Development of High-efficient Illuminated Bio-zeolite Fixed Bed Process for the Anaerobic Digestion of Ammonia-rich Waste

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Hanying ZHENG

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Hanying ZHENG

Abstract

Anaerobic digestion (AD) of livestock waste is an attractive practice as it can solve the problem of waste contamination and produce renewable energy. However, the ammonia which is rich in livestock waste or produced as a metabolic end product during the biodegradation of high nitrogenous compounds, is toxic to methanogens and often causes failure of the whole biological process at high concentration. Several methods, such as ammonia stripping, struvite precipitation or adding ammonia adsorption material, have been developed to alleviate ammonia inhibition. However, all these methods have pose certain limitations like energy intensity and large effluent discharge that are challenging for their practical application. Bedding material fixed-bed reactor by adding absorbent to immobilize microorganism of bioreactor in ammonium rich waste anaerobic digestion can be used to minimize these limitations. Zeolite, could adsorb ammonia and dissociate trace elements like Ca^{2+} and Mg^{2+} (via ion-exchange), provide microbes favorable environment for growth. Anaerobes immobilize on bed materials could create a high cell density and activity for resistance of high ammonia environment. Nevertheless, there is few research investigated the combination effects of suitable bed material and zeolite to mitigate ammonia inhibition in AD process. Meanwhile, zeolite contains trace amount of metal cations showed limited ion-exchange capacity with low ammonium adsorption ability. Modification works on zeolite designed specifically for the high ammonium adsorption capacity and nutritional cations supplement are required. Furthermore, it has been found that intermittent illumination could relieve ammonium inhibition and

enhance methane production. Therefore, a novel bioprocess incorporated optimal bed material fixed modified-zeolite bioreactor with intermittent illumination was developed for alleviating ammonia inhibition to improve the efficiency of ammonium-rich AD.

Firstly, to find the optimal bed material, three typical polymer materials including polymer foaming sponge (PFS), chlorinated polyethylene (CPE) and porous nylon (PN) were investigated. The zeolite fixed bed bioreactors were constructed by suspending these three bedding materials fixed zeolite in the anaerobic digester, respectively. Series batch experiments were operated under ammonium-rich condition (NH_4^+-N: 7,511 mg/L). The CPE fixed zeolite bioreactor 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 operation. Then the CPE fixed zeolite bioreactor was applied in the long-term semi-continuous ammonium-rich. Higher and stable methane concentration and yield during 100 days semi-continuous AD clearly indicated the long-term practical effectiveness of the CPE fixed zeolite bioreactor. Synergy of ammonia adsorption and microorganism immobilization by CPE fixed zeolite contributed to the enhanced anaerobic digestion efficiency. The developed CPE fixed zeolite bioreactor was suggested to be a favorable system for improving the anaerobic digestion efficiency of ammonium-rich livestock wastes.

After that, zeolite optimization was conducted. Oyster shell with high $CaCO_3$ content has high ammonium exchange ability, and lignite contains abundant mineral elements could be a good source for cations supplement. Thus, oyster shell and lignite

were used as modification material to improving cations content and ammonia adsorption ability of zeolite. Additionally, intermittent illumination strategy have been further adopted for developing the high-efficient bioprocess. SEM morphology shows newly synthesized oyster shell and lignite modified zeolite (OLMZ) had porous structure and rough surface, with increased BET surface area compared to raw zeolite (UMZ). After modifying by oyster shell and lignite, metal cations content in newly synthesized OLMZ increased obviously and ammonia adsorption capability of OLMZ was improved by 1.3 folds compared with UMZ. Adsorption kinetics and isotherm results verified ammonia adsorption on OLMZ was dominated by ion exchange with high affinity for ammonia uptake. The results suggested that OLMZ is a much more excellent adsorbent than UMZ on ammonia uptake. As for the AD, the illuminated OLMZ fixed bioreactor process (OLMZ-I) resulted in the highest methane yield of 372 ± 13 mL/g-$DOC_{removed}$, which showed 300% increase compare to the control (124 ± 12 mL/g-$DOC_{removed}$). Correspondingly, metal cations supplement and light stimulation in OLMZ-I showed increased ATP and coenzyme F_{420}, implying high activation of methanogens leading to improved CH_4 production. The large biomass quantity immobilized (Biomass and SEM observation) in the OLMZ-I further supported the enhanced performance of the reactor. Meanwhile, the conductance of sludge in OLMZ-I increased about 3 folds compared to the control, reflecting the electrical communication between anaerobes was strengthened. The synergetic effects of ammonia removal, microbes immobilization, metal cations supplement and accelerated electrical communication between methanogenic system combined with

light stimulation on methanogen activation facilitated the great enhancement of methanogenesis. Therefore, the illuminated OLMZ fixed bioprocess shows great potential in practical application for high-efficient bioconversion of ammonium-rich livestock waste.

Contents

Tables

Figures

Chapter 1 Introduction

1.1 Background

Along with the development of the world economy and the upgrading of the agricultural structure, the modes of livestock and poultry breeding have transformed from former household feeding separately by farmers to intensive factory farm and are mostly centralized in the vicinity of the city. These industrial farms discharge a large amount of livestock manure into the surrounding environment, making the pollution of livestock wastes a prominent environmental problem.

Based on the investigation by the Food and Agriculture Organization of the United Nations (FAO), global demand and production of animal source food have increased about 3 times since 1960 [1]. About 330 million cattle, 1.5 billion pigs, 1 billion goats are breed for livestock product widely every year [2]. China is the largest pork producer in the world, the amounts of hogs breed vary between 490 million and 618 million for 2009–2010, accounting for nearly 42% of global production [3,4]. The total amount of generated livestock wastes was reached to around 40 billion tons annually. In Japan, there are 79 million tons of livestock waste have been produced every year [5]. Such huge amount and continuous generation of livestock wastes have caused growing environmental problems, including the pollution of water, air and soil, as well as the potential hazard to human and animal health.

Livestock wastes have been identified as the largest contributor to water contamination, contributing to nearly 69% of streams and rivers contamination [2]. However, livestock wastes are usually rich in organic carbon, which can be used as a

promising feedstock for bioenergy conversion rather than discharged as a waste. Therefore, the development of high-efficient and environmental-friendly techniques for disposing large quantities of livestock wastes is necessary. Since high concentration of organics contained in livestock waste, to convert such organic wastes into bio-energy is of great importance for waste reduction and resources recycling. Anaerobic digestion is considered as one of the most promising approach in the treatment of livestock waste in recent decades.

1.2 Anaerobic digestion process

Anaerobic digestion is a cost-effective and environmental-friendly technology for the disposal of livestock wastes, due to the reduction of organic wastes and the recovery of bioenergy in the form of H_2 and CH_4 simultaneously [6]. The anaerobic digestion of livestock wastes can reduce approximately 40% volume of the wastes by a biodegradation of the organic substrates into biogas. In addition, the produced biogas is a promising alternative to fossil fuels and the anaerobic digestate can be used as organic fertilizer for planting.

1.2.1 Theory of anaerobic digestion

Anaerobic digestion is multi-step biological process in which the biodegradable organic substrates can be eventually degraded to methane by various microorganisms under anaerobic conditions. Generally, anaerobic digestion process consists of four biochemical reaction steps, including hydrolysis, acidogenesis, acetogenesis and methanogenesis [7], as shown in Fig. 1.1.

In the first step of hydrolysis, complex organic substrates such as carbohydrates, lipids and proteins are hydrolyzed to simple and soluble organic compounds by the hydrolytic bacteria [8]. Then, the acidogenic bacteria metabolize the soluble organics like monosaccharide, fatty acids and amino acids, and produce organic acids. After that, organic acids generated in the acidogenesis step are further degraded by acetogens to H_2, CO_2 and short-chain fatty acids. Finally, methanogenesis play a dominant role in this period. The products of the previous steps, like acetic acid and H_2, can be converted to CH_4 and CO_2 by methanogens [9].

1.2.2 Ammonium inhibition during ammonium-rich livestock waste anaerobic digestion

Over the past decades, anaerobic digestion has been widely employed as a suitable technique for the bioconversion of livestock wastes to bioenergy. However, the anaerobic oxidation of livestock wastes is usually inhibited by excess concentration of ammonia [10]. Ammonia is one of the end products during biodegradation of organic nitrogenous compounds, such as proteins, nucleic acids and urea [11]. Livestock wastes like swine manure contain high amounts of protein. During the hydrolysis step of anaerobic digestion process, fermentative bacteria hydrolyze proteins to polypeptides and amino acids [12]. Then, shorter chains of amino acids are produced as important hydrolysates, and finally degraded to ammonium.

Ammonia inhibition affects the anaerobic digestion process to different levels ranging from inhibited steady state where mainly methanogens are inhibited and

volatile fatty acids (VFAs) are accumulated to severe inhibition affecting all stages of the digestion process [13,14]. Livestock waste is rich in proteineous organic compounds, high concentration of ammonia would accumulate during the biodegradation [15]. The inhibition of high concentration ammonia poses a great challenge to the anaerobic digestion of ammonium-rich livestock wastes. Studies on the ammonia inhibition mechanism and mitigation strategies play an important role in making use of that material in environmental cleaning and bioenergy producing activities.

1.3 Ammonia inhibition problems

1.3.1 Ammonia inhibition mechanism

Anaerobic biodegradation of livestock waste is limited by many factors, especially the ammonium nitrogen concentration [16,17]. Suitable concentration of ammonium worked as N-nutrient source which is necessary for the growth and activity of microorganisms, while excessive amount will induce toxicity to anaerobes, inhibit their activity even leading to the process failure [18]. During the fermentation of livestock waste, most of the nitrogenous organics are hydrolyzed to ammonia nitrogen, and mainly exist in the form of ammonium ion (NH_4^+) and free ammonia (NH_3), only a small amount is taken-up and utilized by the anaerobic microbes for growth. NH_3 decreases the activity of methane synthesis enzymes directly and ammonia molecules could easily penetrate the microbial cell membrane which are rapidly converted into ammonium leading to proton and intracellular pH imbalance [19]. There are two main pathways of methane production in the methanogenesis

process, acetoclastic methanogenesis dominated by acetoclastic methanogens and syntrophic acetate oxidation (hydrogenotrophic methanogenesis) dominated by hydrogenotrophic methanogens, which contribute to 70% and 30% of methane production, respectively [20]. Generally, the inhibitory effect of ammonia on acetoclastic methanogens was more serious than that on hydrogenotrophic anaerobes [10,19]. Angelidaki et al., pointed that high concentration of free ammonia caused disastrous effects on anaerobic microorganisms, particularly methanogens [10]. Hansen et al., reported that high ammonia content leads the methanogens to hydrogenotrophic methanogenesis pathway and the tolerance of hydrogenotrophic to high levels of ammonia can be improved after adaptation, whereas the acetotrophic methanogens are inhibited [14].

1.3.2 Factors affecting the ammonia inhibition

Ammonia inhibition may occur as a result of total ammonium nitrogen (TAN) levels up to 1,500–7,000 mg/L based on the AD condition [15]. Lay et al. have observed a decrease in methanogenic activity by 10% at ammonium concentration of 1,670–3,720 mg N/L, decreased by 50% at 4,090–5,550 mg N/L, and fell to zero when further increased to 5,880–6,000 mg N/L [22]. While in another work, the glucose degradation was decreased by about 70% with TAN concentration at 3,500 mg/L [23].

The concentration of free ammonia depends mainly on three parameters: the total ammonia concentration, temperature and pH, which can be represented as [14,24]:

$$NH_3\,(\text{Free}) = \text{TANX}\left(1 + \frac{10^{-\text{pH}}}{10^{-(0.09018+\frac{2729.92}{T\,(\text{K})})}}\right)^{-1} \quad (1)$$

The free ammonia concentration increases with increasing temperature, and the biogas process becomes more sensitive towards ammonia when the pH value increases [24]. Proper pH control of anaerobic digestion process might alleviate the toxicity of ammonia to the microorganisms [25]. Besides, the acclimation of microbes involved in anaerobic digestion process to adapt to high ammonia concentration could also mitigate the ammonia inhibition [26,27]. Hashimoto reported that the inhibitory concentration of NH_3 enhanced from 2,500 mg/L to 4,000 mg/L by the acclimation of methanogens in anaerobic digestion [28]. Nevertheless, these strategies have not always been followed by a complete recovery of the original methane production. Therefore, ammonia removal by some effective methods is essential to mitigate ammonia inhibition in the anaerobic digestion.

1.4 Methods for ammonium removal

Ammonia inhibition intrudes the anaerobic digestion of ammonium-rich organic substrates and even leads to the process failure, resulting in serious economic losses of biogas plants. The removal of ammonia could enhance the anaerobic digestion performance of ammonium-rich organic wastes. Parkin and Speece found that the ammonium toxicity was reversible, and the anaerobic digestion system could rapidly recover to full biogas production if the ammonium in the supernatant were removed [21]. Recent years, several different strategies including physical, chemical and biological methods have been adopted for ammonia removal.

1.4.1 Physical methods

To remove ammonia from the substrate, many traditional physical methods, such as air stripping and adsorption, have been applied to decrease the inhibition of ammonia.

(1) Air stripping

Air stripping has proved to be a good option for treating different kinds of waste, such as liquid fraction of dewatered sewage sludge and landfill leachate [23,29]. During air stripping, ammonia is transferred from waste stream into the air, and then absorbed by a strong acid solution, thereby generate an ammonium-salt which can be crystallized. In order to obtain high ammonia removal efficiency, the addition of numerous alkali like NaOH is often required in the process to guaranteed high pH (10.5-11.5) [30]. The resulting high concentration of Na^+ might readily influence the metabolism and activity of methanogens. Rinzema et al. have reported that Na^+ concentration of 14 g/L could cause complete inhibition of methanogens in an anaerobic granular biomass at neutral pH and room temperature [31]. When air stripping is performed at high temperature, the high buffering capacity of pig slurry may be able to maintain pH at the favorable value, without alkali addition in the process. However, ammonia air stripping at high temperature may cause a greater fraction of toxic free ammonia and often requires more thermal energy input [30].

