the syringe. The composition of the biogas was analyzed by gas chromatography (GC-8A, SHIMAZU, Japan) according to the description of Zhang et al. [18]. Dissolved organic carbon (DOC) of the digestate was detected using a TOC analyzer (TOC-5000A, Shimadzu, Kyoto, Japan). The adenosine triphosphate (ATP) concentration of the microbes was measured by a Bac Titer-GLoTM Microbial Cell Viability Assay (Promega, USA) according to Zheng et al. [21]. The fixed-bed was taken out after the experiment for observation using a Scanning Electron Microscope (SEM, DS-720, Topcon, Tokyo, Japan). All samples were measured in triplicate.

For the economic analysis, the costs of the adsorbents were split in ingredients cost and electric cost. The prices of the chemicals were provided by the FUJI FILM WAKO Co. (Japan). According to the U.S. Energy Information Administration (EIA) [23], the average market price of electricity in total area of U.S. was 0.1 US$/kWh in April, 2018.

3. Results and discussion

3.1 NH_4^+-N adsorption by different modified zeolite

Figure 1 showed the ammonium adsorption capacity of different adsorbents at the initial concentration of 4.1 g/L. The ELMZ exhibited the highest ammonium adsorption capacity of 55 mg/g, which was 5.9 folds higher than natural zeolite (9.2 mg/g), 1.4 folds of the zeolite modified by calcium chloride (40 mg/g), 1.1 folds of the eggshell-modified zeolite (52 mg/g), respectively. This result indicated that natural materials, eggshell and lignite, may substantially improve the adsorption efficiency of the zeolite. Although, the natural zeolite has been regarded as an effective adsorbent for ammonia removal during the treatment of municipal and other industrial wastewaters [24], the ELMZ in this study proves to be a much more efficient adsorbent than the natural zeolite. Some previous works listed in Table 2 also indicated that the eggshell-and lignite-modified zeolite showed a much stronger ammonium adsorption capacity compared to other modified zeolite adsorbents with cation-modified zeolites, and it revealed the next highest compared to the ELMZ. Hydrated aluminum-oxide modified natural zeolite had an ammonium adsorption capacity of 30 mg/g at an initial ammonium nitrogen concentration of 5000 mg/L or about half that of the ELMZ [25]. The NaA-modified zeolite demonstrated ammonium adsorption capacity of 44 mg/g or about 80 % that of the ELMZ [26]. However, the NaA adsorbent was tested at an ammonium concentration only around 10 % of the ELMZ and hydrated aluminum adsorbent. Therefore, ELMZ seems to be the most efficient adsorbent for ammonia removal during anaerobic digestion of nitrogen-rich substrates.

The cost for the use of different adsorbents presented in the previous studies, and the cost for ELMZ in this study was included in Table 2 was estimated to 0.95 US$/kg. Compared to other adsorbents, the natural material modified adsorbents showed relatively low costs (i.e. < 1.0 US$/kg) due to the wide-distribution of raw materials, but all of them revealed considerably lower adsorbent capacities. ELMZ exhibited the highest capacity of ammonia adsorption with relatively cost. Thus, ELMZ could be a promising choice for the practical application of ammonia removal with high efficiency and cost effective.

3.2 Adsorption kinetics

Figure 2 showed the similarity curves and coefficients of the ELMZ for the kinetic models. For the pseudo-first-order model in Fig. 2A, the correlation coefficient was only around 0.9695, the experimental ammonium adsorption capacity (55 mg/g) was much higher than the theoretical data (32 mg/g), and suggesting a poor fit between the model and the experiment. Subsequently, the ammonium adsorption process by ELMZ did not follow the pseudo-first-order kinetics. In the case of the pseudo-second-order model with the correlation coefficient (R^2 = 0.996) as shown in Fig. 2B, there was no significant difference between the experimental adsorbed masses at equilibrium (55 mg/g) and the theoretical data, indicating that the ammonium uptake process on the ELMZ well followed the pseudo-second-order kinetics. Accordingly, this adsorption process of ELMZ likely is subjected to be a chemical process, dominated by ion-exchange.

