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Development of a bio-zeolite fixed-bed bioreactor for mitigating ammonia inhibition of anaerobic digestion with extremely high ammonium concentration livestock waste

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HIGHLIGHTS

- A novel bio-zeolite fixed bioreactor was developed to mitigate ammonia inhibition.
- Ammonia adsorption and microbe immobilization contributed to enhanced efficiency.
- Chlorinated polyethylene (CPE) is more suitable to fix zeolite in the bioreactor.
- Fabriform/porous structures make CPE the best material for microbe immobilization.
- The developed bioreactor is promising for treating ammonium-rich livestock wastes.

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ABSTRACT

A novel bio-zeolite fixed-bed bioreactor combining with physicochemical and biological methods for mitigating ammonia inhibition was developed to improve the anaerobic digestion of ammonium-rich livestock wastes. The fixed-bed bioreactor was constructed by zeolite that was fixed using three different kinds of polymer materials including chlorinated polyethylene (CPE), polymer foaming sponge (PFS) and porous nylon (PN). Using ammonium-rich swine wastes (NH_4^+-N: 7511 mg/l) as substrate, a series of batch anaerobic fermentation experiments were carried out under 35 °C. The bioreactor suspending with CPE fixed zeolite showed the shortest start-up period (2 days) and the highest methane production (about 96 times higher than that of the Control) after 30-days anaerobic digestion. Furthermore, the higher and stable methane concentration and yield during 100-day of semi-continuous anaerobic digestion clearly indicated the long-term practical effectiveness of CPE-fixed zeolite bioreactor. Synergy of ammonia adsorption and microorganism immobilization by CPE fixed zeolite contributed to the enhanced anaerobic digestion efficiency. The developed novel CPE fixed zeolite bioreactor was suggested to be a favorable system for improve the anaerobic digestion of ammonium-rich agricultural wastes.

1. Introduction

Nowadays, the generation of livestock wastes constitutes a growing problem due to their negative environment impacts, such as pathogenic, contamination of soil, water and air without treatment. Livestock wastes have been identified as the major source of water contamination, accounting for 69% of contaminated streams and rivers was caused by these wastes [1]. Nevertheless, there exist many high-concentration biodegradable nutrients in the livestock wastes, so that the methane can be obtained via the biodegradation of such kind of wastes. Anaerobic digestion is usually considered as a promising option for the treatment of livestock wastes, because it cannot only solve the problem of waste contamination and reduce green house gas emission, but also obtain energy, economic and social benefits. However, anaerobic digestion of ammonium-rich livestock wastes is seriously inhibited by the ammonia generated during the biodegradation of organic nitrogenous compounds such as proteins, nucleic acid and amino acids [2]. The inhibition of high-concentration ammonia proposes a great challenge to anaerobic digestion of ammonium-rich livestock wastes.

Ammonia inhibition is often been observed in the anaerobic digestion of ammonium-rich livestock wastes like swine manure, which resulted in a low methane production and a high VFA level in the effluent. Moreover, it has been found that free ammonia is

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the active form causing ammonia inhibition [3]. It may diffuse passively into bacterial cells, leading to the proton imbalance and interfere with the metabolic enzymes of microorganisms [4]. The high concentration of ammonia is the major causes of digester upset or failure [5]. The methanogen activity was reported to decrease by 10% at ammonia concentrations of 1670–3720 mg N/l, decreased by 50% at 4090–5550 mg N/l, and dropped to zero at 5880–6000 mg N/l [6]. Removing ammonia during the anaerobic digestion has attracted considerable interest in recent years. Many physicochemical and biological methods have been developed to alleviate the inhibition of ammonia, such as ammonia striping [7,8], adding ammonia-adsorption materials [9–11], struvite precipitation [12,13] and anaerobic ammonia oxidization [14,15]. However, since their complex process, low efficiency and high operation costs, these methods are usually unsatisfactory to the practical application for ammonia removal. To improve the efficiency of anaerobic digestion, there is considerable interest in developing novel high-efficient bioreactor for the removal of ammonia.

Adding ammonia-adsorption materials such as bentonite, activated carbon and zeolite in the bioreactor has been demonstrated to be a promising option for enhancing the efficiency of anaerobic digestion. Among these adsorbents, zeolite which has high adsorption capacity, dissociation of Na^+, Ca^{2+} and Mg^{2+} cations and favorable characteristics for microorganism adhesion has been widely used for ammonia extraction in the anaerobic digestion [16]. In addition, Sasaki et al. [17] found that adding bed material such as carbon fiber textiles enables stable proliferation of acetoclastic methanogens via preventing ammonia inhibition in thermophilic bioreactors which includes high levels of ammonia. Meanwhile, the bed material as a decisive factor in the system's performance can determine the biomass retention capacity [18]. The characteristics of bed materials for immobilization have a significant influence on methane production [19]. The adherence and metabolites of microbes mainly depends on the physicochemical characteristics of fixed bed materials like specific surface area, porosity, surface roughness and pore size [19,20]. Many researchers have already revealed the importance of fixed bed materials to microbes in the anaerobic digestion system. Therefore, the combination of physical adsorption and microorganism's immobilization appears to be a promising technology for ammonia removal during the anaerobic digestion process. A fixed zeolite bioreactor using porous nylon bag packed zeolite has been developed by Wang et al. [11], and successfully used for the anaerobic digestion of ammonium-rich swine waste by shorten the start-up period and effectively increase methane production. The fixed zeolite could effectively remove ammonium in the bioreactor thereby enhance the anaerobic digestion performance. However, the author just used one kind bed material for fixing zeolite in the bioreactor and did not investigate the influence of microbes exerted by the bed material. Besides, there are few study have focused on the combination mode of zeolite with different bed materials, which is of great significance in a fixed zeolite bioreactor. Thus, this study attempted to develop a novel high-efficient bioreactor that consists of suitable bedding material and zeolite for the anaerobic digestion of ammonium-rich livestock wastes.