(2) Adsorption

Adding ammonia-adsorption materials in the anaerobic digestion process could effectively remove ammonia. Zeolite is now considered as the most effective and

practical ammonia absorbent, due to its high capacity for ammonia adsorption, wide distribution and low-cost. It has been widely used as an ion exchanger for ammonia extraction in anaerobic digestion due to the presence of Na^{+}, Ca^{2+} and Mg^{2+}cations in its crystalline structure [32]. Borja et al. have reported that adding zeolite could effectively remove ammonia and improve the methane production rate in the anaerobic digestion of cow manure [33]. Milán et al. have found that the anaerobic digestion process was favored by the addition of natural zeolite at doses between 2 g/L and 4 g/L [34]. With properties of ionic exchange and microbes fixing, zeolite seems to be a promising ammonia adsorbent and microorganism carrier for mitigating the ammonia inhibition in the anaerobic digestion of ammonium-rich livestock wastes.

1.4.2 Chemical methods

Struvite precipitation is commonly known as an efficient chemical method to remove and recover nitrogen. The removal of N by struvite precipitation ($MgNH_4PO_4$ $6H_2O$) can not only mitigate the ammonia inhibition of the anaerobic digestion process, but also reduce the amount of sludge need to be disposed from the treatment facilities [35]. It has been applied to treat different types of wastewaters, such as swine waste, agro-industrial effluents [36,37].

Uludag-Demirer et al. coupled the anaerobic digestion of dairy manure and controlled struvite precipitation in the same reactor, by adding 1,750 mg/L of $MgCl_2 \cdot 6H_2O$, up to 19% extra chemical oxygen demand (COD) and almost 11% extra NH_3 removals were achieved relative to the control [38]. The reduction of

NH_4^+concentrations below 10 mg/L is possible through adding 0.06 mol/L Mg^{2+} for struvite formation in anaerobically digested dairy manure, and the corresponding NH_4^+ removal efficiencies reached to 95% [35]. Nevertheless, it has been revealed that the deposition of struvite crystalline coated the digested sludge lines and even led to pipe blockages in an activated sludge plant equipped for phosphate and ammonia removal [36,39]. Therefore, despite the high efficiency ammonia removal by struvite precipitation can be obtained, operational problems like pipe blockage prevents the wide commercialization of this chemical method.

1.4.3 Biological methods

In the past few years, some novel biological processes such as anaerobic ammonium oxidation (Anammox) and microorganisms immobilization have been developed to mitigate ammonia inhibition in the anaerobic digestion process.

(1) Anammox

Anammox is a lithoautotrophic biological conversion process, mediated by a group of *Planctomycete* bacteria, the stoichiometry of Anammox reaction is given in Eq. (3)-(5) [40].

$$NH_4^+ + NO_2^- \rightarrow N_2 + 2H_2O \quad (2)$$

$$CO_2 + 2NO_2^- + H_2O \rightarrow CH_2O + 2NO_3^- \quad (3)$$

$$CO_2 + 1.32NO_2^- + 0.066HCO_3^- + 0.13H^+ \rightarrow$$

$$0.066CH_2O_{0.5}N_{0.15} + 1.02N_2 + 0.26NO_3^- + 2.03H_2O \quad (4)$$

Yang et al. employed simultaneous ammonium and sulfate removal Anammox process in an anaerobic digestion bioreactor filled with granular activated carbon, and

the removal efficiency of ammonium was up to 97% [41]. However, the Anammox bacteria are very sensitive, the process may be inhibited by NO_2^- concentration around 0.1g N/L in a continuous operation and 50% inhibited by NO_2^- concentration at 0.35 g N/L [42]. In addition, high cost, poor regeneration and uncertainty of outcome are some of the frequently encountered limitation in the application of this method.

(2) Microorganism immobilization

There are lots of studies have revealed the importance of fixing microbes on bed materials in the anaerobic digestion. Sasaki et al. have used the carbon fiber textiles bed material for decrease ammonia inhibition in thermophilic methanogenic bioreactors, indicated that adding bed material enables stable proliferation of acetoclastic methanogens via preventing ammonia inhibition in thermophilic bioreactors that include high levels of ammonia [43]. The characteristics of bed materials have a significant influence on methane production, and the adherence and metabolisms of the microbes are largely depend on the fixed-bed material's physicochemical characteristics like specific surface area, porosity, surface roughness and pore size [44]. However, the immobilization of microorganism on bed material cannot remove ammonia from the anaerobic digesters directly. Therefore, it is necessary to combine the microorganism immobilization on bed material and ammonia removal by zeolite adsorbent for mitigating ammonia inhibition in anaerobic digestion of ammonium-rich livestock wastes.

1.4.4 The bedding material fixed-zeolite bioreactor

It might be a promising technology that physical adsorption combined with

microorganisms immobilization for ammonia removal in the anaerobic digestion process. Wang et al. have developed a fixed zeolite bioreactor using porous nylon bag packed zeolite for the anaerobic digestion of ammonium-rich swine waste, which can increase methane production effectively [45]. It suggesting that the fixed zeolite could effectively adsorb ammonia and the bed material fixed zeolite system could immobilize microbes then enhances the anaerobic digestion performance. However, the author just used one kind of bed material for zeolite fixing in the bioreactor and did not investigate the influence on microbes exerted by different bed materials, which is of great significance in a fixed zeolite bioreactor. Besides, the commercial A-3 zeolite contains trace amount of metal cations which was found to have limited ion-exchange capacity with low ammonium-selective adsorption ability, modification studies are required in order to make it commercially viable for industrial applications. Therefore, to develop a novel high-efficiency bioreactor for ammonium-rich livestock wastes fermentation, it is necessary to investigate a suitable bedding material and implement some modification works on zeolite designed specifically for the high ammonium adsorption capacity and nutritional metal cations supplement.

1.5 Oyster shell and lignite

Oyster shell contains large quantities of calcium salts is ubiquitous in oyster farming and generally treated as waste. In China, random disposal of large amount of oyster shell has imposed a series of severe environmental problems, effective handling of oyster shell has become an urgent issue [46]. It is mainly made of calcium carbonate and calcium phosphate that accounted for 80-95% total dry weight, trace

elements, vitamins, as well as amino acids [47]. The high content of $CaCO_3$ empowered the waste oyster shell with high ammonium exchange ability [48]. Thus, the oyster shell has a high potential to be used as modification material for improving the adsorption ability of zeolite.

Additionally, lignite is a widely distributed coal resource at total amount of 2,600 billion tons over the world [49]. Due to the deficiency like high moisture, low heat value and easy spontaneous combustion, it is not an ideal fuel compared to the other energy sources. Whereas, the abundant mineral elements in lignite could be a good source to provide micronutrients for the microbes in ammonium-rich anaerobic bioreactor. Micronutrients are essential for metabolic activity of the microorganism, deficit of micronutrients is expected to cause reduced anaerobic digestion performance. However, the anaerobic treatment process with high feedstocks of manure are often found to be deficient in micronutrients [50]. The abundant nutrients in lignite could be a good source. In addition, lignite may work as the electron exchange conduit in the digester for inducing the direct interspecies electron transfer (DIET) between syntrophic microbial species. DIET is defined as a microbial syntrophy in which free electrons transfer between cell-to-cell contact without being shuttled by interspecies electron carrier such as reduced hydrogen or formate during the fermentation. Recent discoveries revealed that DIET is the principal mode of interspecies electron exchange in degrading wastes into methane and the addition of conductive materials, such as biochar, magnetite and granular activated carbon, can significantly accelerate methane production [51]. Until now, an ideal adsorbent, which is expected to

selectively uptake the ammonium, supplement the nutritional elements as well as induce the electron communication between methanogen commnities is still unachieved. Therefore, zeolite optimization was carried out by integrating the oyster shell and lignite as modification material for enhancing the efficiency and economy of bed material fixed zeolite bioreactor during the ammonium-rich anaerobic digestion.

1.6 Intermittent illumination assisted fermentation

Recent years, a new strategy has been proposed that suitable intermittent illumination process is beneficial for anaerobic digestion, and methane production could be significantly increased under lightening [41,52]. Higher methane production have been obtained under continuous incandescent illumination than that of the dark reactors [53]. Yang et al. have reported that appropriate light stimulating could accelerate the activation of the CH_3-S-CoM reductase for methane generation [54]. Moreover, Zhang et al. investigated the intermittent illumination in an ammonium-rich anaerobic digestion, and the suitable light stimulation enhanced the bio-methane production which is found to be an effective approach to relieve ammonium inhibition in converting ammonium-rich organics to methane [55]. In my research, I have adopted the optimal condition of illumination with the light intensity of 100 ± 10 μmol/m^2/s at 60 min/d for integrating of light stimulation and the novel bioreactor with bed material fixed optimized zeolite for ammonium-rich anaerobic digestion.

1.7 Objectives and thesis outline

The introduction part has concluded that anaerobic digestion is a promising

technology for the treatment of livestock wastes. However, the presence of excessive ammonium during the biodegradation of livestock wastes containing high-concentration nitrogenous organic compounds posed a serious inhibition problem in practical anaerobic digestion plants. The main purpose of this study is to development of a novel high-efficiency bioprocess by incorporating bed material fixed modified zeolite bioreactor with intermittent illumination condition to resolve the ammonia inhibition for ammonium-rich anaerobic digestion. The specific contents are listed as follows:

Chapter I

Giving a brief background and introduction of the research field. Also, explaining some basic insights that are progressed until now.

Chapter II

Explore the optimal bedding material for the development of novel high-efficiency bed material fixed zeolite bioreactor for mitigating ammonia inhibition in the ammonium-rich anaerobic digestion.

Chapter III

Optimize the zeolite to high cation exchanger ammonium adsorbent and investigate the feasibility and possible benefits of combining the novel optimized zeolite fixed bioreactor with intermittent illumination for high efficient ammonium-rich anaerobic digestion.

Chapter IV

Summarize the results and conclusions of this research and propose some

prospective ideas for future research.

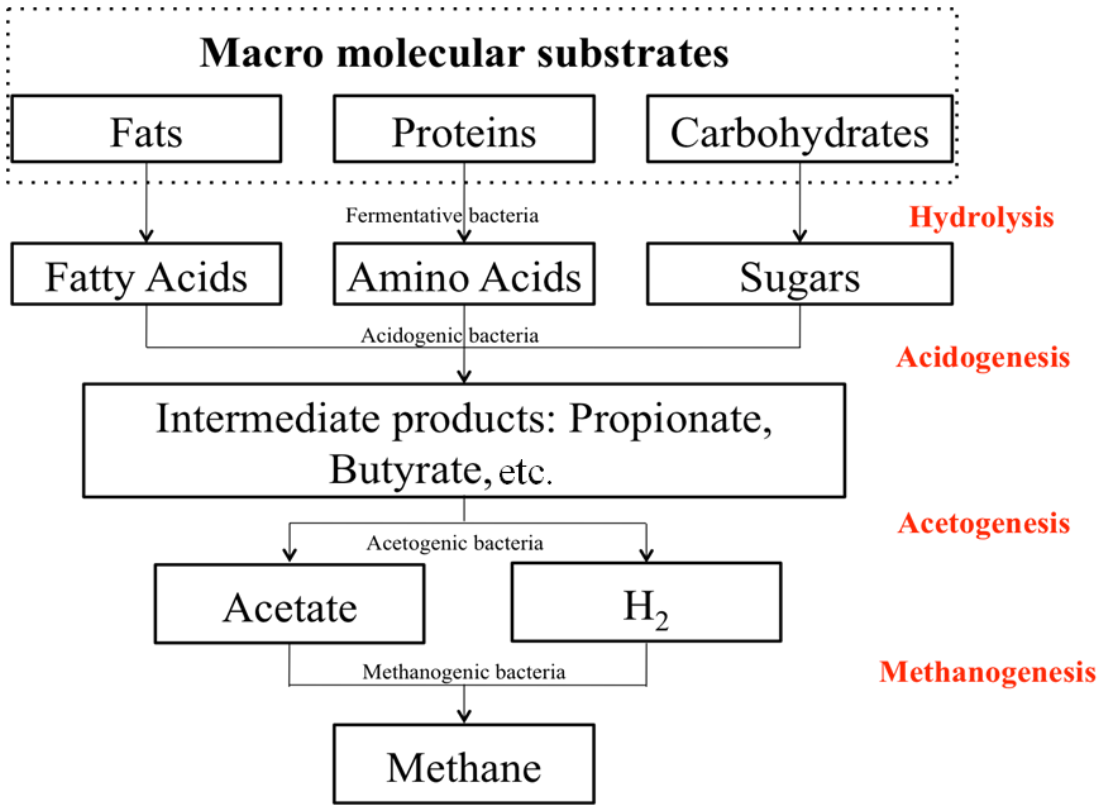


Figure 1.1 Schematic of anaerobic digestion organic substrate [7].