The intraparticle diffusion model presented in Fig. 2C, revealed two intercepting lines in the plot of Q_t to $t^{1/2}$, representing that adsorption process on the ELMZ involved two steps. The first step was ascribed to the boundary layer diffusion of the ammonium ions during the first 60 minutes, while the second part attributed to intraparticle diffusion effects and a gradual equilibrium state. An intraparticle diffusion would be regarded as the sole rate-controlling step, if the plot passed through the origin. Otherwise, the boundary layer diffusion would influence the adsorption [27]. As shown in Fig. 2C, the plot of Q_t versus $t^{1/2}$ did not pass through the origin, demonstrating that the intraparticle diffusion is not the sole rate-limiting step for the adsorption process of ELMZ. Zhao et al. [20] also explained similar phenomenon on the intraparticle diffusion model of the ammonium uptake process for a novel modified ceramic adsorbent. Comparing to the pseudo-second-order model, the intraparticle model showed lower correlation coefficient, indicating that the rate-limiting step was dispersed to the pseudo-second-order model.

3.3 Adsorption isotherms

To clarify the adsorption mechanism of the adsorbate with the ELMZ, the linearized forms of Langmuir and Freundich isotherms were analyzed. The coefficients were listed in Table 3 calculated by experimental data according to the Langmuir and Freundlich isotherm. The results exhibited that the Freundlich model (R^2 = 0.987) provided better fit compared to the Langmuir model (R^2 = 0.945). Moreover, the q_m coefficient of 156 mg/g obtained by the Langmuir equation was much higher than the measured value (55 mg/g). These results suggested that adsorption in the ammonium solution by ELMZ occurred as heterogeneously by multilayer sorption but not as monolayer sorption. Similar result achieved by Lei et al. [11] for a microwave-treated Chinese zeolite showed the same equilibrium pattern. In addition, the empirical parameter $1/n$ within the boundaries $0<1/n<1$ indicated a favorable adsorption for ammonium [28]. Thus, the $1/n$ value of 0.56 achieved in this present study, within the suitable range demonstrates a favorable ammonium adsorption capability by the ELMZ.

3.4 Characterization of the eggshell and lignite modified zeolite (ELMZ)

The results of BET and SEM-EDS analysis were presented in Table 4 and Fig. 3, which showed that both of specific areas of ELMZ (15 m^2/g) and the eggshell modified zeolite (15 m^2/g), were around 2.0 folds higher of the natural zeolite (8.0 m^2/g). This indicated that eggshell could be regarded as a favorable porogen. The SEM photographs in the Fig. 3 A displayed the surface morphologies of the adsorbents. Compared to the other zeolite samples, the ELMZ and the eggshell-modified zeolite possessed rougher surfaces and larger pore diameters for their porous structure with irregularly distributed channels. These features likely improved reactivity of these materials, which may contribute to their higher adsorption capacity presented in the Fig. 1.

Moreover, Fig. 3A revealed that the ELMZ adsorbent contained several different cations, e.g. Na^{+}, K^{+}, Ca^{2+} and Mg^{2+}. Earlier, these cations were used in adsorbent-modification processes, in which especially the Ca^{2+} have exhibited high ability for ammonium exchange [20]. Figure 3B showed that the content of Ca^{2+} in ELMZ was around 10 folds higher than that in the natural zeolite, while there were no significances differences of Na^{+}, K^{+} and Mg^{2+} contents between natural zeolite, eggshell-modified zeolite and ELMZ. This result is consistent with the results obtained from the adsorption kinetics showing that ammonium adsorption was dominated by ion-exchange processes. The ion-exchange reaction is regarded as a stoichiometric process, in which one equivalent of an ion in the solid phase is replaced by an equivalent ion from aqueous solution according to the following reaction:

$$Ca^{2+} (S) + 2NH_4^{+} (L) \rightleftharpoons 2NH_4^{+} (S) + Ca^{2+} (L) \quad (7)$$

where S and L represent the solid and liquid phases, respectively. The equation 7 showed that ammonium ion in the liquid phase was removed by the calcium content in the ELMZ. According to Le Chatelier`s principle, the higher the calcium in the adsorbent, the more ammonium ion removed from the liquid phase [20]. Therefore, the high calcium content in ELMZ accelerated the reaction from left to right, contributing to the higher ammonium adsorption capacity. These results indicated that ELMZ had a high ion-exchange efficiency, due to its highest amount of Ca^{2+} content and abundant existence of trace elements (Fig. 3B).

The cations Na^{+}, K^{+}, Ca^{2+} and Mg^{2+} released from the zeolites may improve the metabolic activity of the microorganism performing the anaerobic digestion. Especially, the calcium has been shown to promote anaerobic digestion process and improve the methane production upon supplementation by calcium salt [6]. Hence, the ELMZ may improve the anaerobic digestion not only by its ammonium removal capacity, but also by the release of calcium, which further promotes its practical application during the anaerobic digestion treatment.

3.5 The performance of ELMZ fixed-bed during ammonium-rich anaerobic digestion

The bioreactor with ELMZ showed the highest methane concentration (63 %) and it reached a stable level from day 11 (Fig. 4A), demonstrating more efficient performance than other bioreactors under high ammonium concentration environment. According to Fig. 4A, compared to the control bioreactor, the usage of ELMZ shortened the lag-phase of the anaerobic process and provide more stable methane production. Similar results have been reported by Zheng et al. [21] that the NH_4^{+}-N concentration could be decreased and the lag-phase could be shorted by the adsorbent fixed-bed, resulting in the effective improvement of the methane production. It indicated that the ELMZ could contribute to highly efficient anaerobic digestion process under ammonium-rich environment.

The methane yield of the bioreactors is shown Fig. 4B. The bioreactor with ELMZ obtained the highest methane yield of 250 mL/g-$DOC_{removal}$, 7 folds higher than the bioreactor with natural zeolite (32 mL/g-$DOC_{removal}$). The methane yield decreased in the order as ELMZ > Eggshell > $CaCl_2$ > Natural zeolite. The eggshell-modified zeolite and the $CaCl_2$-modified zeolite was 5 folds and 4 folds higher methane yield than that of the natural zeolite. The addition of trace elements during the modification process of the adsorbents may benefit the growth of microorganism and improve the methane production, which was supported by the content of trace elements as shown in Fig. 3. This result also supported the findings that the utilization of ELMZ showed a better performance than other adsorbents during anaerobic digestion with high concentration of ammonium nitrogen.

Figure 4C showed the NH_4^+-N concentration of bioreactors during the anaerobic digestion. The bioreactor with ELMZ had the lowest ammonium nitrogen concentration throughout the anaerobic digestion process, indicating the highest ammonium adsorption ability among the adsorbents, which was in accordance with the result of ammonium adsorption capacity as shown in Fig.1. The NH_4^+-N concentration decreased at first due to ammonium adsorption on the adsorbent. Then the NH_4^+-N concentration increased in all bioreactors after 8 days due to the syntrophic reaction of the microorganism in the ammonium-rich environment and the desorption of ammonium from the adsorbents. Similar patterns were observed in the previous study, supporting that the addition of the adsorbents improves the adsorption efficiency of ammonia during the initial phase of the anaerobic digestion [21]. This result showed that the ELMZ could afford promising environment for the microbes to endure the ammonium-rich condition successfully.