In this work, three novel fixed-bed systems were constructed using different bed materials sewed with zeolite. Different from Wang et al. [11], the contact rate between fixed-zeolite and digestate was greatly increased to improve the adsorption efficiency and create a favorable environment for the formation of bio-zeolite in this present study. Extremely high-concentration ammonium swine wastes (NH_4^+-N: 7511 mg/l) were used as the substrate, and four batch anaerobic fermentation experiments under 35 °C were performed using the developed novel fixed-bed bioreactor system. Different stable polymers including polymer foaming sponge (PFS), chlorinated polyethylene (CPE) and porous nylon (PN) were used as bed materials. The main cellular morphologies present in the biofilms of three different bed materials and zeolite were observed by scanning electron microscopy (SEM). The objectives of this work are: (1) development of a high-efficient adsorption and fermentation bioreactor, and (2) incubation of new functional microorganisms for anaerobic biodegradation of ultra-high ammonium-rich livestock wastes. This present study will provide a valuable platform for improving the biogas production efficiency and advance anaerobic digestion engineering.

2. Materials and methods

2.1. Bed materials

One of the most pressing concerns in using bed material fixed-zeolite system for anaerobic digestion of ammonium-rich substrate is selection of the most appropriate material for zeolite fixation. In order to improve the performance of anaerobic digestion of ammonium-rich livestock wastes, on one hand, the ammonium removal efficiency is of great importance and indispensable should be ensured by the sufficient contact between the zeolite absorbent and the digestate. On the other hand, the quantity of immobilized methanogens should be increased as the immobilized microbes have higher activity and tolerance to unfavorable environmental conditions. Thus, the selection of an appropriate bed material is very important to ensure the system's effectiveness and success.

In this present study, three different polymer materials including polymer foaming sponge (PFS), chlorinated polyethylene (CPE) and porous nylon (PN) purchased from a 100-yen shop in Tsukuba (Ibaraki, Japan) were selected as bed materials. The specific structure and porosity characteristics as well as low cost (i.e. 1.0, 1.5 and 1.2 dollars/m^2 of PFS, CPE and PN, respectively) of these polymer materials make them the chosen bed materials to construct the novel fixed zeolite system. Prior to use, the bed materials were firstly washed with distilled water to remove any non-adhesive impurities, and then dried in an oven at 105 °C for 12 h.

2.2. Seed sludge and swine manure

In order to obtain an extremely high-concentration ammonium condition, the stale pig manure was used in this experiment. The fresh manure had been kept at 4 °C for more than 2 years after been taken from a pig farm located in Ibaraki prefecture, Japan. The ammonium concentration was more than 7500 mg/l in this batch. Before inoculums, the mixture of 400 ml digested sludge and a trace mineral solution (200 ml/l) was put into an anaerobic reactor (500 ml). The trace mineral solution contains $FeSO_4{\cdot}7H_2O$ (1.11 g/l), $MgSO_4{\cdot}7H_2O$ (24.65 g/l), $CaCl_2{\cdot}2H_2O$ (2.94 g/l), NaCl (23.4 g/l), $MnSO_4{\cdot}4H_2O$ (111 mg/l), $ZnSO_4{\cdot}7H_2O$ (28.8 mg/l), $Co(NO_3)_2{\cdot}6H_2O$ (29.2 mg/l), $CuSO_4{\cdot}5H_2O$ (25.2 mg/l), $Na_2MoO_4{\cdot}2H_2O$ (24.2 mg/l) and H_3BO_3 (31.0 mg/l). After 1 day, 0.5 g glucose was added to the reactor every other days. The cultivation experiment was carried out at 35 °C for 15 days. The characteristics of swine manure and seed sludge used in this experiment are listed in Table 1.

2.3. Anaerobic digestion experiment

2.3.1. Batch anaerobic digestion of extremely high ammonium concentration swine wastes

A series of 300 ml fermenter bottles (SIBATA), each with 200 ml working volume, were used as the bioreactors. As shown in Fig. 1, four different bioreactors including a bioreactor without the fixed

Table 1
Characteristics of seed sludge and swine waste used in the experiments after dilution with deionized water.

Parameters	Digested sludge	Swine wastes
Chemical oxygen demand (COD, mg/l)	6500 ± 78	41,000 ± 62
Total nitrogen (TN, mg/l)	5489 ± 82	39,402 ± 103
Total solid (TS, mg/l)	13,292 ± 125	33,900 ± 178
Volatile solid (VS, mg/l)	9500 ± 71	27,150 ± 132
Ammonium nitrogen (NH_4^+–N, mg/l)	1547 ± 25	7511 ± 51
pH (–)	7.1	7.2

zeolite system as control and fixed zeolite system bioreactor immobilized respectively by PN (R_1), PFS (R_2) and CPE (R_3) zeolite were developed.

Each bioreactor contained 200 ml of diluted swine waste including 20% (w/w) digested sludge. To create an extremely high ammonium condition for methane fermentation of livestock waste, the initial ammonium concentration and pH of substrate was adjust to 7511 mg/l by adding NH_4Cl and 7.0 using 1 M NaOH and HCl, respectively. In the fixed zeolite bioreactor, 2 g/l zeolite A-3 was fixed in the bed materials and suspended in the diluted substrate. The dosage of zeolite is 10 g/l-reactor. Nitrogen gas was injected into each bioreactor to keep an anaerobic condition for methane fermentation. After that, the anaerobic digestion experiments were conducted in batch mode at 35 °C. At a given time interval, samples were periodically withdrawn from the sampling port on each bioreactor for the measurement of pH and ammonium concentration. A syringe attached to the reactor was used to collect the daily biogas production. The anaerobic digestion experiments were performed in duplicate, and the average values were used for analysis.