Chapter 2 Exploration of optimal bed material for developing high-efficient zeolite fixed bioreactor for mitigating ammonia inhibition

2.1 Introduction

The combination of microorganism immobilization and ammonia adsorption is a promising technology for mitigating ammonia inhibition in the anaerobic digestion of ammonium-rich livestock wastes. Wang et al. have developed a fixed zeolite bioreactor using porous nylon bag packed zeolite for the anaerobic digestion of ammonium-rich swine waste, which can decrease the start-up time and increase methane production effectively [45]. Bedding material is a crucial factor in determining the efficiency and success of the novel fixed zeolite system. The characteristics of bed materials have a significant influence on methane production and the adherence of microorganisms as well as determine the biomass retention capacity [56]. It has been demonstrated that metabolites of the microbes are largely depend on the fixed-bed material's physicochemical characteristics like specific surface area, porosity, surface roughness and pore size [44,57]. There are lots of studies have revealed the importance of fixed bed materials to microbes in the anaerobic digestion [56]. Several authors have investigated a large variety of bed materials be used in treat different types of wastewater, such as polyurethane foam (PU), loofah sponge, vegetal carbon (VC), ceramic, synthetic pumice (SP), and recycled low-density polyethylene (PE) [57–59]. Yang et al. have reported that different supports provide different specific conditions for the adhesion of distinct

microorganism types [56]. The above researches indicated that the materials with characteristics like high porosity, roughness and big specific surface area are more favorable for microorganism immobilization. Meanwhile, it is necessary that the materials should be low-cost, easily obtained and high-efficiency for practical use. Nevertheless, there is no research focused on investigating the combination 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 bed material and zeolite for the anaerobic digestion of ammonium-rich livestock wastes. Therefore, in this Chapter, three typical and stable polymers materials including polymer foaming sponge (PFS), chlorinated polyethylene (CPE) and porous nylon (PN) were used as bed material. Three fixed-bed systems were constructed using different bed materials fixed with zeolite, respectively. Different from Wang et al. [45], the contact rate between fixed zeolite and fermentation broth was greatly increased to improve both the ammonium adsorption effect of zeolite and microbes attachment on zeolite in this present study. The objectives of this research are: (1) Explore a suitable bedding material for zeolite immobilization to development of novel bed material fixed zeolite bioreactor, (2) Investigate the efficiency and performance of the developed bedding material fixed zeolite bioreactor in anaerobic biodegradation of high organic loading and extremely high-concentration ammonium livestock wastes.

2.2 Materials and methods

2.2.1 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 broth. 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, to select an appropriate bed material is very important to ensure the effectiveness and success of the combination system.

In this present study, three different polymer materials including polymer foaming sponge (PFS, Hiroshima, Japan), chlorinated polyethylene (CPE, Osaka, Japan) and porous nylon (PN, T&T, Wakayama, 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 stable 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℃for 12 h.

2.2.2 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℃ for more than 2 years after been taken from a pig farm located in Ibaraki prefecture, Japan. The ammonium concentration was more than 7,500 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 $FeSO_4 \cdot 7H_2O$ (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). After 1 day, 0.5 g glucose was added to the reactor every other day. The cultivation experiment was carried out at 35℃ for 15 days. The characteristics of swine manure and seed sludge used in this experiment are listed in Table 2.1.

2.2.3 Batch anaerobic digestion of extremely high ammonium concentration swine wastes

A series of 300 mL fermenter bottles (SIBATA, Saitama, Japan), each with 200 mL working volume, were used as the bioreactors. As shown in Fig. 2.1, four different bioreactors including a bioreactor without the fixed zeolite system as control and fixed zeolite system bioreactor immobilized respectively by PN (R_{PN}), PFS (R_{PFS}) and CPE (R_{CPE}) 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 adjusted to 7,511 mg/L by adding NH_4Cl and 7.0 using 1 M NaOH and HCl, respectively. In the fixed zeolite bioreactor, 10 g/L A-3 zeolite was fixed in the bed materials and suspended in the diluted substrate. 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℃. 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 triplicate.

2.2.4. 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℃. The CPE-fixed zeolite bioreactor and the control 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 4,000 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 rates (OLRs) and hydraulic retention time (HRT) as listed in Table 2.2. The sampling and biogas collection methods were the same as those mentioned in Section 2.2.3.

2.2.5 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℃ 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. [56].

2.2.6 Analytical methods

The yield and composition of the produced biogas were measured everyday. Two mL of digestate was sampled every other day. The total solid content (TS), volatile solid content (VS) and ammonium nitrogen (NH_4^+-N) were determined according to standard methods [60]. 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™ Microbial Cell Viability Assay (Promega, Wisconsin, USA). The composition of the produced biogas was analyzed by gas chromatography (GC-8A, Shimadzu, Kyoto, Japan) using a machine equipped with a thermal conductivity detector (80℃) and a Porapak Q column (60℃). Nitrogen was used as the carrier gas. The sample was centrifuged at

10,000 rpm for 10 min (25℃) to allow precipitation of the microbes after been taken. The supernatant was used to measure the dissolved organic carbon (DOC) with a TOC analyzer (TOC-V CSN, Shimadzu, Kyoto, Japan).

2.3 Results and discussion

2.3.1 The characteristics of the bed materials

Fig. 2.2 shows microscope images of the three kinds of bed materials. The fabriform structure of PN (Fig. 2.2a), the porous structure of PFS (Fig. 2.2b), and fabriform and porous structure of CPE (Fig. 2.2c) 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 broth with sufficient space in contact with each other [45,56]. 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 smooth surface. The large quantity of pore could provide zeolite and broth 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 are 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.

2.3.2 Reactor performance

The methane concentration and cumulative methane production are presented in Fig. 2.3a, and Fig. 2.3b, respectively. As seen from Fig. 2.3a, compared to the control, three fixed zeolite bioreactors have showed higher methane content during 30 days 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 [61]. 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. 2.3b, the cumulative methane production was attained from CPE-fixed zeolite bioreactor, and decreased in the following order: R_{CPE} > R_{PN} > R_{PFS} > Control. The cumulative methane production of R_{CPE} increased gradually during 30 days 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 83% (Fig. 2.3a).

The higher methane content and accumulated methane production in R_{CPE} indicated that using CPE fixed zeolite system achieved amore 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 contributed to the enhancement of methane production [45]. 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 [56].

2.3.3 NH_4^+-N concentration variation

Fig. 2.4 shows the variation of ammonium concentration in the four bioreactors during 30 days 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 showed an increasing tendency in all bioreactors after 22 days. During days 15–22, the ammonium content in R_{PN}, R_{PFS} and R_{CPE} were ranged from 4,000 to 5,000 mg/L, while this value remained around 7,000 mg/L in the control reactor. 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. 2.3). The better mitigation of ammonia inhibition and higher methane production in R_{CPE} were partially attributed to effective ammonia removal by the CPE fixed zeolite system. The similar result has been observed by Resch et al. who reported that an effective reduction of ammonium could shorten start-up period and improve anaerobic digestion performance [62]. 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. 2.3, 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. 2.2, which might contributed to different quantity and activity of immobilized microorganisms in bioreactors. 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 R_{PN}, R_{PFS} and R_{CPE} started to increase due to continuous generation of ammonia during the biodegradation of nitrogenous organics in swine wastes (Fig. 2.4). The same phenomenon was also observed by Halim et al. [63]. The increasing ammonium concentration led to the fluctuation of methane production in the fixed zeolite bioreactors after day 25 (Fig. 2.3). The total NH_4^+-N concentration in the three fixed zeolite bioreactors of PN, PFS and CPE and the control bioreactor increased respectively to 8,140, 7,851, 7,439, 8,565 mg/L at the end of 30 days anaerobic digestion. However, the performance of CPE fixed zeolite reactor has almost not been affected (Fig. 2.3a).

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 [64]. 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, the CPE fixed zeolite bioreactor remained stable and successful [65].

2.3.4 SEM observation

Fig. 2.5 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 reactor, 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 microbes 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 coccal methanogens closely resembling to *Methanosarcina* predominated on the bed materials, especially on the CPE [66–68]. 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. 2.2). 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 [65]. 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% [69]. 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.

2.3.5 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 [70]. 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 microbes were successfully immobilized in the 3 bed materials fixed zeolite bioreactors corresponding to the SEM observation results (Fig. 2.6a). ATP concentration on the surface of the bed material fixed zeolite systems were measured. The ATP value in the CPE fixed zeolite bioreactor is much higher than that in the other two reactors (Fig. 2.6b). 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 [71]. 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 further study.

2.3.6 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. 2.7 and 2.8. As shown in Fig. 2.7a, 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 0.15–0.51 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. 2.7b. During the first HRT (35 d) at an OLR of 0.15 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, increased 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. 2.8 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-$DOC_{removed}$ in the second HRT (14 d) and 212 mL-CH_4/g-$DOC_{removed}$ 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 days 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.

2.4 Summary

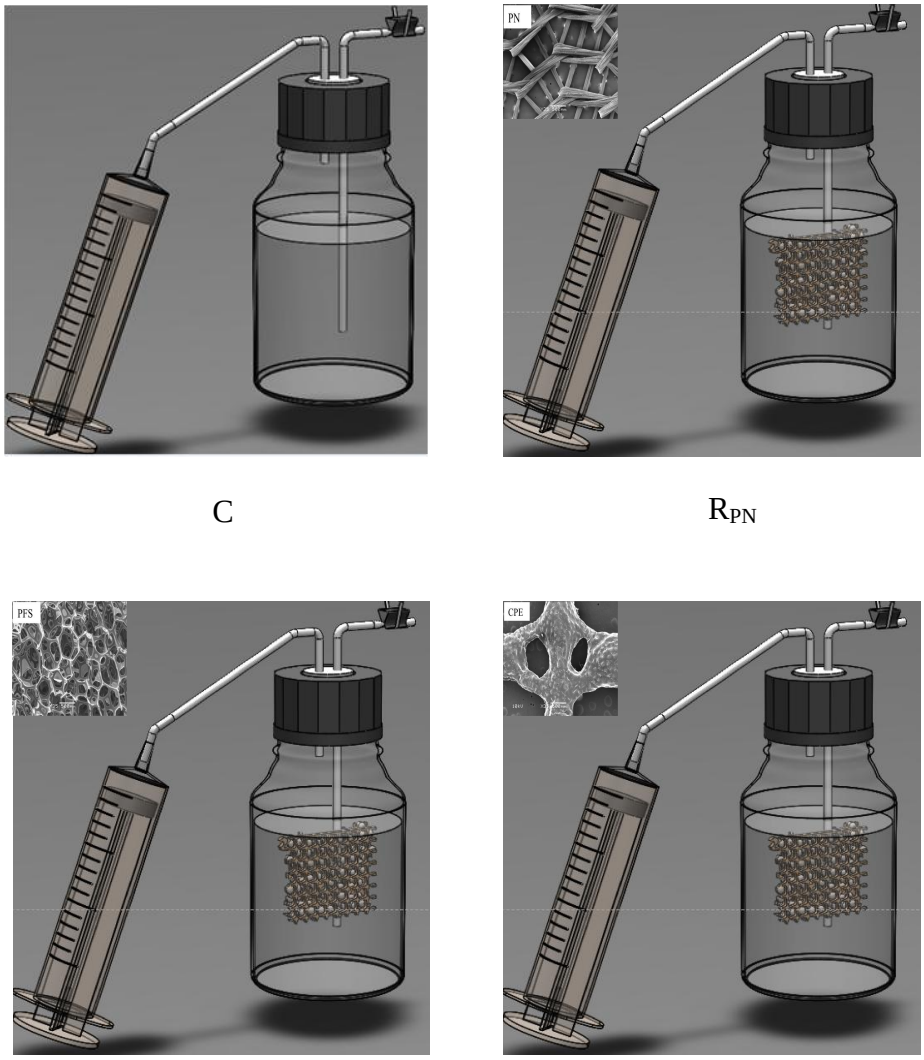
In this chapter, the effect of combines the bedding material and zeolite to develop a fixed zeolite bioreactor in the anaerobic digestion of ammonium-rich swine wastes was investigated. A novel CPE fixed zeolite system bioreactor, which can efficiently mitigate the ammonia inhibition and improve the anaerobic digestion performance was developed. The synergy of ammonia adsorption and immobilized microorganisms by chlorinated polyethylene fixed zeolite system contributed to the enhancement of anaerobic digestion efficiency. It can be concluded from the results that: (1) Of the assayed bedding supports, fabriform and porous structure of CPE has proven to be the optimal bed material for microorganism immobilization in the fixed zeolite bioreactor on anaerobic digestion of extremely high-concentration ammonium livestock wastes. (2) Synergy of ammonia adsorption and microorganism's immobilization by CPE fixed zeolite system contributed to the enhanced anaerobic digestion efficiency. (3) 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.

Table 2.1 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)	6,500 ±78	41,000 ±62
Total nitrogen (TN, mg/L)	5,489 ±82	39,402 ±103
Total solid (TS, mg/L)	13,292 ±125	33,900 ±178
Volatile solid (VS, mg/L)	9,500 ±71	27,150 ±132
Ammonium nitrogen (NH_4^+-N, mg/L)	1,547 ±25	7,511 ±51
pH	7.1	7.2

Table 2.2 The hydraulic retention time (HRT) and organic loading rate (OLR) in the semi-continuous anaerobic digestion

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

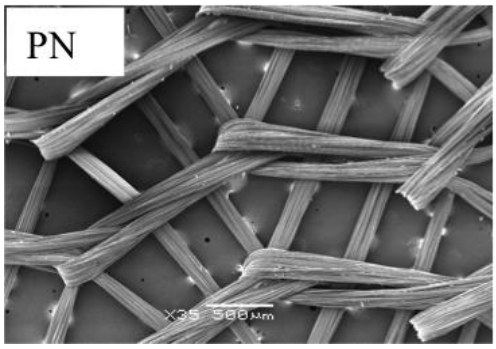


C R_{PN}

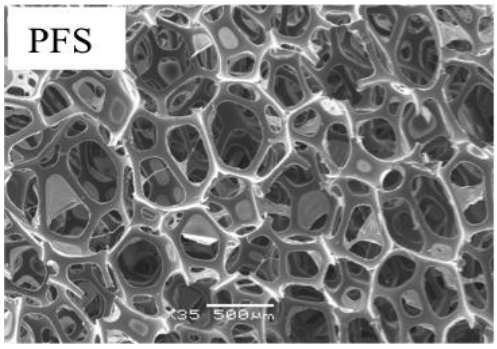
R_{PFS} R_{CPE}

Figure 2.1 Schematic of the bioreactors: control (C), porous nylon (PN) fixed zeolite system (R_{PN}), polymer foaming sponge (PFS) fixed zeolite system (R_{PFS}), and chlorinated polyethylene (CPE) fixed zeolite system (R_{CPE}).