3.6 Quantity and activity of the immobilized anaerobes on the ELMZ fixed-bed bioreactor

Figure 5 shows the colonization of microorganisms on adsorbents in different bioreactors. The quantity of microorganisms immobilized on ELMZ were the highest among all the adsorbents. This result certified that the amount of microorganisms on the ELMZ was the highest among all the adsorbents. The ELMZ could work as adsorbent at the very beginning of the anaerobic digestion process as shown in Fig. 4C. After the ammonium desorption process reached to the equilibrium stage, the main function of the bio-adsorbent transferred into providing places for microorganism multiplication. Thus, the abundant trace element content of the novel zeolite adsorbent serves as micronutrient supplementary for the microorganisms and provides suitable conditions for the immobilization of microorganisms. Besides, compared to the other adsorbents the large surface area, rough surface and large pore size of the ELMZ offered better immobilization conditions for the microorganisms (Table 4). It was reported that a system with immobilized anaerobe had a higher tolerance to severe environment of anaerobic digestion [21].

The amount of immobilized biomass on the surface of adsorbents and bedding materials shown in Fig. 6A verified the result observed from the SEM photographs. The ELMZ bioreactor immobilized the highest amount of biomass, which was 4 folds higher than that of the control bioreactor and 2 times of that of the $CaCl_2$-modified and eggshell-modified zeolite. The three modified adsorbents containing more trace elements immobilized more microorganisms, and the abundant content of trace element may benefit the growth of microorganism. Especially the ELMZ showed the highest content of trace elements corresponding to the highest amount of immobilized biomass, leading to higher bio-methane production from ammonium-rich waste.

The adenosine triphosphate (ATP) concentration of the experimental reactors was shown in Fig. 6B. The bioreactor with ELMZ achieved the highest value of 0.12 μmol/L, which was 1.2, 1.4 and 3 folds higher than that of the eggshell-modified zeolite, $CaCl_2$-modified zeolite and natural zeolite, respectively. Higher ATP value achieved by ELMZ bioreactor indicated higher activity of microorganism than the bioreactors with other adsorbents. The activity of the microorganisms is a critical factor to evaluate the efficiency of anaerobic reactors [18]. The enhanced biogas production of the bioreactor with the modified bio-adsorbents could be reinforced by the higher activity of the microbes. The high calcium content and diversity of cations species of ELMZ may also have affected the efficiency by providing essential nutrients for the microbial metabolism, which was reflected by the higher ATP value under this treatment.

According to the result of adsorption kinetics and isotherms (Fig.2), ammonium adsorption of ELMZ was dominated by ion-exchange processes, where the ammonium ions were removed from the liquid, while the ions like calcium were released. Ca^{2+} is a fundamental nutrient for the metabolic activity of the microorganism and reported to promote the performance of anaerobic digestion. Ca^{2+} likely stabilize enzymatic activity of microbes, contributing to alleviate the ammonia inhibition [29]. Therefore, Ca^{2+} dissociation from the ELMZ likely enhanced the ammonium-rich anaerobic digestion efficiency in our study. Also, the trace elements released from the ELMZ could serve as micronutrients for the microorganisms during the anaerobic digestion. Bougrier et al. [12] reported that abundance of micronutrients which is required by the microorganisms to sustain their basic activity and improve their performance and, thus, contribute to efficient and stable anaerobic digestion. The ATP results supported the result of NH_4^+-N concentration as shown in Fig. 4C. Thus, the effective adsorbent created a lower NH_4^+-N concentration environment and improved microorganism multiplication, leading to higher methane yield.

Therefore, the results of anaerobic digestion in the bioreactor with ELMZ proved that, as a bio-adsorbent, the ELMZ could successfully applied in ammonium-rich anaerobic digestion to improve the performance of the bioreactors. This due to its: (1) ammonia uptake; (2) cation dissociation, especially the Ca^{2+} may serve as nutritious source for the microorganisms; (3) effective microorganism immobilization. The ELMZ bio-adsorbent not only worked as an ammonium adsorbent, but also functioned as a bed material for microorganism to immobilize. Furthermore, this study also offered a unique viewpoint to treat the eggshell and lignite in an eco-friendly and cost-effective method.