2.3.2. Semi-continuous anaerobic digestion of ammonium-rich swine wastes using the developed CPE-fixed zeolite bioreactor

The semi-continuous experiments on the anaerobic digestion of swine wastes were carried out at 35 °C. The CPE-fixed zeolite bioreactor and a 250 ml Duran bottle (SIBATA) with 200 ml working volume were used as the anaerobic fermenters. In the beginning, each bioreactor contained 200 ml of synthetic medium including 20% (w/w) digested sludge. The initial ammonium concentration was 3984 mg/l and the pH was adjusted to 7.0 using 1 M NaOH and HCl. After start-up, the bioreactors were fed with synthetic medium at different organic loading rate (OLR) and hydraulic retention time (HRT) as listed in Table 2. The sampling

Table 2
The hydraulic retention time (HRT) and organic loading rate (OLR) in the semi-continuous anaerobic digestion of swine wastes.

Operation periods (d)	HRT (d)	OLR (g-DOC_{added}/l-reactor/d)
0–37	35	0.15
38–68	14	0.51
69–100	7	1.01

and biogas collection methods are the same as those mentioned in Section 2.3.1.

2.4. Scanning electron microscope

The bed materials and the main cellular morphologies present in the fixing material and zeolite were observed using a scanning electron microscope (SEM, DS-720, Topcon, Tokyo, Japan). At the end of fermentation, the fixed zeolite systems were removed from the bioreactor, respectively. The bed materials and zeolite were washed using buffer solution, and then 2.5% (v/v) glutaraldehyde solution was used to fix the microorganisms about 2 h. After that, the samples were desalted with ultrapure water for six times and refrigerated at −80 °C about 2 h. Finally, the samples were dried for 24 h in a freeze dryer (FD-5, RIKAKIKAI, Tokyo, Japan). Before the SEM observation, the samples were coated with Pt powder. The pretreatment of samples was referred to Yang et al. [19].

2.5. Analytical methods

The yield and composition of the produced biogas were measured everyday. 2 ml of digestate was sampled every other day. The chemical oxygen demand (COD), total solid content (TS), volatile solid content (VS) and ammonium nitrogen (NH_4^+-N) were determined according to standard methods [21]. The pH was measured everyday by a pH meter (TES 1380). The activity of microorganisms was indicated by the adenosine triphosphate (ATP) concentration, which itself was determined using a Bac Titer-Glo™ Micro-bial Cell Viability Assay (Promega, USA). The composition of the produced biogas was analyzed by gas chromatography (GC-8A, SHIMAZU, Japan) using a machine equipped with a thermal conductivity detector (80 °C) and a Porapak Q column (60 °C). Nitrogen was used as the carrier gas. The sample was centrifuged at 10,000 rpm for 10 min (25 °C) to allow precipitation of the microbes after been taken. The supernatant was used

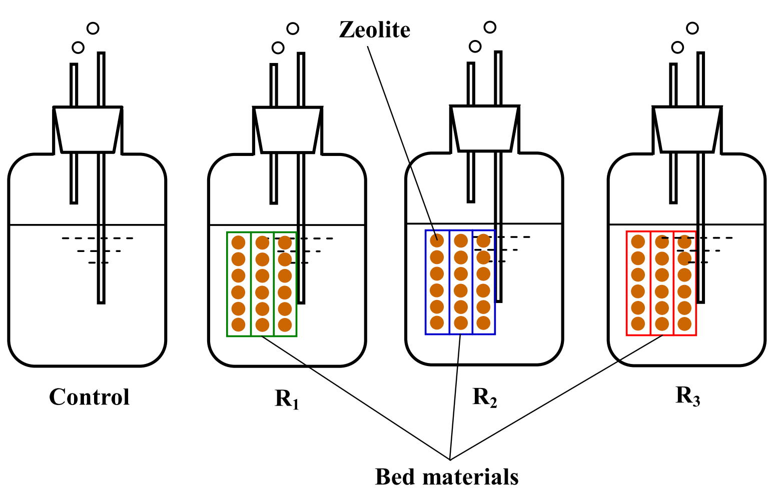

Fig. 1. Schematic of the bioreactors: control, porous nylon (PN) wrapped zeolite system (R1), polymer foaming sponge (PFS) wrapped zeolite system (R2), and chlorinated polyethylene (CPE) wrapped zeolite system (R3).

to measure the dissolved organic carbon (DOC) with a TOC analyzer (TOC-V CSN, Shimadzu, Kyoto, Japan).

3. Results and discussion

3.1. The characteristics of the bed materials

Fig. 2 shows microscope images of the three kinds of bed materials. The fabriform structure of PN (Fig. 2a), the porous structure of PFS (Fig. 2b), and fabriform and porous structure of CPE (Fig. 2c) were observed. It has been found that the surface of CPE is roughness with irregular defined channels and the aperture is very large. The rough surface with irregular channels might make CPE a very suitable material for immobilizing microorganisms and the large pore size provides zeolite and digestate with a sufficient space in contact with each other [11,19]. Nevertheless, the PFS and PN did not present adequate characteristics such as roughness and large surface area. PN is a fiber-like material with many pores and smoothly surface. The large quantity of pore could provide zeolite and digestate with sufficient contact area while the smooth surface could be not suitable for microbe's attachment. In terms of PFS, it has large porosity, but we have observed the pores is not connected to each other, hence the zeolite might work ineffectively. Meanwhile, the smooth surface of PFS make it unfavorable for microbes immobilization. On the whole, considering the physical characteristics of these three materials, CPE was supposed to be the most suitable and promising material for fixing zeolite to form a novel fixed zeolite system.