(a)



(b)



(c)

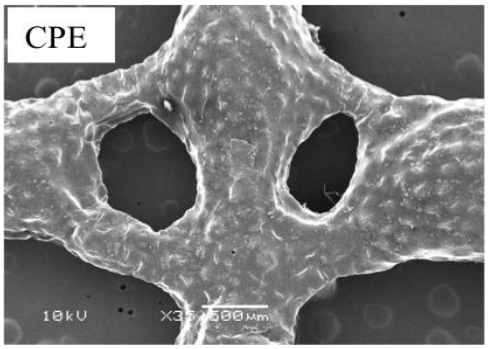


Figure 2.2 SEM images of bed materials: (a) porous nylon (PN), (b) polymer foaming sponge (PFS), and (c) chlorinated polyethylene (CPE).

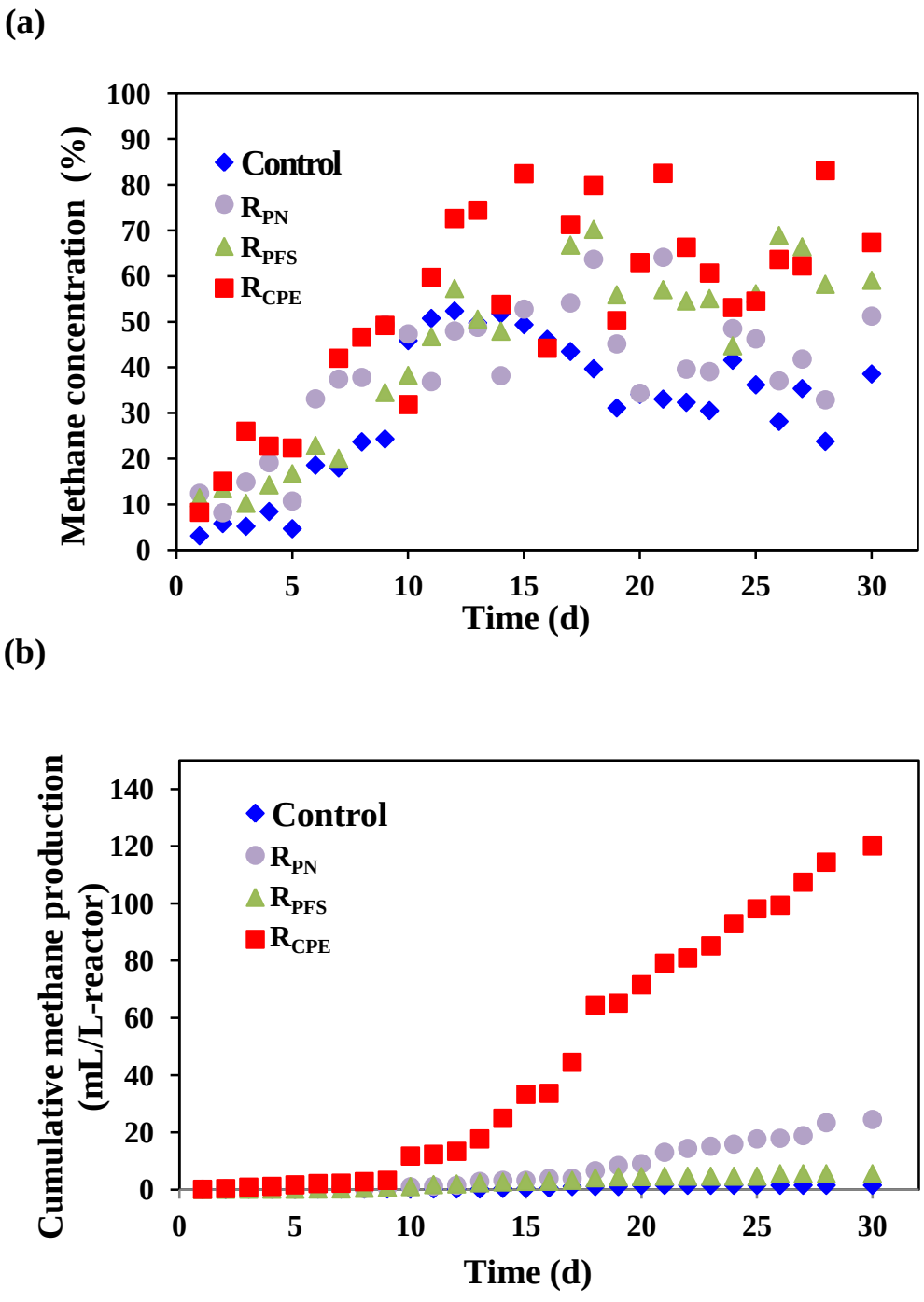


Figure 2.3 Changes of (a) methane concentration and (b) cumulative methane production in four bioreactors: control, PN fixed zeolite system (R_{PN}), PFS fixed zeolite system (R_{PFS}), and CPE fixed zeolite system (R_{CPE}).

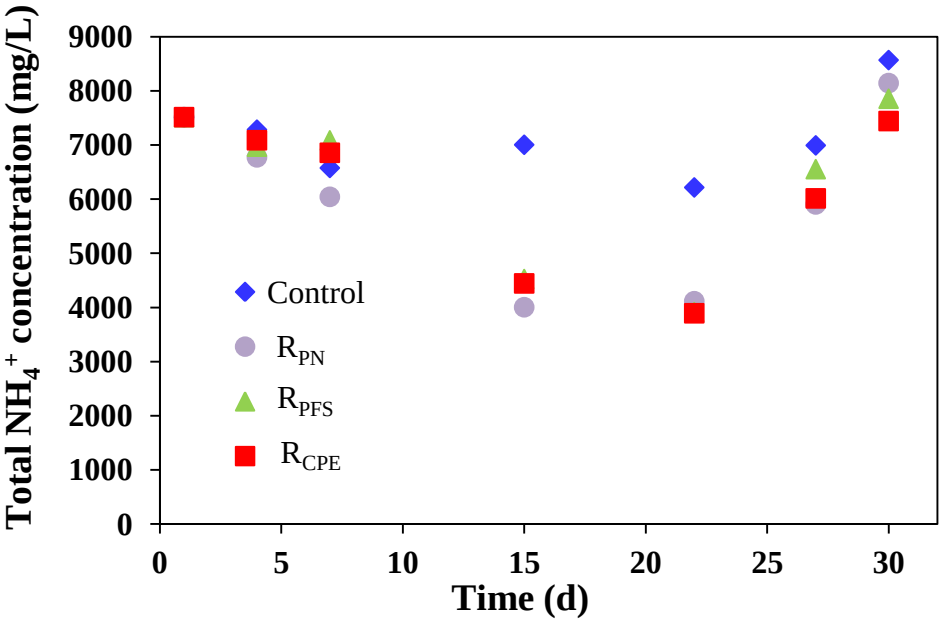


Figure 2.4 Changes of total ammonia nitrogen concentration in four bioreactors: control, PN fixed zeolite system (R_{PN}), PFS fixed zeolite system (R_{PFS}), and CPE fixed zeolite system (R_{CPE}).

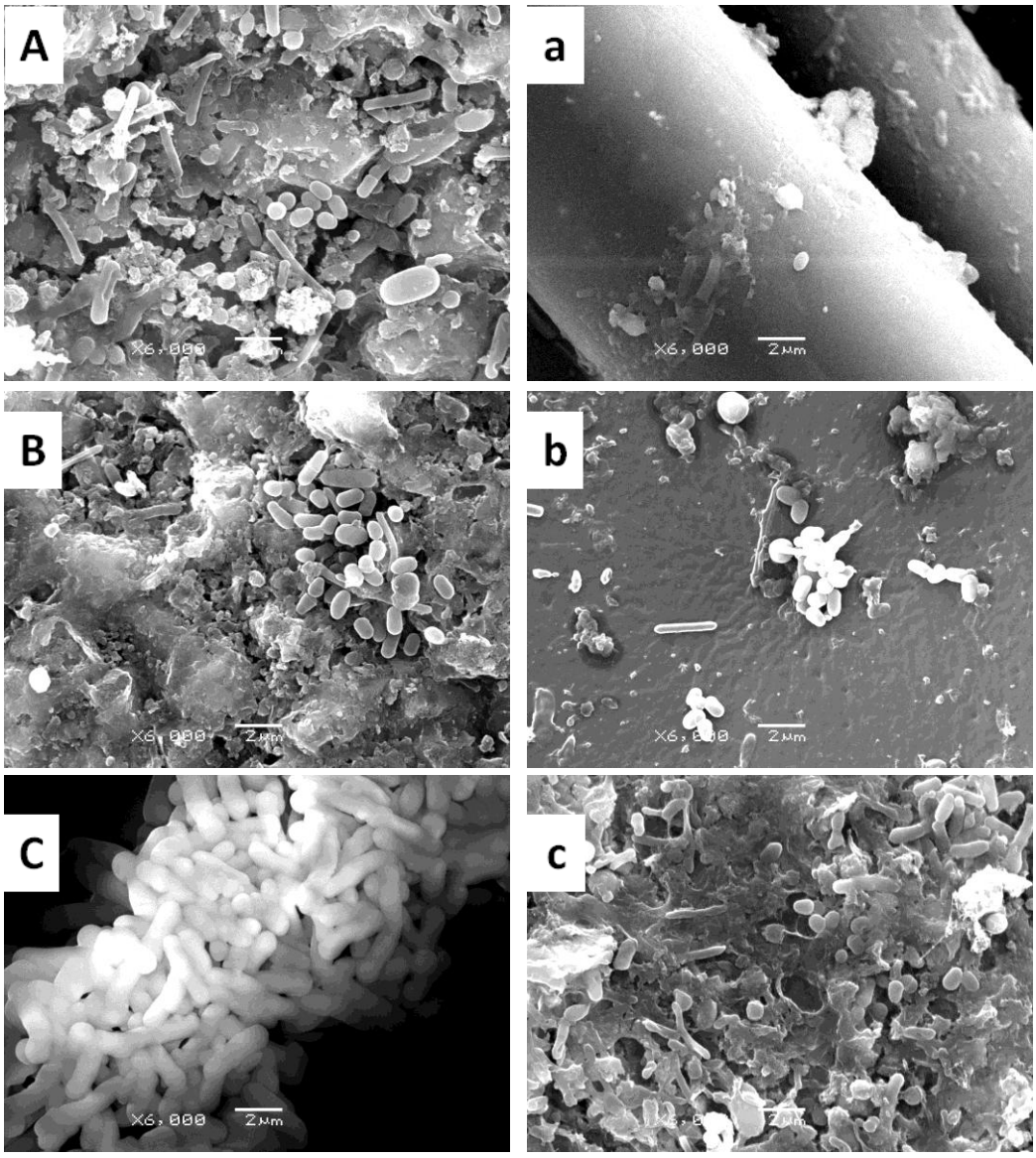


Figure 2.5 SEM images of anaerobic microbes immobilized on the surface of zeolite and bed materials. (A) PN-zeolite, (a) PN, (B) PFS-zeolite, (b) PFS, (C) CPE-zeolite, (c) CPE, 6,000×.

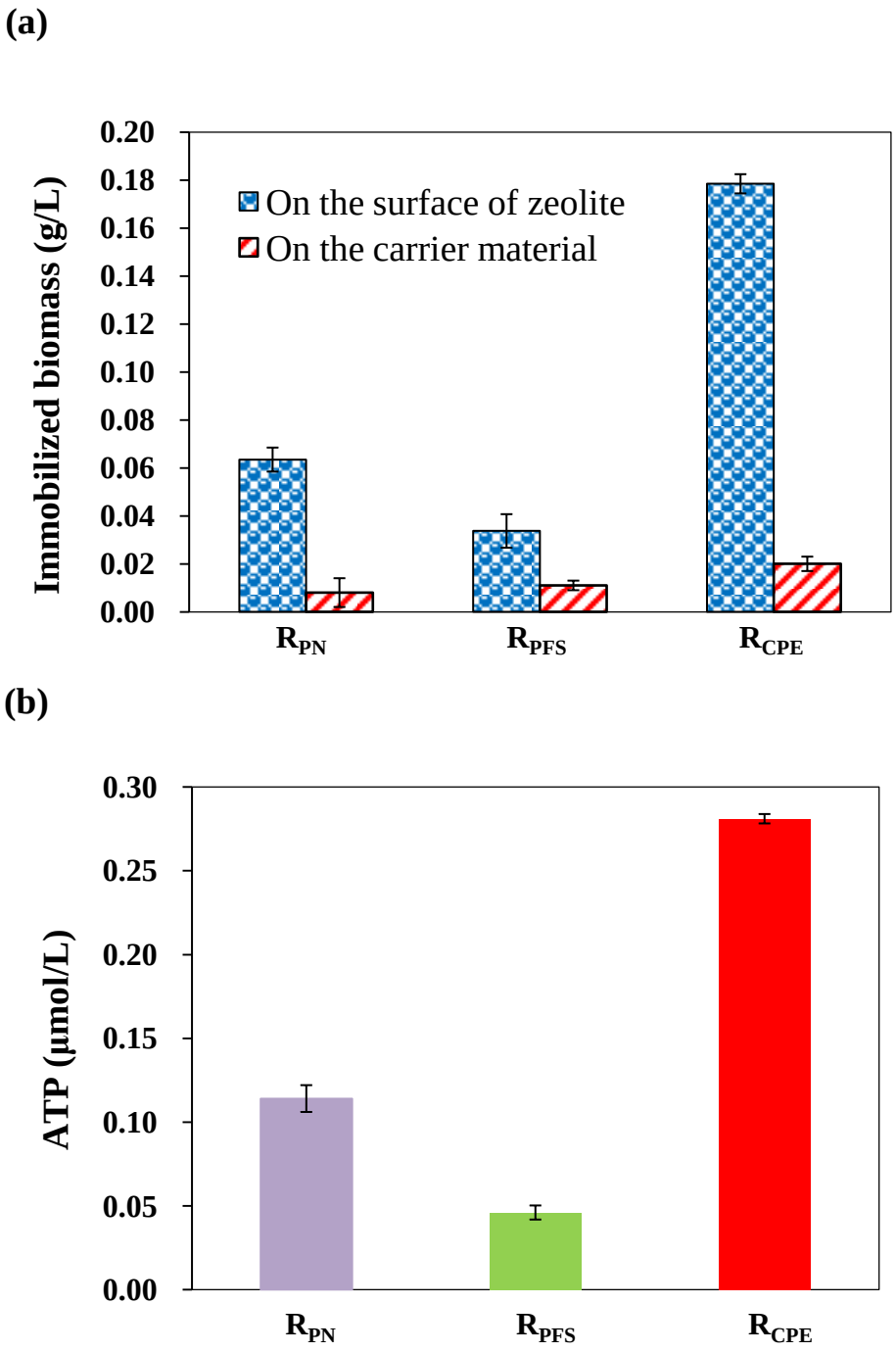


Figure 2.6 (a) Biomass immobilized on the surface of zeolite and bed materials in three bioreactors: PN fixed zeolite system (R_{PN}), PFS fixed zeolite system (R_{PFS}), and CPE fixed zeolite system (R_{CPE}); (b) ATP values of anaerobes attached in fix-bed bioreactors at the end of the experiment. The bars designate standard deviations (95% confidence, t-test).