4. Conclusions

In this study, a novel bio-adsorbent, eggshell-and lignite-modified zeolite (ELMZ) was developed for bio-methane recovery from ammonium-rich waste. ELMZ showed very high ammonium adsorption capacity. The abundant content of cations, especially the Ca^{2+}, likely benefited the metabolic activity of microorganisms, which would contribute to high methane production during the anaerobic digestion system. Furthermore, the excellent performance achieved during the anaerobic digestion system, indicates that the ELMZ could be a feasible bio-adsorbent for the improvement of anaerobic digestion with ammonium-rich waste. This study also provides a promising way for recycling treatment of the waste eggshell and lignite cost-effectively and eco-friendly in the future.

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Figure Legends

Figure 1 Ammonia adsorption capacity of zeolite modified with different adsorbent materials at initial NH_4^+-N concentration of 4.1 g/L.

Figure 2 (A) The plot of Lagergren`s pseudo-first-order kinetics; (B) the plot of the Ho`s pseudo-second-order kinetics and (C) the plot of the intra-particle diffusion model of eggshell-and lignite-modified zeolite adsorbents.

Figure 3 (A) SEM-EDS analyses and (B) element contents of three differently modified adsorbent and unmodified zeolite.

Figure 4 (A) Methane concentration; (B) Methane yield and (C) Ammonium nitrogen concentration in bioreactors with four different adsorbents including an unmodified zeolite during anaerobic of an ammonia-rich substrate with an initial NH_4^+-N of 4.1 g/L.

Fig. 5 SEM photographs of the microbes immobilized on the surface of different zeolite adsorbents, 4000×. (A) Control (Natural zeolite); (B) $CaCl_2$ modified zeolite; (C) Eggshell modified zeolite; (D) ELMZ.

Figure 6 (A) Biomass immobilized on the surface of adsorbents and bedding materials in different bioreactors; (B) ATP value in the bioreactors with different zeolites during anaerobic digestion of ammonia-rich substrate at an initial NH_4^+-N of 4.1 g/L.

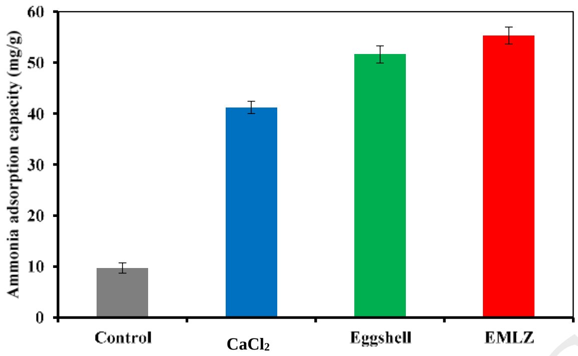

Ammonia adsorption capacity (mg/g)
60
50
40
30
20
10
0
Control
CaCl₂
Eggshell
EMLZ

Figure 1

(A) (B) (C)

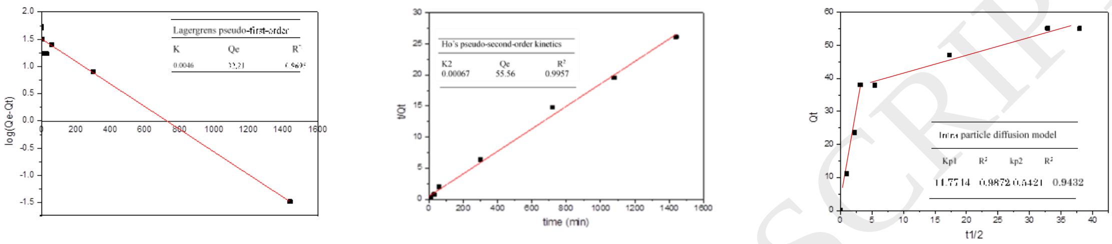

Lagergrens pseudo-first-order
K
Qe
0.0046
log(Qe-Qt)
Ho's pseudo-second-order kinetics
K2 Qe R²
0.00067 55.56 0.9957
t/Qt
time (min)
Intra particle diffusion model
Kp1 R² kp2 R²
11.7714 0.9872 0.5421 0.9432
Qt
t1/2