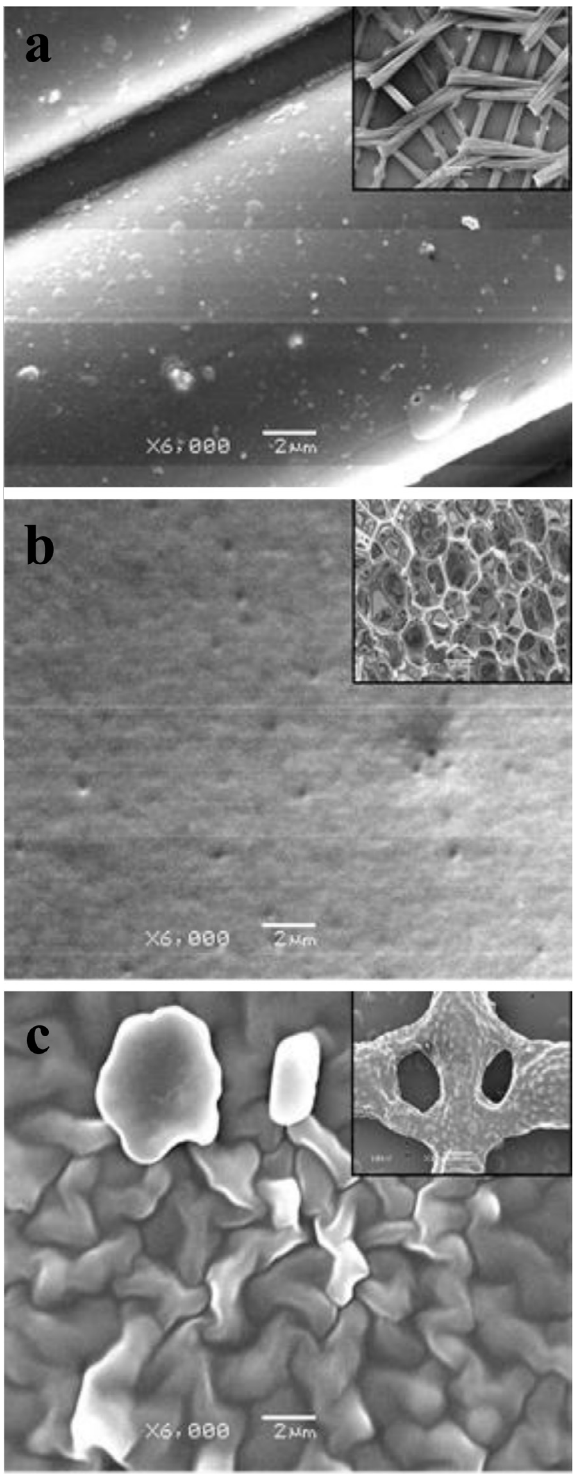

Fig. 2. SEM images of bed materials: (a) porous nylon (PN), (b) polymer foaming sponge (PFS), and (c) chlorinated polyethylene (CPE).

3.2. Reactor performance

The methane concentration and cumulative methane production are presented in Fig. 3a and b, respectively. As seen from Fig. 3a, compared to the control, three fixed zeolite bioreactors have showed higher methane content during 30-day anaerobic digestion. Among the three bioreactors, CPE fixed zeolite reactor had a better performance than the two others with regard to methane content. The highest average methane content of 80% was observed for CPE fixed zeolite reactor, while that of the other reactors were only ranged between 45% and 60%. Methane concentration is a representative parameter for anaerobic digestion process monitoring, which can be used to imply the steady balance of methane and carbon dioxide produced by the methanogenesis and acetogenesis [22]. Therefore, a high content of methane can indicate that the stable and balance of the methanogenesis and acetogenesis process, and if the methane content is low, means that there are some inhibition decreased the methanogenic activity. As shown in Fig. 3b, the cumulative methane production was attained from CPE-fixed zeolite bioreactor, and decreased in the following order: $R_3 > R_1 > R_2 >$ Control. The cumulative methane production of R_3 increased gradually during 30-day anaerobic digestion and obtained the maximum quantity of 139 ml/l-reactor at the end of the experiment corresponding to the methane concentration increased to 82.51% (Fig. 3a).

The higher methane content and accumulated methane production in R_3 indicated that using CPE fixed zeolite system achieved a more stable and better-performing anaerobic digestion of livestock waste with extremely high ammonium concentration. In CPE-fixed zeolite bioreactor, the partial removal of ammonia that is the main inhibitor to microorganisms [11] contributed to the enhancement of methane production. In addition, the CPE fixed zeolite system provided a favorable environment for the adherence of microbial consortium, resulting to the enhanced performance of anaerobic digestion related to microbes [19].

3.3. NH_4^+-N variation

Fig. 4 shows the variation of ammonium concentration in the four bioreactors during 30-day anaerobic digestion of the ammonium-rich swine wastes. The ammonium concentration of the three fixed zeolite bioreactors decreased at the beginning of the experiment, and then shows an increasing tendency in all bioreactors after 22 days. During days 15–22, the ammonium content in R1, R2 and R3 were ranged from 4000 to 5000 mg/l, while this value remained around 7000 mg/l in the control reactor.