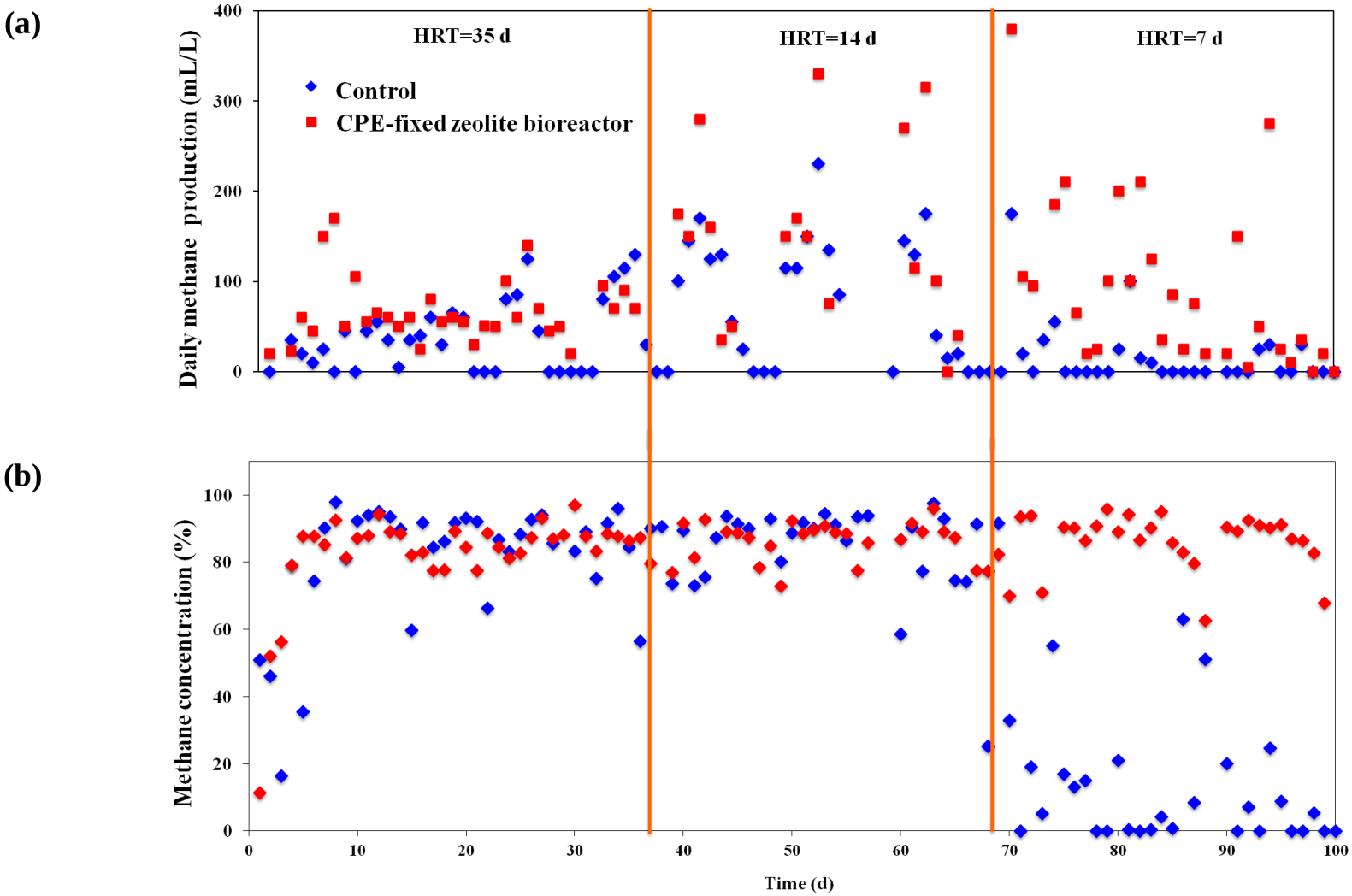


Figure 2.7 The variation of daily methane production (a) and methane concentration (b) during 100 days semi-continuous anaerobic digestion of ammonium-rich swine wastes.

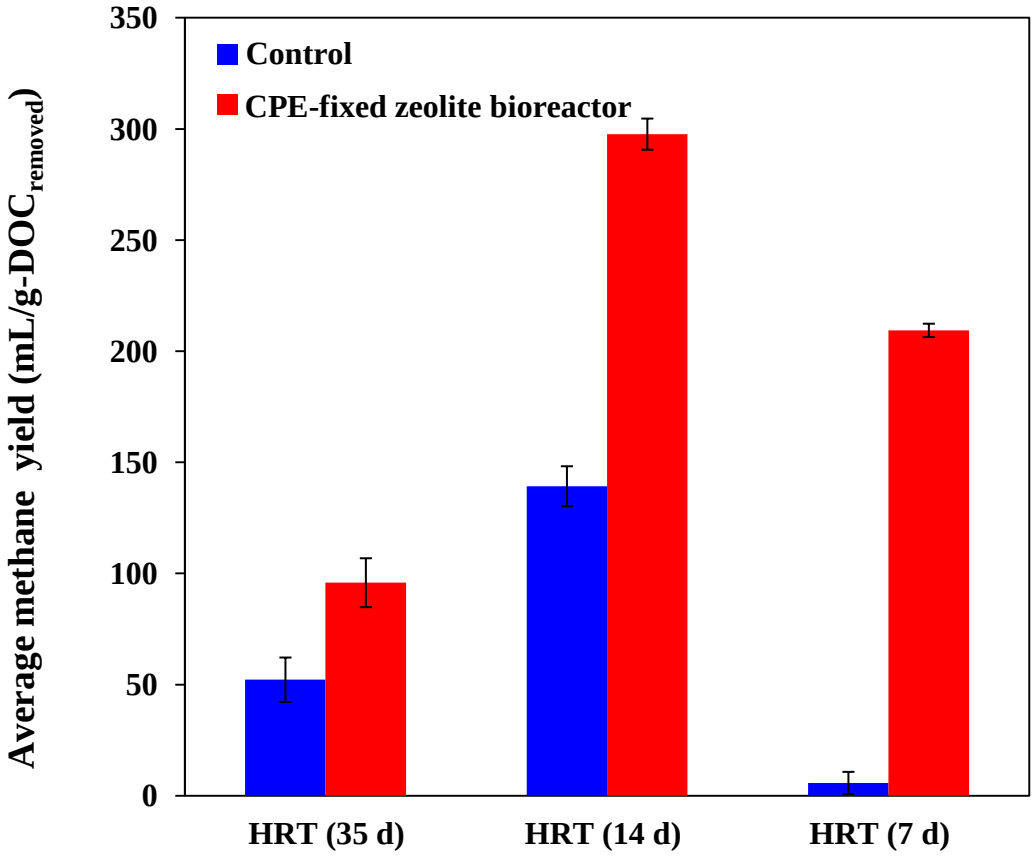


Figure 2.8 The average methane yield under different hydraulic retention time (HRT) and organic loading rate (OLR) during 100 days semi-continuous anaerobic digestion of ammonium-rich swine wastes. The bars designate standard deviations (95% confidence, t-test).

Chapter 3 Study on the optimization of CPE fixed zeolite bioreactor and coupled with light stimulation for high efficient ammonium-rich anaerobic digestion bioprocess

3.1 Introduction

From the results of chapter 2, we have developed a novel bioreactor constructed by suspending the bed material CPE (Chlorinated polyethylene) fixed zeolite in the fermentation liquid. In applying to anaerobic digestion of extremely high ammonium concentration manure, it has shown outstanding performance in mitigating ammonia inhibition and improving methane yield. Zeolite adsorbed ammonia and dissociated trace elements of Ca^{2+}, K^{+} and Mg^{2+}, which restrained the ammonia toxicity in the bioreactor. Meanwhile, large amount of anaerobes immobilized on the CPE fixed zeolite system created a high cell density and activity for resistance of high ammonia environment. However, the A-3 zeolite contains trace amount of metal cations which was found to have limited ion-exchange capacity with low ammonium-selective adsorption ability, more studies are required in order to make it commercially viable for industrial applications. Therefore, it is necessary to implement some modification works on zeolite designed specifically for the high ammonium adsorption capacity and nutritional cations supplement.

As introduced in chapter 1, oyster shell contains large quantities of calcium salts has a high potential to be used as modification material for improving the adsorption ability of zeolite. Meanwhile, the variety and large amount of mineral elements like

Ca^{2+}, Mg^{2+}, Fe^{3+} maintained in the lignite can provide essential nutrients for the growth of microorganisms [50]. Additionally, lignite may also work as the electron transfer conduit in the digester for inducing electron communication between methanogens communities. Until now, an ideal adsorbent, which is expected to effectively and selectively uptake the ammonium, supplement nutritional elements as well as accelerate the electron transfer between methanogenic communities is still unachieved. Therefore, zeolite optimization was carried out by integrating the oyster shell and lignite as modification materials for enhancing the efficiency and economy of CPE fixed zeolite bioreactor during the ammonium-rich anaerobic digestion.

On the other hand, previous studies have reported that intermittent illumination is beneficial for activating the methanogens, and methane production could be significantly increased under lightening [41,52]. The suitable illumination stimulation has found to be an effective approach to relieve ammonium inhibition in converting ammonium-rich organics to methane [55]. Under investigation, we have adopted the intermittent illumination condition with the light intensity of 100 ± 10 μmol/m^2/s at 60 min/d for integration of light stimulation and the novel bioreactor with CPE fixed modified zeolite for ammonium-rich anaerobic digestion [72].

Therefore, the aim of this Chapter was to develop an effective process by incorporating the fixed oyster shell-lignite modified zeolite bioreactor with intermittent illumination condition for high efficient ammonium-rich livestock waste fermentation. The major objectives including: (1) Optimize the zeolite to high cation exchanger,

evaluate and compare the ammonium adsorption capability of the explored adsorbents using adsorption kinetics and equilibrium isotherm; (2) Investigate the feasibility and possible benefits of combining the novel optimized zeolite fixed bioreactor with intermittent illumination for high efficient ammonium-rich anaerobic digestion; (3) Preliminarily reveal the underlying interaction mechanism of the integrated bioprocess system.

3.2 Materials and methods

3.2.1 Preparation of adsorbents

The modification of zeolite was performed. The analytical grade A-3 zeolite in powder form was purchased from Sigma-Aldrich, and was used as the precursor material. Oyster shell and lignite were used as modification additives, montmorillonite and starch were added as adhesives. Oyster shell and lignite were finely grounded and sifted to a grain size of 105 μm for further use. Initially, different amount of zeolite, modification additives and adhesives were blended homogeneously. The ingredients of the four adsorbents are as follows: Oyster and lignite modified zeolite (OLMZ): 7.6 g of zeolite, 0.9 g of oyster shell, 0.5 g of lignite, 0.5 g of montmorillonite and 0.5 g of starch. Oyster shell modified zeolite (OMZ): 8.1 g of zeolite, 0.9 g of oyster shell, 0.5 g of montmorillonite and 0.5 g of starch. Lignite modified zeolite (LMZ): 8.5 g of zeolite, 0.5 g of lignite, 0.5 g of montmorillonite and 0.5 g of starch. Unmodified Zeolite (UMZ): 9.0 g of zeolite, 0.5 g of montmorillonite and 0.5 g of starch. After that, about 100 mL of ultra-pure water was added to each mixture with constant stirring at room

temperature to make a paste. A glass tube with inner diameter of 8 mm was used to mould the pastes into uniform cylinders and shaped to balls manually. Finally, the spherical-shaped adsorbents were dewatered, oven-dried at 105℃ for 24 h and then calcined at 600℃ for 1.5 h in a muffle furnace to obtain the porous structure and stable configuration. After cooled to room temperature, the adsorbents were stored in dry chamber for subsequent experiments.

3.2.2 Ammonia adsorption evaluation of adsorbents

3.2.2.1 Adsorbents structure and surface morphology

To characterize the modified zeolite adsorbents, the surface area and the total pore volume of the synthesized adsorbents were determined using Brunauer-Emmett-Teller (BET) specific surface analysis device (Coulter SA-3100, Beckman, USA). The morphology images and metal elements identification in each adsorbent were analyzed by scanning electron microscopy (SEM, JSM-6700F, JEOL, Japan) and Electron Probe Micro Analyzer (EPMA, JXA-8530F, JEOL, Japan), respectively.