Figure 2

(A) (B)

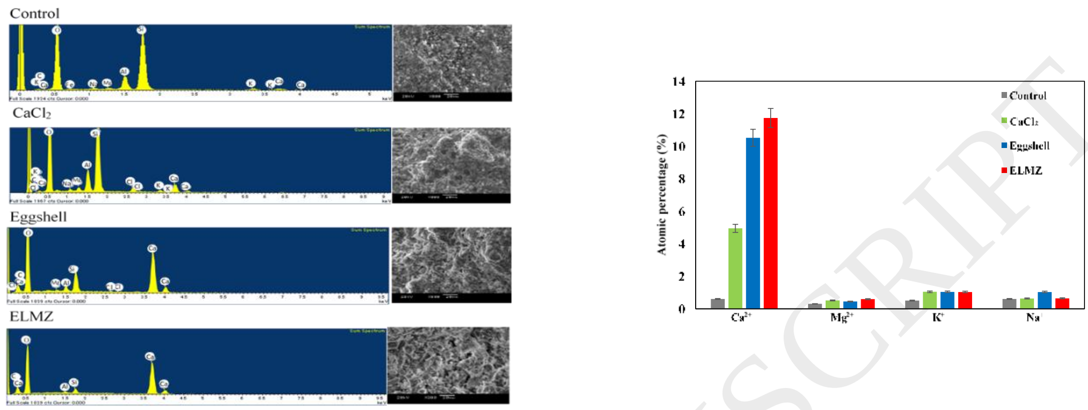
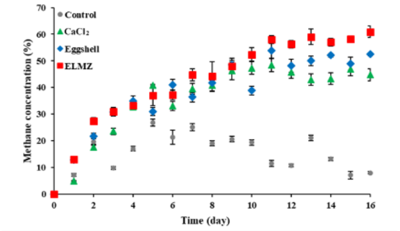
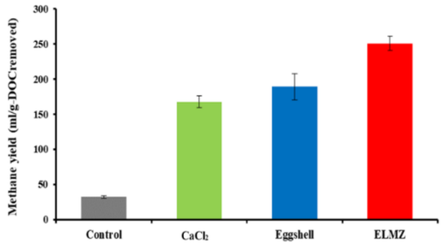

Control
CaCl2
Eggshell
ELMZ
Atomic percentage (%)
0
2
4
6
8
10
12
14
Control
CaCl2
Eggshell
ELMZ
Ca2+
Mg2+
K+
Na+

Figure 3

(A)


Control
CaCl2
Eggshell
ELMZ
Methane concentration (%)
Time (day)

(B)


Methane yield (ml/g-DOCremoved)
Control
CaCl2
Eggshell
ELMZ

(C)

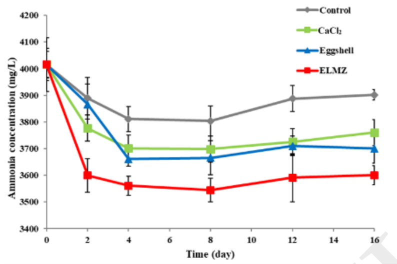

Control
CaCl2
Eggshell
ELMZ
Ammonia concentration (mg/L)
Time (day)