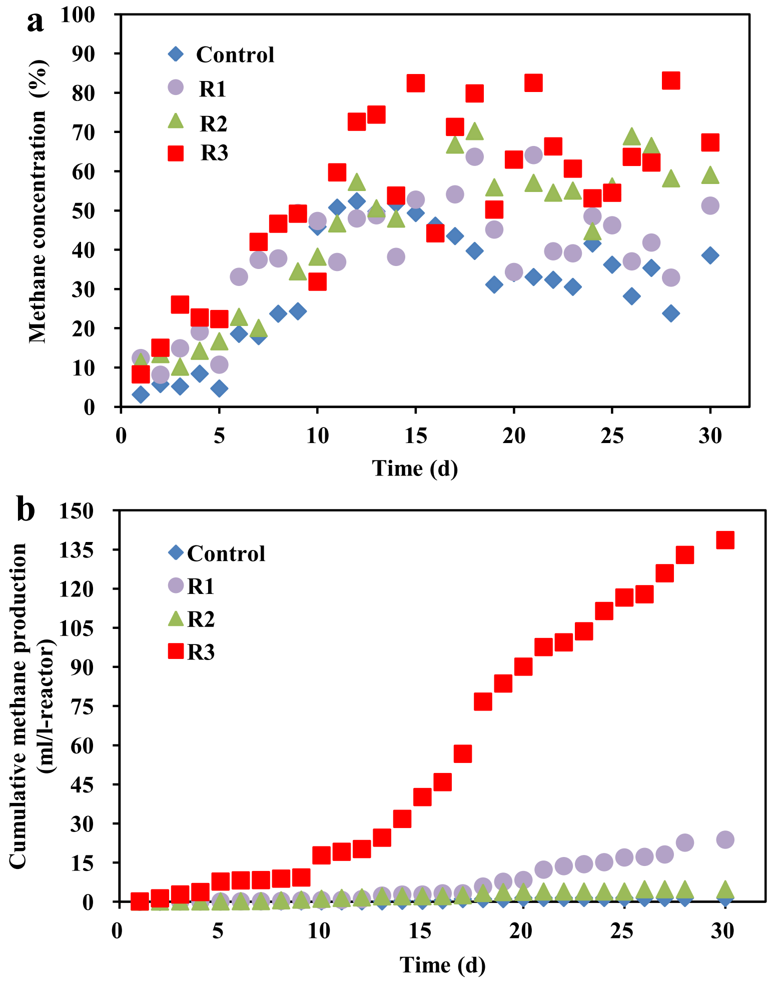

Fig. 3. Changes of (a) methane concentration and (b) cumulative methane production during 30-day mesophilic (35 °C) anaerobic digestion of ammonium-rich swine wastes in four bioreactors: control, PN wrapped zeolite system (R1), PFS wrapped zeolite system (R2), and CPE wrapped zeolite system (R3).

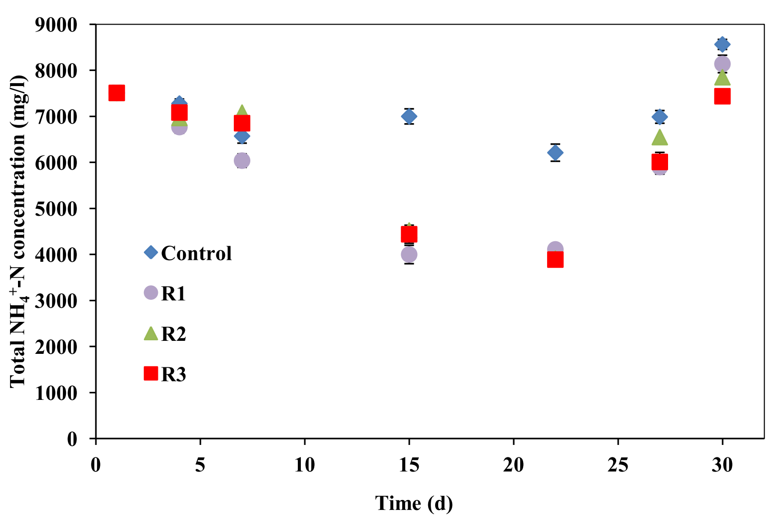

Fig. 4. Changes of total ammonia nitrogen concentration in four bioreactors: control, PN wrapped zeolite system (R1), PFS wrapped zeolite system (R2), and CPE wrapped zeolite system (R3).

The decreased NH_4^+-N content in fixed zeolite bioreactors alleviated the ammonia inhibition, and resulted to the improvement of methane production (shown in Fig. 3a and b). The better mitigation of ammonia inhibition and higher methane production in R_3 were partially attributed to effective ammonia removal by the CPE fixed zeolite system. The similar result has been observed by Yang et al. [15] and Resch et al. [23] who reported that an effective reduction of ammonium could shorten start-up period and improve anaerobic digestion performance. Although the quantity of ammonium adsorbed by three fixed zeolite systems is almost the same because the amount of added zeolite was equal in all the reactors, the anaerobic digestion performance of each bioreactor was different. As shown in Fig. 3a and b, CPE fixed zeolite reactor obtained the best performance than the other two fixed zeolite reactors. It achieved the highest average methane content of 80%, while that of the others only ranged from 45% to 60%. The bedding materials (PN, PFS and CPE) possess significant distinct surface morphology and inner structure, as seen from the SEM images in Fig. 2, which contributed to the different quantity and activity of immobilized microorganisms in each bioreactor. The fabriform and porous structure of CPE compared with PN and PFS is more suitable for immobilizing biomass in the zeolite-fixed bioreactor. Besides, the higher methane concentration in CPE-fixed zeolite bioreactor can also be attributed to the better buffer capacity of its liquid mixture.

After day 22, when the ammonium adsorption on fixed zeolite reached equilibrium, the ammonium concentration in R1, R2 and R3 started to increase due to continuous generation of ammonia during the biodegradation of nitrogenous organics in swine wastes (Fig. 4). The same phenomenon was also observed by Halim et al. [24]. The increasing ammonium concentration leads to the fluctuation of methane production in the fixed zeolite bioreactors after day 25 (Fig. 3a). The total NH_4^+-N concentration in the three fixed zeolite bioreactors of PN, PFS and CPE and the control bioreactor increased respectively to 8140,7851, 7439, 8565 mg/l at the end of 30-day anaerobic digestion. However, the performance of CPE fixed zeolite reactor has almost not been affected (Fig. 3a).

During the start-up period (0–15 days), the CPE fixed zeolite system decreased the ammonium concentration and provided a relative favorable environment for microorganism's growth. It was well known that the formation of biofilm usually needs 2–3 weeks [25]. Therefore, the biofilm has formed by microbes attached on the CPE bed material and zeolite within 20 days in this present experiment. Since biofilm has good resistance to the unfavorable environment even the ammonium concentration increased [26], the CPE fixed zeolite bioreactor remained stable and successful.