3.2.2.2 Ammonium adsorption experiments

Batch adsorption experiments were performed to identify the ammonium nitrogen sorption capability of the synthesized adsorbents. The ammonium solution (4,000 mg/L) was prepared by dissolving NH_4Cl in deionized water, and the synthesized adsorbents were immersed in the solution with dosage of 10 g/L, respectively. The removal studies were carried out in a shaker controlled at 155 rpm at 35℃ for 24 h. After the

equilibrium was reached, the sorbent was separated by centrifugation (10,000 rpm/min) and filtered. The quantity of adsorbed ammonium nitrogen was calculated according to the following equation:

$$Q = \frac{(C_0 - C_{eq})V}{m} \quad (1)$$

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

3.2.2.3 Adsorption kinetics and isotherm models

The adsorption equilibrium studies were carried out to determine the adsorption capacity for removal of ammonium from aqueous phase by synthesized adsorbents. There are two important mathematical models, known as the adsorption kinetic and isotherm models. Adsorption kinetics are essential for better elaborate the reaction mechanism of ion exchange process during the adsorption. Adsorption isotherm analysis is necessary to evaluate ammonium sorption potential on the synthesized adsorbents.

For the kinetics analysis, two typical kinetic models were employed to analyze the NH_4^+-N adsorption experimental data, including the Lagergren's pseudo-first-order and Ho's pseudo-second-order kinetic model. Equations (2) and (3) presented the integration of the Lagergren's pseudo-first-order and Ho's pseudo-second-order kinetic equations [73,74]:

$$\log(q_e - q_t) = \log q_e - \frac{k_1}{2.303} t \quad (2)$$

$$\frac{t}{q_t} = \frac{1}{k_2 q_e^2} + \frac{1}{q_e} t \quad (3)$$

where k_1 (l/m) and k_2 (g/(min·mg)) are the rate constant of the pseudo-first-order and the pseudo-second-order kinetic model, respectively. q_t is the adsorbed ammonium quantity at time t (mg/g), q_e the NH_4^+-N uptake mass at the equilibrium time and t is the contact time (mg/g).

The isotherm experiments were performed at 35 ℃ with various initial ammonium nitrogen concentration from 1,000 mg/L to 6,000 mg/L. The amount of adsorbent taken was 10 g/L and all the experiments were continued for 24 h. The ammonium removal capacity of the four adsorbents were determined and analyzed by Langmuir and Freundlich isotherms. The linearized form of the Langmuir isotherm equation is characterized by the following equation:

$$\frac{C_e}{q_e} = \frac{1}{b q_m} + \frac{C_e}{q_m} \quad (4)$$

The rearranged forms of the Freundlich isotherm equation is as follows:

$$\ln q_e = \ln k_f + \frac{1}{n} \ln C_e \quad (5)$$

where q_e is the ammonium uptake amount of adsorbent at equilibrium stage (mg/g), q_m is the maximum adsorption ability (mg/g), c_e is the equilibrium concentration of the ammonium nitrogen in the liquid phase (mg/L), b is the Langmuir constant representing the energy of adsorption (L/mg), k_f (mg/g) is a Freundlich constant related to adsorption capacity, and the 1/n is an empirical parameter reflecting the adsorption intensity, differs between materials.

3.2.3 Anaerobic digestion experiments

3.2.3.1 Seed sludge and substrate

In this study, the fresh digested sludge was collected from a municipal sewage treatment plant located in Ibaraki prefecture, Japan. Before the fermentation experiment, 400 mL of digested sludge was put into a 35 ℃ anaerobic reactor (500 mL) for cultivation, 0.5 g/L glucose was added on alternate days for two weeks.

According to the previous study, the synthetic medium was prepared by mixing a total of sodium acetate (2.5 g/L) and glucose (2.5 g/L) in a medium containing KH_2PO_4 (16 mg/L), yeast extract (200 mg/L) and trace mineral solution (200 mL/L) to obtain a substrate with organic loading at 20 g-DOC/L. In order to create a high ammonium condition (4,000 mg/L), NH_4Cl was added.

3.2.3.2 Fermentation experiments

The batch anaerobic fermentation was performed in Schott Duran bottle (300 mL, SIBATA, Saitama, Japan) reactor capped with rubber stopper of 200 mL working volume at 35 ℃. There are two perforated outlets on the stopper, one is connected to the syringe (60 mL) to collect the produced biogas mixture and the other is for liquid digestate sample. A volume of 1 mL biogas samples was taken every day for composition analysis and 2 mL digestate was sampled third a week for later chemical analysis. According to our previous research, chlorinated polyethylene (CPE) was used as the bed material to fix the adsorbent (10 g/L), and the illumination condition (100 ±

10 μmol/m^2/s, 60 min/d) was applied to Illumination group for higher methane recovery during the ammonium-rich anaerobic digestion [72,75].

The schematic diagram of anaerobic digester is shown in Fig. 3.1. Each bioreactor contained 80% (w/w) substrate, 20% (w/w) digested sludge and varied kind of adsorbents depending on the test condition, respectively. The digester control only contained substrate and inoculum sludge; the experimental digesters under dark condition with CPE fixed adsorbents were designated as OLMZ, LMZ, OMZ, and UMZ, respectively. While in further added with light illumination (Incandescent lamps, LW110V60W, Mitsubishi Oshram, Tokyo, Japan) at intensity of 100 ± 10 μmol/m^2/s, 60 min/d, the illumination group were named as OLMZ-I, LMZ-I, OMZ-I, and UMZ-I, respectively. The initial concentration of ammonium nitrogen was set at 4,000 mg/L and the pH was 7.0 ±0.3. All experiments were conducted in triplicate.

3.2.4 Sludge conductance

Gold electrode conductivity measurement was used to evaluate the conductivity of sludge samples according to Zhao et al. [76]. The schematic diagram of gold electrode for conductance measurement is shown in Fig. 3.2, two gold electrodes fixed on a nonconductive glass substrate, separated by a 1.0 mm nonconductive gap, and the sludge samples were placed on the gold electrodes across the non-conductive gap. The collected sludge samples were centrifuged at 8,000 rpm for 5 min, and washed by NaCl solution (0.1 mol/L) three times, then were placed on the two-gold electrodes covering the non-conductive gap. After that, an electrochemical measurement system (HZ-7000,

Hakuto, Fukushima, Japan) was used to provide a voltage ramp from −0.3 V to 0.3 V with measurement intervals of 0.025 V. The time-averaged current at each applied voltage was recorded to create the current-voltage curve. The conductance of sludge was calculated using the following equation:

$$\sigma = \frac{L}{RS} \quad (6)$$

where σ is the sludge conductivity (S/m), R is the reciprocal of the slope from the current-voltage curve (Ω), L is the width of the gap with values of 1×10^{-3} m, and S is the cross-sectional area of 2.4×10^{-6} m^2.

3.2.5 Analytic methods

The produced biogas was collected by the syringe connected to the digester and the volume was calculated using the scale on the syringe. The biogas composition was quantified using a gas chromatography (GC-8A, Shimadzu, Kyoto, Japan) machine equipped with a thermal conductivity detector (80 ℃) and a Porapak Q column (60 ℃). Argon was used as the carrier gas. The pH was monitored with a pH meter (TES 1380, Mettler-toledo, Tokyo, Japan). Dissolved organic carbon (DOC) content of aqueous phase was measured using a TOC analyzer (TOC-Cvpn, Shimadzu, Kyoto, Japan). Ammonium concentration was determined using the Nesslerization spectrophotometry method (420 nm) recorded in China State Environmental Protection Administration (HJ 535-2009) as the standard method. The ATP and coenzyme F_{420} value were used to reflect the activity of microbes, ATP was measured by the BacTiter-Glo™ Microbial Cell Viability Assay (Promega, Wisconsin, USA) and the F_{420} was tested using

Shimadzu UV-1600 spectrophotometer according to the method recorded by Cheng et al. [77]. The cellular morphological images of the microorganism immobilized on the surface of the fixed adsorbent units were observed by a scanning electron microscope (SEM, JSM-6700F, JEOL, Japan). The immobilized biomass was measured at the end of fermentation. The adsorbent fixed system was removed from the bioreactor, washed by the distilled water to detach the biomass. The biomass sample was dried at 105 ℃ for 24 h and then calcined at 600°C for 1 h (Muffle furnace, F-1404-T, Tokyo Garasu Kikai, Tokyo, Japan) [78], then cooled down in a desiccator and weighed. The cations composition of the four synthesized adsorbents after the digestion process were examined by Electron Probe Micro Analyzer (EPMA, JXA-8530F, JEOL, Japan).

3.3 Results and discussion

3.3.1 Characterization of synthesized adsorbents

The SEM morphologies indicating the general textural property and surface structure of the four synthesized adsorbents are shown in Fig. 3.3. The unmodified zeolite showed porous structure with rough micro-surface and irregular layer channels (Fig. 3.3a), which is in line with previous study [79]. In comparison, the surface morphology of the other three modified zeolite adsorbents (Fig. 3.3b-d) was relative coarser with larger apertures. Whereas, the OLMZ which modified with oyster shell and lignite simultaneous possessed highly developed porous structure and roughest surface. The BET analysis results of the four adsorbents are presented in Table 3.1. An increase in the value of BET surface area was observed for all the three modified

adsorbents, with the OLMZ got the largest BET surface area and total pore volume. Comparing to the UMZ, the specific surface area of OLMZ increased by 31% and OLMZ achieved the highest enhancement in terms of surface area and porosity. Therefore, these features might be responsible for increasing the workability of OLMZ for ammonium exchange with higher ability and the attachment of microorganisms at the same time.

After that, the ammonium adsorption capacity of the four synthesized adsorbents was tested and the results are depicted in Fig. 3.4. The ammonium adsorption capacity decreased in the following order: OLMZ > OMZ > LMZ > UMZ. OLMZ showed approximately 30% higher maximum adsorption capacity compared to UMZ, evidencing that OLMZ is a much more excellent adsorbent than UMZ as for ammonia uptake. This could be attributed to the abundant metal cations embedded after oyster shell and lignite modification, especially the Ca^{2+}, making the OLMZ a good exchanger for NH_4^+ in fermentation broth. Zeolite has been widely used as ion-exchange media and adsorbents in large amount of researches [80]. NH_4^+ present in the solution could be replaced by similarly charged ions like Ca^{2+} present in zeolite. According to the ion exchange order, cations including Na^+, Ca^{2+}, Mg^{2+}, Fe^{3+}, with lower exchange order than NH_4^+ are able to participate in this process [81]. Table 3.2a showed the main elements content ratio (%) of the four adsorbents through EPMA spectra analysis. It reveals that the major elements exist in UMZ were silicon, aluminum, and oxygen with a trace amount of metal cations such as Na^+, Ca^{2+} and Mg^{2+}, in consistent with the

natural zeolite which is an aluminosilicate mineral. These loosely bound cations Ca^{2+}, K^{+} and Mg^{2+} could be exchanged by NH_4^{+} in water [82]. While, due to low concentration of exchangeable ions content, unmodified zeolite has limited ion exchange capacity. For the OLMZ, the chemical composition of the major components presented does not show much prominent change, but a major wt% gain was observed for metal cations, particularly Ca^{2+}, followed by Fe^{3+} and Mg^{2+}. The Ca^{2+} contained in the OLMZ increased by nearly 63 folds as compared to the precursor zeolite. These inserted cations may have created some new sites for exchange, hence increasing NH_4^{+} adsorption capability after modification. The Ca^{2+} has been recognized as a prominent cation for NH_4^{+} exchange and the overall stoichiometry predicted for the ion exchange reaction mechanism can be explained as equation 7 (S and L are the acronyms of solid and liquid, respectively.) [73]. The replacement of ammonium from aqueous solution was accompanied by the dissociation of Ca^{2+} as ion exchanger from OLMZ, and based on the Le Chatelier's principle, increasing the Ca^{2+} content in the adsorbent would induce the ammonium transforming from liquid to solid [80]. Zhang et al. reported that ammonium could be recovered by ion exchange reaction with Ca^{2+} modified zeolite, and the recovery rate was based on the concentration of Ca^{2+} modified on the zeolites [48]. Thus, the high Ca^{2+} and other metal cations maintenance of OLMZ with enhanced ion exchange capacity contributed to high efficiency ammonium adsorption capability (Fig. 3.3).

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

On the other hand, it is well known that calcium, iron, potassium and magnesium

are essential nutrients required for the growth and activity of microbes [83]. Calcium could be a catalyst for promoting anaerobic digestion process with high methane production [84]. Iron has been proved to be able to enhance the growth of methanogenic bacteria [85]. As various metal cations were detected, such as Ca^{2+}, Mg^{2+}, Fe^{3+}, Na^{+}, OLMZ seems to be an ideal nutritional support for the anaerobes during the fermentation.

3.3.2 The ammonia adsorption kinetics and isotherm analysis of adsorbents

Kinetic models including the Lagergren's pseudo-first order equation and the Ho's pseudo-second order equation model were employed to explain the time-dependence of the NH_4^+ adsorption on the four adsorbents. The results are summarized in Table 3.3. As can be seen that all the adsorbents fit best for the pseudo-second-order equation, exhibited that ammonium adsorption on the four adsorbents was dominated by ion-exchange process [80,86]. In case of the OLMZ, the correlation coefficient of the pseudo-second-order model reached to 0.9985 and the theoretical data (99 mg/g) showed good coherence with the practical ammonium uptake masses at equilibrium (100 ± 2 mg/g). Nevertheless, for the pseudo-first order model, the correlation coefficient was very low (0.4393) and the theoretical value (6.295 mg/g) was extraordinarily lower than the experimental ammonium adsorption capacity (100 ± 2 mg/g), indicating a poor fit between the model and the experimental data. As a result, the ammonium adsorption on OLMZ is likely to be dominated by a chemical process with ion-exchange and OLMZ had much higher ion exchange capacity (Fig. 3.3 and

Table 3.2) than the pristine zeolite. Consistently, the similar results of kinetic models analysis have been observed for the other three adsorbents (OMZ, LMZ and UMZ) because the zeolite was used as the basal material. However, among all the adsorbents, OLMZ has a significantly higher pseudo second-order rate constant (K_2) implying a better ion exchange kinetics, hence, showed higher exchange speed and affinity for ammonium.