Figure 4

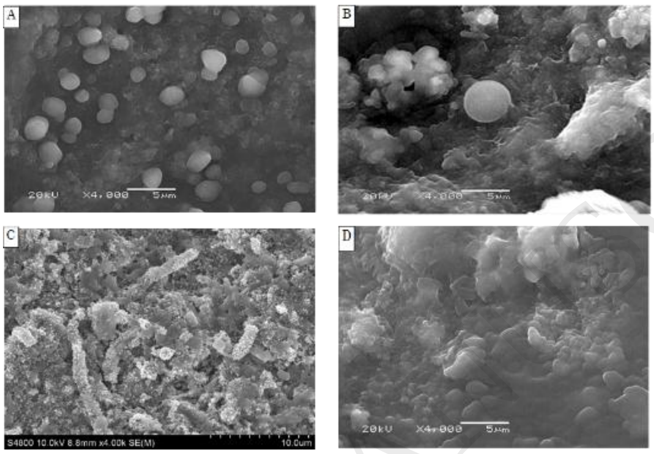
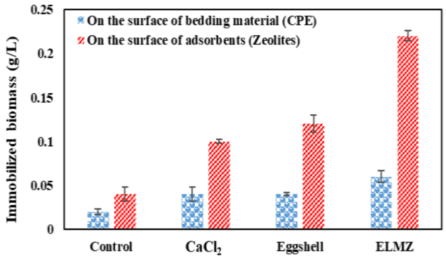

A
B
C
D
20kU X4,000 5μm
S4800 10.0kV 6.8mm x4.00k SE(M)
10.0um
20kU X4,000 5μm

Figure 5

(A)



(B)

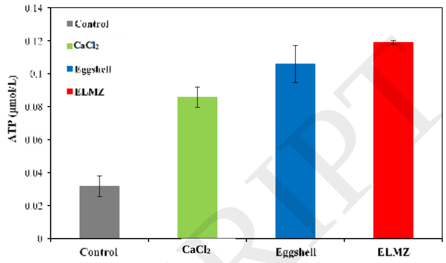


Figure 6

Table 1 Characteristic of seed sludge used in the experiments after dilution with deionized water.

Parameters	Digested sludge
Dissolved organic carbon (DOC, g/L)	6.4 + 0.2
Total nitrogen (TN, g/L)	5.5 + 0.2
Total solid (TS, g/L)	14 + 0.4
Volatile solid (VS, g/L)	9.0 + 0.4
Ammonium nitrogen (NH_4^+-N, g/L)	1.5 + 0.0
pH	7.0 + 0.1

***** Standard deviation; n=3

Table 2 Comparison between various zeolite adsorbents used for ammonium nitrogen removal.

Adsorbent	Initial NH_4^+-N concentration (mg/L)	Adsorbent dosage (mg/L)	Adsorbent capacity (mg/g)	Cost (US$/kg)	Refs. **
ELMZ	4100	10	53	0.95	This study
Silicate-carbon modified zeolite	5	--*	0.12	0.68	[10]
Hydrated aluminum	5000	10	30	2.6	[25]

oxide modified natural zeolite					
NaA zeolite from haslloysite mineral	300	4	44	2.7	[26]
Microwave-treated zeolite	200	3	23	2.3	[11]
Natural calcium-rich zeolite	25	10	4.0	0.6	[30]

*Data has not been mentioned clearly.

**The price of the chemicals was provided by the FUJI FILM WAKO Co. (Japan). The electricity price was used the data provided by US energy information administration.

Table 3 Langmuir and Freundlich isotherm constants for the ammonium adsorption for eggshell and lignite modified zeolite (ELMZ).

Langmuir isotherm			Freundlich isotherm		
b (L/mg)	q_m (mg/g)	R^2	k_f	1/n	R^2
1.4×10^{-4}	156	0.945	0.77	0.56	0.987

Table 4 Surface area characterization of different zeolite adsorbents.

	BET surface area (m^2/g)	One point BET surface area (m^2/g)	T-plot surface area (m^2/g)	Correlation Coefficient
Control	8.0	7.3	8.4	0.999
$CaCl_2$	8.0	7.2	7.6	0.999
Eggshell	15	15	15	0.999
ELMZ	15	15	15	0.999