3.4. SEM observation

Fig. 5 shows the SEM images of the bed materials and zeolite with microbes. As seen from this figure, the microbes were successfully immobilized on the three bed materials and zeolite. The quantity of microbes attached on the novel system follows the order of CPE fixed zeolite > PN fixed zeolite > PFS fixed zeolite. The highest quantity and diversity of morphologies among the three fixed zeolite systems was found in the CPE fixed zeolite

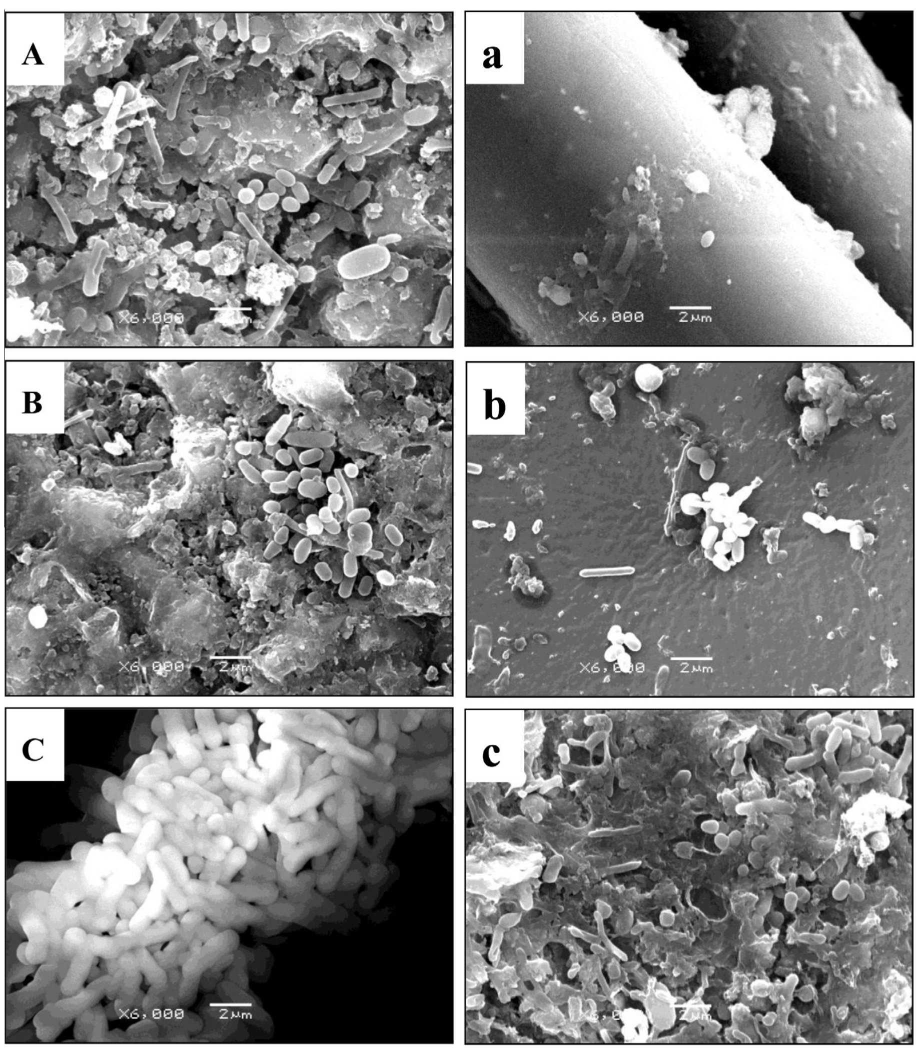

Fig. 5. SEM images of anaerobic microbes immobilized on the surface of zeolite and bed materials. (A) PN-zeolite, (a) PN, (B) PFS-zeolite, (b) PFS, (C) CPE-zeolite, (c) CPE, 6000×.

reactor. It is clear that the majority of microbes immobilized on the surface of zeolite other than bed materials, and the diversity of morphologies can be observed. The diversity of methanogens plays an important role in the anaerobic digestion performance. The SEM images revealed that different species of bed material gives specific conditions for the adherence of distinct microbe quantity and types, the immobilized biofilm on the bed material fixed zeolite bioreactors were primarily composed of coccus, coccobacillus of Methanosarcina-like bacteria, short rods Methanosaeta-like cells and long rods of Methanobacterium-like cells. Short rods Methanosaeta-like cells and long rods of Methanobacterium-like cells prevailed on the zeolite, while coccalmethanogens closely resembling to *Methanosarcina* predominated on the bed materials, especially on the CPE [27–29]. This is probably due to the fabriform and porous structure of CPE offered sufficient contact possibility to zeolite and microbes, and the roughness surface of CPE and zeolite have provided the microorganisms with favorable condition to immobilize on (Fig. 2). Therefore, the bioreactor with CPE fixed zeolite system showed the highest tolerance to high-concentration ammonium environment. Compared to a suspended growth system, the system with immobilized cells has a better tolerance to higher levels of toxic material [26]. In addition, by the immobilization of hemicellulolytic bacteria on trace metal activated zeolite, the biogas production from hemicellulose-rich substrates was greatly enhanced by 53% [30]. It has been verified in this present experiment that the performance of CPE fixed zeolite reactor remained stable even with ammonium concentration increased again after 20 days. The adsorption of ammonia by the fixed zeolite and the large amount of microorganisms attached on the surface of CPE fixed zeolite system contributed to the improvement of the anaerobic digestion performance of ammonium-rich swine wastes. This result can be further confirmed by the biomass and ATP amount of microbes attached on three fixed-bed systems, which will be illustrated in the next section.

3.5. Microorganism quantity and activity

Biomass can reflect the quantity of the microorganisms, while ATP is an indicator of metabolically active cells or an index of microbial density. Therefore, ATP and biomass are both important parameters that could reflect the performance of anaerobic digestion [31]. In this present study, biomass was determined on the surface of bed materials and zeolite of three fixed zeolite systems at the end of anaerobic digestion, respectively. The results exhibited that the biomass value is small on bed materials but the quantity on the surface of zeolite is much higher that corresponding to the SEM observation results (Fig. 6a).