Equilibrium studies are essential for explaining the mechanism of the interaction between NH_4^+ and adsorbents during the adsorption process. The isotherm experiments were carried out for various initial concentration of ammonia. The linear form of Freundlich and Langmuir models were used to model the experimental data, the plotted equilibrium isotherms values obtained for the four adsorbents are compared in Table 3.4. As shown in Table 3.4, for all the four adsorbents, the higher correlation coefficient was obtained for Freundlich isotherm, meanwhile, the obvious difference was observed between the q_m calculated by Langmuir model and the adsorption experimental value (Fig. 3.3), which suggested that Freundlich isotherm is a better fitted equilibrium model than Langmuir to describe the adsorption process. Freundlich isotherm assumes that adsorption of adsorbate onto adsorbent is multilayer. It was proved that ammonium removal from the aqueous phase by all the four adsorbents occurs on a heterogeneous surface by multilayer sorption. The empirical parameter 1/n within the boundaries $0 < 1/n < 1$ for all adsorbents in which case the isotherms are considered favorable. In addition, the Freundlich constant k_f is an index to the sorption capacity of sorbent. The

k_f value of OLMZ (3.150) is obvious higher than in the case of the other 3 adsorbents suggesting its higher sorption capacity and affinity for NH_4^+. Therefore, the specialized high ammonium uptake ability and enriched metal cations content (Table 3.2a) could make the OLMZ an ideal alternative to UMZ for the anaerobic digestion of ammonium-rich livestock waste.

3.3.3 Performance of anaerobic digestion

Adsorption results verified the OLMZ with enriched metal ions content is beneficial for NH_4^+ exchange and microbe nutrition supplementation. To examine the feasibility of OLMZ for mitigating ammonia inhibition on bio-methane conversion, batch fermentation experiments were conducted. Furthermore, refer to our previous study, illumination strategy was further applied to investigate the combined effect of intermittent light stimulation and the novel OLMZ fixed bioreactor for higher methane production.

Fig. 3.5 shows the anaerobic digestion performance of the four adsorbents fixed bioreactor under illumination and dark condition, respectively. Fig. 3.5a revealed the methane concentration of all bioreactors. Starting from the first day, the methane content in OLMZ-I increased gradually to the maximum (93%) on the 4^{th} day and stably maintained at 80–90% until the end of the experiment, whereas the concentration in control was much lower ranged at 40% to 60% with obvious fluctuation. Methane content is a typical parameter that can be used to evaluate the stability and health of the anaerobic digestion process. Bioreactor with high methane concentration suggests a

steady balance of methane and carbon dioxide produced during methanogenesis and acetogenesis processes, while the low methane content means inhibited activity in the microbial communities [61]. The superior methane concentration result indicating that the ammonia inhibition was successfully mitigated in the OLMZ-I. Fig. 3.5b exhibited the methane yield in terms of DOC removal efficiency for the bioreactors. The statistical analysis suggested that the differences between OLMZ-I and other treatment groups were significant since the *p* values were all lower than 0.05, indicating that the methane yield of OLMZ-I was significantly higher than the other treatment bioreactors. OLMZ-I presented the best performance of methane yield at 372 ± 30 mL/g-$DOC_{removed}$, which increased by 300% compared to the control under ammonium-rich condition (p=0.0002). Without illumination assistant, all the bioreactors each were fixed with the corresponding adsorbents achieved obviously higher methane yield than the control, decreasing in the following order: OLMZ > LMZ > OMZ > UMZ > control. This result supported that OLMZ is more effective than the other 3 adsorbents for high-efficient ammonium-rich fermentation. Moreover, by way of light stimulating, remarkable enhancement of methane yield was presented, the methane yield increased by 54% to 69% for all the experimental bioreactors, which the OLMZ-I contributed the highest methane yield promotion of 69% compare to the OLMZ. These results indicated that the integration of OLMZ fixed bioreactor and intermittent illumination has a positive effect on the ammonium-rich anaerobic digestion. OLMZ, with efficient ammonia removal ability and abundant metal-nutrients content, could alleviate the ammonia

toxicity and improve the growth of microorganism [83]. Zhang et al. reported that intermittent light stimulation could improve the bio-methane conversion of high ammonium concentration anaerobic digestion [55]. However, under higher ammonium concentration condition (4000 mg/L), CH_4 yield of OLMZ-I (372 ± 30 mL/g-$DOC_{removed}$) obtained in the present study was significantly higher compared to their research (3000 mg/L, 283 mL/g-$DOC_{removed}$). The synergetic effects of the light activation and essential metal nutrients supplementary from OLMZ that might be able to induce the activity of microbes during their metabolisms. In conclusion, the results presented that the synthesized OLMZ adsorbent is a potential material to replace bare zeolite for constructing the fixed adsorbent bioreactor for high-efficient ammonium-rich anaerobic digestion. Besides, the integration of illumination strategy could additionally increase the methane yield compared to the condition in dark.

Fig. 3.5c reveals the variation on the ammonium concentration with digestion time of the bioreactors. Compared with the control reactor, ammonia nitrogen accumulation was all relieved in adsorbents applied bioreactors. OLMZ-I brought a slightly higher ammonia removal compare to the other adsorbents fixed bioreactors during the anaerobic process, indicating that the highest ammonium adsorption capacity of OLMZ-I within the experimental reactors. The ammonium concentration of all treatments decreased during the first 2 days of fermentation, due to the adsorption of ammonium where zeolite adsorbents served as a physico-chemical sink. About 10 days later, the values of ammonium content showed marginal increase, which might be

ascribed to the desorption of ammonium from the adsorbents and the syntrophic reaction of the microorganism in the ammonium-rich environment. In general, the ammonium content value in OLMZ-I was always lower than the other bioreactors. The result is in consistent with the ammonia adsorption capacity analysis where adsorbent OLMZ attained the highest ammonia uptake ability (Fig. 3.4). The high efficient ammonia removal ability of OLMZ-I probably could promote the anaerobic digestion process for methane production, this could be justified by a previous study which observed positive effect of activated carbon for anaerobic stabilization of post-hydrothermal liquefaction wastewater obtained from swine manure [87]. The high-efficient performance of OLMZ-I presented the highest and stable methane content and yield with greater ammonia removal efficiency than the other bioreactors demonstrated that it might be a favorable bioreactor for anaerobic digestion of ammonium-rich livestock waste. As microorganism plays an important role in bio-methane conversion, to understand the different performances of these reactors, further investigation of the microbial activity and quantity in reactors was conducted.

3.3.4 Activity and quantity of microorganisms

The OLMZ-I presented highly efficient methane production under ammonium-rich anaerobic digestion process. As microorganism is the key factor in determining the performance of the bioreactor, the activity and colonization of microorganisms on adsorbents fixed system were investigated. Biomass and ATP are two typical parameters commonly used for evaluating the performance of anaerobic

digestion, coenzyme F_{420} is an essential enzyme for methane bioconversion and frequently used to directly reflect the activity of methanogens [88,89].

Obviously, Fig. 3.6a shows the highest ATP concentration was obtained by the OLMZ-I arriving at 4.3 ± 0.2 μmol/L, which is 5.4 folds of the control at the mid-term (day 8) showed stable performance, suggesting that microorganism activity was effectively enhanced in OLMZ-I. At the beginning of day 2, all the bioreactors added fixed adsorbents system showed higher ATP result than the control. OLMZ-I at 0.8 ± 0.08 μmol/L contributed to the best performance. From day 2 to day 8, ATP value in all the bioreactors enhanced due to the adaptation of anaerobes, while higher ATP value was observed in OLMZ-I. The ATP was improved by more than 400% in OLMZ-I (4.3 ±0.2 μmol/L) compared with the control (0.8 ±0.03 μmol/L). This enhancement can be attributed to the fact that the OLMZ fixed system aggregated large amount of anaerobes providing essential metal nutrients for the microbial metabolism, thus improved their activity. At the end of fermentation, the ATP values declined in all the bioreactors, which might be relevant to the deficient in substrate, due to the nutrient substances were consumed by methanogenic activity. ATP concentration is an index of metabolically active cells as well as the microbial density, a large amount of microbes would contribute to the high concentration of ATP. The results suggested that OLMZ-I stimulated the activities of microbes and thus boosted the production of methane.

Also, the coenzyme F_{420} exhibited similar tendency as the ATP concentration. The OLMZ-I reactor detected obvious higher F_{420} concentration than the other conditions

throughout the whole fermentation period, and higher value was shown under light condition than the dark (Fig. 3.6b). Without illumination aided, the F_{420} value was increased in all the experimental groups compare to the control and the OLMZ achieved the highest. After light irradiation, 15% to 43% increase in F_{420} concentration was detected within all experimental groups, which indicates that methanogen activity was enhanced under light stimulation. Specifically, the OLMZ-I exhibited obvious higher F_{420} concentration compare to the other parallel bioreactors. At the stable operation period (the 8^{th} day), the difference enlarged as OLMZ-I with 1.27 ± 0.06 μmol/L F_{420} value achieved 6.85 folds higher F_{420} than the control, suggesting that greater improvement of methanogen metabolism obtained through integrating the OLMZ fixed system and light stimulation. Afterwards, the F_{420} value decreased in all the bioreactors and the difference between them was narrowed due to limited substrate available at the end of anaerobic digestion. Although ATP can reveal the performance of whole microbes evolved in the biodegradation, the activity of methanogens can be explained more effectively by F_{420} than by ATP value. F_{420} concentration could reveal the efficiency of hydrogenotrophic methanogenesis directly [90]. Higher coenzyme F_{420} concentration represents higher conversion rate from organic matters to methane [91]. Besides, previous studies reported that appropriate light stimulating could accelerate the activation of the CH_3-S-CoM reductase for methane generation [72]. Accordingly, the incorporation of OLMZ fixed system and intermittent illumination strategy could effectively improve the activity of methanogens and accelerate the

methanogenesis.

In methanogenic syntrophic communities, electrons and protons flow from one organism to the other with or without by shuttle components. The electrical communication between methanogenic communities is considered as an important factor to promote biomethane conversion [92]. Therefore, the conductivities of sludge samples were detected when all of the reactors performed stable and high methanogens activity. It should be noted that the conductivity of sludge in OLMZ-I was 12.40 ± 0.18 μS/cm, which was 2.75 folds higher than that of the control (Fig. 3.7). The OLMZ and OLMZ-I in their respective groups (dark and illumination) all exhibited apparent higher conductivity compared to the others, corresponding to better bio-methane conversion. However, the incorporation of intermittent illumination strategy (OLMZ-I) further improved conductance by 24% compared to OLMZ in dark. Recent years, several anaerobes have showed electron transfer capacity, methanogenic gathering was found to be an electrically conductive process. The electron exchange within the methanogenic system could be conducted by direct interspecies electron transfer (DIET) or interspecies hydrogen/formate transfer [93]. DIET seems to be a more effective syntrophic metabolism for higher methane production compared to interspecies hydrogen/formate transfer. Through DIET, free electrons could transport from the electrogenic bacteria to the electrophic methanogen without being shuttled by medium of reduced molecules like molecular hydrogen or formate and require less energy consumption [94]. The augmented sludge conductance value in the OLMZ-I suggested

the high efficiency of electron transfer between methanogenic communities. It could be speculated that the integrating of fixed OLMZ and light illumination could improve the DIET-based syntrophy which contributes to higher sludge conductivity for enhancement of anaerobic digestion process [76].

In addition, in order to measure the quantity and show morphology of attached microbes, biomass quantity analysis and SEM observation were conducted at the end of fermentation. As shown in Fig. 3.8, the highest biomass quantity was attached in OLMZ-I which showed 59% higher value than UMZ. Biomass is quantity of microorganisms, a high value of immobilized biomass indicates a high amount of microbes attached. Immobilized anaerobes have a high resistance to ammonia inhibition [75]. It can be inferred that the majority of anaerobes have successfully aggregated on the surface of the CPE fixed OLMZ system in OLMZ-I. The SEM observation of immobilized microbes could be the strong evidence. The SEM graph approved that methanogens had preferable attachment on the OLMZ adsorbent compare to the other adsorbents. As seen from the Fig. 3.9, it is clear that high amount of microbes were successfully immobilized on the fixed adsorbents in all the bioreactors. Nevertheless, OLMZ-I attached the highest quantity of microorganisms, corresponding to the biomass results (Fig. 3.8). The rough surface, large porosity and abundant metal cations content of OLMZ contributed to the enhanced immobilization of microorganisms in OLMZ-I. Additionally, in the OLMZ-I, short rods and cocci shaped *Methanosarcina*-like and *Methanosaeta*-like cells have been found on the

surface of OLMZ adsorbent, the *Methanosarcina*-like microbes attached closely and abundant [95,96]. *Methanosarcina soligelidi* has been found to be the most tolerant acetoclastic methanogens at extreme ammonia levels in anaerobic digestion (greater than 7 g NH_4^+-N/L) in previous study [97]. Besides, *Methanosarcina* and *Methanothrix* are typical methanogen species have been proved to join the DIET process which reduces CO_2 to CH_4 via receiving the electrons from other species utilizing acetate, butyrate and propionate directly [96,98]. The immobilization of those ammonia-tolerant and potential electerophic methanogens (DIET partner) was supposed to be the supporter of enhanced biomethane conversion in OLMZ-I.