ATP concentration on the surface of the zeolite which immobilized highest quantity of microbes was measured. The ATP value on

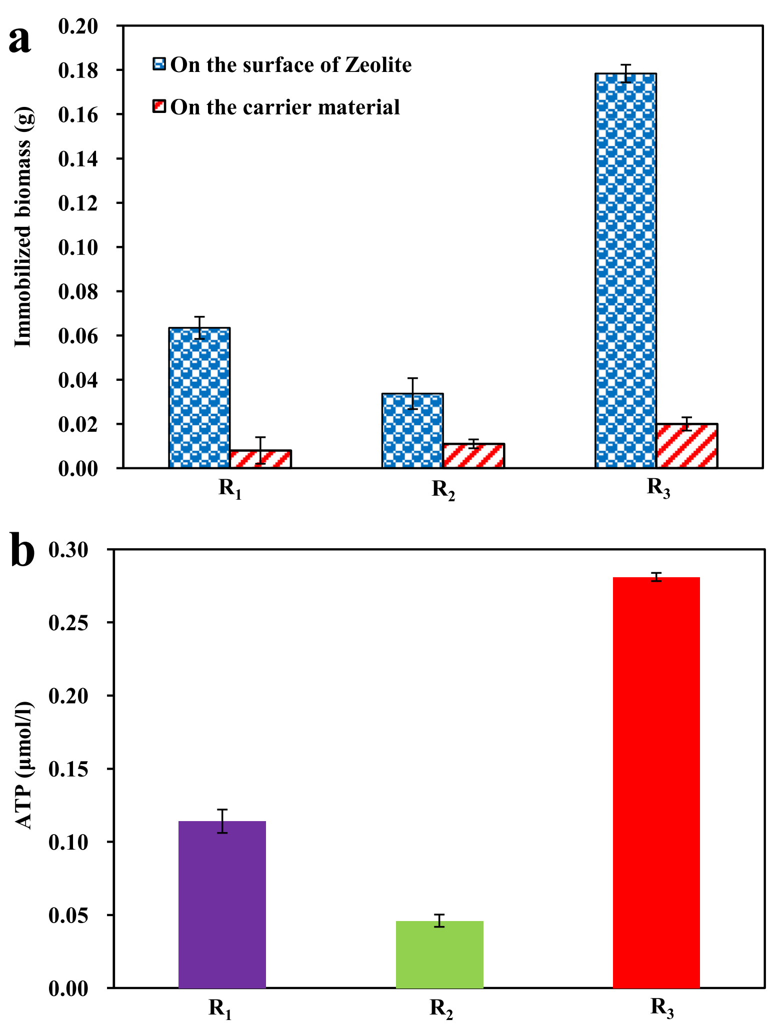

Fig. 6. (a) Biomass immobilized on the surface of zeolite and bed materials in three bioreactors: PN wrapped zeolite system (R1), PFS wrapped zeolite system (R2), and CPE wrapped zeolite system (R3); (b) ATP values of anaerobic microbes attached on the zeolite in fix-bed bioreactors at the end of the experiment. The bars designate standard deviations (95% confidence, *t*-test).

the surface of zeolite in the CPE fixed zeolite bioreactor is much higher than that in the other two reactors (Fig. 6b). ATP is an indicator of metabolically active cells and an index of microbial density, a large quantity of microbes contribute to the higher concentration of ATP [32]. Therefore, this result indicated higher activity levels of microorganisms attached on the CPE fixed zeolite system.

These high activity and quantity of the immobilized microbes attached on the surface of the zeolite fixed by CPE indicated that CPE is a promising and suitable carrier for microbes. The immobilized microbes, which have a high tolerance to toxic environments, produced positive impact on the anaerobic digestion of extremely high-concentration ammonium swine wastes. The SEM observations, biomass quantity and ATP concentration provided strong supports to explain the better performance and high efficiency of CPE fixed zeolite system bioreactor. Therefore, the CPE fixed zeolite system bioreactor showed the best performance with regard to the anaerobic digestion of extremely high-concentration ammonia swine wastes.

In order to investigate the long-term effectiveness of this CPE fixed zeolite bioreactor, a continuous anaerobic digestion operation should be carried out in the further study.

3.6. Semi-continuous anaerobic digestion of ammonium-rich swine wastes using CPE-fixed zeolite bioreactor

In this present experiment, semi-continuous anaerobic digestion of swine wastes was performed to investigate the long-term practical effectiveness of CPE-fixed zeolite bioreactor. The results are illustrated in Figs. 7 and 8.

As shown in Fig. 7a, the CPE-fixed zeolite bioreactor was beginning to produce methane at the second day, while the start-up period needs four days in the Control. As low organic loading rate (OLR) of 0.12–0.51 g-DOC_{added}/l-reactor/d in the first and second period, methane production in the bioreactors showed a fluctuation along with feeding substrate. Methane production increased when the synthetic medium was added, and then decreased after the carbon source was consumed. When the OLR increased to 0.51–1.01 g-DOC_{added}/l-reactor/d in the second and third periods, the methane production in CPE-fixed zeolite bioreactor increased and was obviously higher than that in the Control. This result indicated that the better tolerance of immobilized anaerobes to high OLR and NH_4^+-N concentration contributed to the higher methane production in CPE-fixed zeolite bioreactor.

The variation of daily methane concentration in CPE-fixed zeolite bioreactor and the control under different OLR and HRT are shown in Fig. 7b. During the first HRT (35 d) at an OLR of 0.12 g-DOC_{added}/l-reactor/d, the CPE-fixed zeolite bioreactor compared with the control achieved higher and more stable methane concentration. When increasing the OLR to 0.51 g-DOC_{added}/l-reactor/d under subsequent HRT (14 d), the CPE-fixed zeolite bioreactor remained stable methane concentration around 90%, while that in the control exhibited a slight decrease and fluctuation. Further increasing the OLR to 1.01 g-DOC_{added}/l-reactor/d in the third HRT of 7 d, methane concentration in the control decreased dramatically, whereas that in the CPE-fixed zeolite bioreactor remained as high as 80–90%. The CPE-fixed zeolite bioreactor obtained higher and more stable methane concentration, even under short HRT, high OLR and NH_4^+-N conditions. The result showed that CPE-fixed zeolite system could effectively enhance methane concentration in the bioreactor, and this enhancement depended on the specific immobilization mode of zeolite by CPE bed material.

Fig. 8 shows the average methane yield in the bioreactors during three HRTs. In the first HRT (35 d) at OLR of 0.15 g-DOC_{added}/l-reactor/d, the average methane yield in CPE-fixed zeolite bioreactor was slight higher than that in the control. CPE-fixed zeolite bioreactor achieved obviously higher average methane yields of 297 ml-CH_4/g-DOC_{added} in the second HRT (14 d) and 212 ml-CH_4/g-DOC_{added} in the third HRT (7 d), which was respectively 3.1 and 2.8 times of that in the control. The higher average methane yield in CPE-fixed zeolite bioreactor can be ascribed to the higher biomass concentration on the CPE fixed zeolite system during the anaerobic digestion of ammonium-rich swine wastes. Therefore, the higher methane concentration and yield during 100-day semi-continuous anaerobic digestion clearly indicated that CPE-fixed zeolite bioreactor is a suitable and promising option for the anaerobic biogas production from ammonium-rich livestock wastes.

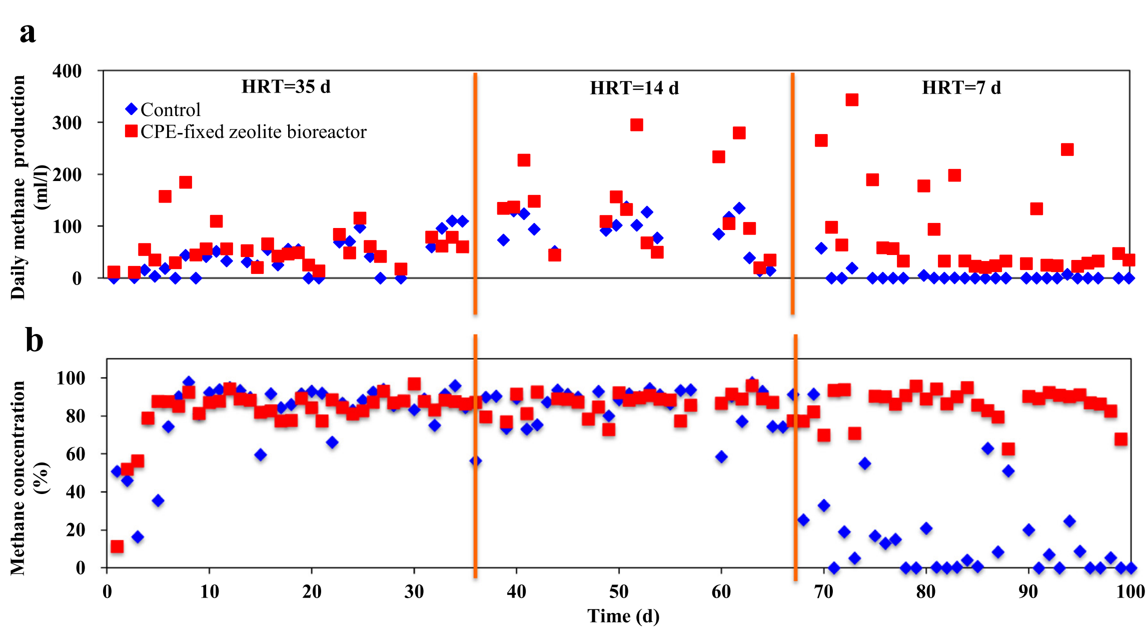

Fig. 7. The variation of daily methane production (a) and methane concentration (b) during 100-day semi-continuous anaerobic digestion of ammonium-rich swine wastes.

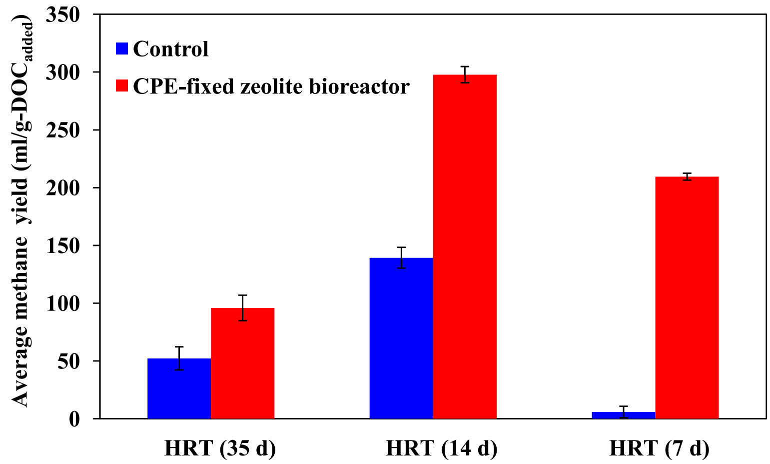

Fig. 8. The average methane yield under different hydraulic retention time (HRT) and organic loading rate (OLR) during 100-day semi-continuous anaerobic digestion of ammonium-rich swine wastes.

4. Conclusions

A novel fixed zeolite bioreactor was developed to improve the anaerobic digestion performance of ammonium-rich swine wastes via effectively mitigating the ammonia inhibition. Fabriform and porous structure of CPE has proven to be the best bed material for microorganism immobilization in the fixed zeolite bioreactor on anaerobic digestion of extremely high-concentration ammonium livestock wastes. Synergy of ammonia adsorption and microorganism's immobilization by CPE fixed zeolite system contributed to the enhanced anaerobic digestion efficiency. The developed high-efficient CPE fixed zeolite bioreactor is suggested to be a favorable system for enhancing the anaerobic digestion efficiency of ammonium-rich agricultural wastes.

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