The high activity and quantity of methanogens are probably due to promotion of microbes immobilization on the OLMZ adsorbent as well as the activation of coenzyme F_{420} by the illumination [55]. The rough surface, large pore size and surface area make the OLMZ a favorable fixing material for multiplication of microorganisms. The high concentration of essential metal cations in OLMZ adsorbent provided sufficient nutrients for microbes growth, contributed to improving both the density and activity of microbes. Meanwhile, DIET-based syntrophy of methanogenesis was accelerated due to the anaerobes aggregation and abundant metal cations in OLMZ. Therefore, the ATP, F_{420} value, sludge conductance, biomass quantity and SEM morphologies indicated that OLMZ adsorbent is a promising and suitable alternative for unmodified zeolite. The high metabolic activity and quantity of the microbes immobilized in the OLMZ-I bioreactor provided strong support to demonstrate better performance and high

efficiency of OLMZ-I bioreactor with regard to the ammonium-rich anaerobic digestion, and the mechanism will be further illustrated in the next section.

3.3.5 Proposed mechanisms of the illuminated fixed OLMZ bioprocess

The results and discussions presented above proposed that OLMZ is a promising alternative material for developing the high-efficiency adsorbent fixed bioreactor and the biomethane conversion could be further enhanced with intermittent light activation supporting. In order to make further application, it is necessary to illustrate the mechanism involved in the integration of OLMZ fixed system and light stimulation on mitigating the ammonia inhibition during the ammonium-rich anaerobic digestion.

Fig. 3.10 showed the schematic of the mechanisms involved in the novel illuminated OLMZ fixed bioreactor process. Five important functions in the process could enhance the biomethane conversion. Firstly, ammonium adsorption occurred in the start-up period. According to the adsorption analysis results, ammonium adsorption on the OLMZ adsorbent was dominated by ion-exchange process, large amount of ammonia was adsorbed along with release of cations like Ca^{2+}, Na^{+}, K^{+} and Fe^{3+} into the liquid gradually (Table 3.2). By modification of oyster shell and lignite, considerable amount of metal ions were embedded in the raw zeolite, especially Ca^{2+} which has high affinity for NH_4^{+} exchange [48]. Thus, the ion-exchange capability of OLMZ was significantly enhanced, exhibited excellent adsorptive capability and selectivity for ammonia due to the large ion exchange capacity and its high-porosity property. Meanwhile, adequate amount of micronutrients is essential for the

microorganisms to sustain their basic activity and enhance their environmental adaptability that could contribute to efficient and stable anaerobic digestion performance [99,100]. McCarty et al. and Chen et al. found that Ca^{2+} worked as an antagonistic ion which enables stable enzyme activity of anaerobic microbes and alleviation of ammonium toxicity [101,102]. Therefore, we can infer that the ammonia inhibition was effectively mitigated by the OLMZ adsorption at the start-up phase, providing the microorganisms with relatively suitable environment for reproduction, and the exchanged cations in the bioreactor act as micronutrient elements source which can improve the activity of microbes as well.

Secondly, the physical structure of OLMZ is favorable for microorganism attachment and the adequate micronutrients content make the OLMZ attractive to microbes. When the ammonium adsorption on OLMZ reached to its equilibrium state, the main function of OLMZ altered in anaerobic digestion process. OLMZ played an important role in the immobilization of microbes. The rough surface and porous structure properties favor the microorganisms granulation on the OLMZ (Fig. 3.8), and the high metal cations content (Table 3.2) is appealing to microbes [75]. Usually, it takes 2–3 weeks for anaerobes to form the biofilm [64]. Thus, after the adsorption of ammonium saturated, biofilms were gradually formed on the fixed OLMZ system (Figs. 3.8 and 3.9) in this present experiment. The biofilms aggregated with large quantity and various species of microbes have a higher activity and tolerance to ammonia toxicity during the anaerobic digestion [75].

Thirdly, oyster shell is rich in Ca^{2+} and lignite contains various metal cations, through the modification, abundant resources of metal cations were embedded into the OLMZ that could benefit the growth of microorganisms. The EPMA results showed the cations content in OLMZ before and after anaerobic digestion process (Table 3.2). The main cations including Fe^{3+}, K^{+}, Ca^{2+}, Na^{+} and Mg^{2+} decreased by 100%, 98%, 95%, 86% and 46%, respectively, were assimilated by anaerobes which showed excellent performance on CH_4 conversion (Fig. 3.5). The supplementation of above-mentioned cations could increase microbial activity and promote the immobilization of microorganisms. This result was consistent with that obtained in previous study by Bougrier et al., who reported that abundant micronutrients supplied to the microorganisms could maintain their activity and improve their performance for an efficient and stable anaerobic digestion [103].

Fourthly, together with the microorganism immobilization and metal cations maintenance, it could also facilitate the electron transfer between the microorganisms. The aggregation of syntrophic bacterial and archaea organisms maximizes the contact of anaerobes which favors the DIET process and OLMZ itself contains metal cations could also be a carrier for the electron transfer. We have observed obvious higher conductance values of sludge in OLMZ-I compared to that in the other bioreactors indicating the electron transfer activity was significantly promoted in OLMZ-I (Fig. 3.7). This inferred that the promotion could be attributed to the microbes attached on the CPE fixed OLMZ system that could substitute for some biological connectors to

induce DIET-ability between the electrophic methanogens. Rotaru et al. has proved that the direct cell-to-cell contact is an essential route for DIET process [98]. Similarly, Wang et al. also reported that the addition of biochar could improve conductivity and selectively enrich the potential DIET microbes for enhancing the DIET process [93]. In this study, the aggregation of microbes and the presence of abundant metal cations on OLMZ could enhance the electron transport during the biomethane conversion.

Finally, with the integration of light illumination, the performance of OLMZ-I was further improved compare to the OLMZ. The higher methane production in OLMZ-I might be due to the synergetic effects between these two strategies. Under the light irradiation, the essential reductase (F_{420}) responsible for catalyzing methane production during the methanogenesis process have been activated contributing to advanced methane production activity [55]. In addition to light stimulation, with the fixed OLMZ system, anaerobes immobilized on the OLMZ that the activity and electron communication have been boosted. Meanwhile, the OLMZ with various metal cations sustained could be a good nutrients resource for microbes growth. Thus, through integrating the OLMZ fixed system and intermittent light strategy, the novel process presented facilitated methanogenesis activity, contributing to high tolerance to harsh ammonia inhibition environment as well as much better methane production during the ammonium-rich anaerobic digestion. The synergetic effects of ammonia removal, microbes immobilization, metal cations supplement and accelerated electrical communication between methanogenic system combined with light stimulation on

methanogen activation facilitated the great enhancement of methanogenesis in OLMZ-I bioreactor. In order to exactly elucidate the synergistic effects of illuminated OLMZ fixed bioreactor process and illustrate the responses of methanogens, microbial community analysis should be performed in the future experiment.

3.4 Summary

In this chapter, a novel CPE fixed oyster shell and lignite modified zeolite (OLMZ) bioreactor incorporated with intermittent illumination process was developed for mitigating ammonia inhibition to improve the efficiency of ammonium-rich anaerobic digestion. OLMZ illustrated advanced ammonium adsorption capacity and abundant metal cations content, and effectively mitigated the negative impacts of ammonia and improved the microorganism growth. In addition, light stimulation enhanced the activity of methanogens for bio-methane conversion in the CPE fixed OLMZ bioreactor. Synergy of ammonia removal, metal ions supplement, microorganism immobilization, electrical communication and methanogens light activation by OLMZ-I contributed to the improved anaerobic digestion efficiency. Therefore, the novel CPE fixed OLMZ bioreactor coupled with intermittent light stimulation bioprocess strategy shows great potential for pollution control and resources recovery for improving the biomethane conversion of ammonium-rich waste.

Table 3.1 Surface area characterization of the four adsorbents

Adsorbent	BET surface area (m^2/g)	Total pore volume (m^3/g)
UMZ	1.812	0.0164
OMZ	1.980	0.0131
LMZ	1.946	0.0117
OLMZ	2.371	0.0178

Table 3.2 The main elements content ratio (wt%) of four adsorbents before (a) and after (b) the fermentation

Elements	K^{+}	Ca^{2+}	Na^{+}	Mg^{2+}	Fe^{3+}	Al^{3+}	Si^{4+}	O
Before fermentation (a)								
UMZ	4.95	0.12	4.28	0.087	0.067	17.30	13.18	36.62
OMZ	4.68	8.08	4.78	0.094	0.11	15.06	12.87	29.21
LMZ	4.77	1.29	3.53	0.10	0.16	14.83	13.39	32.13
OLMZ	3.78	7.53	4.35	0.16	0.19	13.24	12.43	27.96
After fermentation (b)								
UMZ	0.78	0.019	0.90	0.01	0.00	15.15	12.78	38.90
OMZ	0.93	0.66	0.26	0.19	0.00	13.96	12.44	35.65
LMZ	0.82	0.39	0.79	0.046	0.073	10.03	11.24	37.92
OLMZ	0.92	0.69	0.52	0.017	0.019	15.88	14.72	40.24
UMZ-I	0.53	0.011	0.61	0.067	0.081	17.92	11.06	39.33
OMZ-I	0.41	0.26	0.46	0.31	0.087	15.89	11.66	36.08
LMZ-I	0.19	0.22	0.054	0.071	0.061	13.32	14.53	39.44
OLMZ-I	0.09	0.35	0.62	0.087	0.00	12.95	14.69	43.76

* Standard deviation, n=3. Data are calculated from average value without showing the standard deviation.

Table 3.3 Ammonia adsorption performance fitting results by adsorption kinetics models for the four adsorbents

Adsorbent	Ho's pseudo-second-order kinetic model			Lagergren pseudo-first-order kinetic model		
	K_2 (g/(mg·min))	q_e (mg/g)	R^2	K_1 (1/min)	q_e (mg/g)	R^2
UMZ	0.000126	78	0.9933	0.00031	6.282	0.4432
OMZ	0.000496	92	0.9947	0.00322	5.647	0.5668
LMZ	0.000496	89	0.9980	0.00230	5.862	0.9193
OLMZ	0.000838	99	0.9985	0.00069	6.295	0.4393

Table 3.4. Ammonia adsorption performance fitting results by Freundlich and Langmuir isotherm models for the four adsorbents

Adsorbent	Freundlich isotherm model			Langmuir isotherm model		
	K_f	1/n	R^2	b (L/mg)	q_m (mg/g)	R^2
UMZ	1.128	0.3616	0.9895	7.99×10^{-4}	63	0.9259
OMZ	1.763	0.4280	0.9908	4.91×10^{-4}	61	0.8167
LMZ	1.617	0.3575	0.9916	6.83×10^{-4}	48	0.8316
OLMZ	3.150	0.3351	0.9944	7.32×10^{-4}	86	0.9166

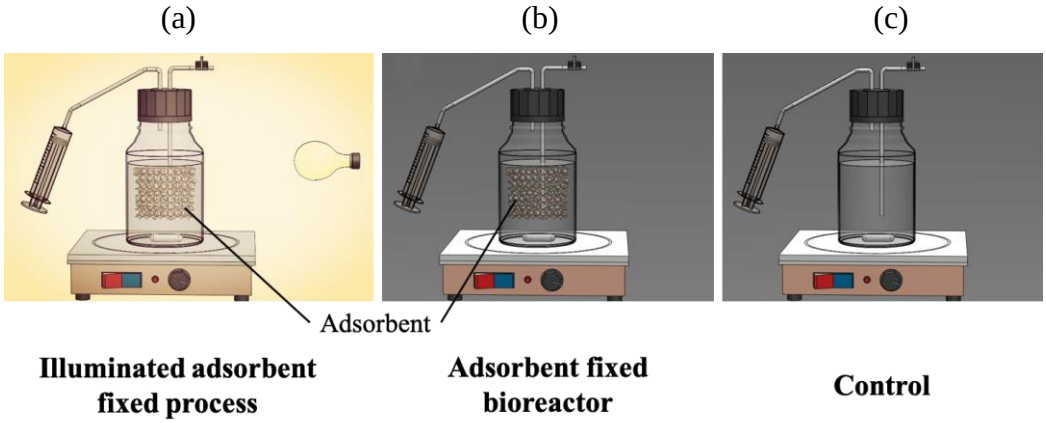


Figure 3.1 Schematic of the bioreactors: (a) illuminated adsorbent fixed process, (b) adsorbent fixed process and (c) control.

A - Gold electrode
B - Glass substrate
C - Anaerobic sludge

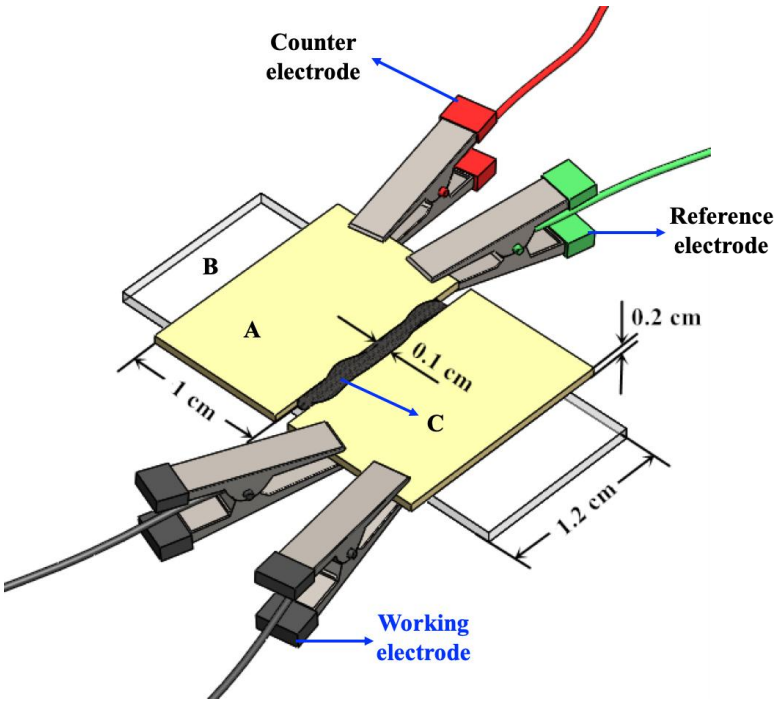


Figure 3.2 Schematic diagram of gold electrode for conductance measurement.

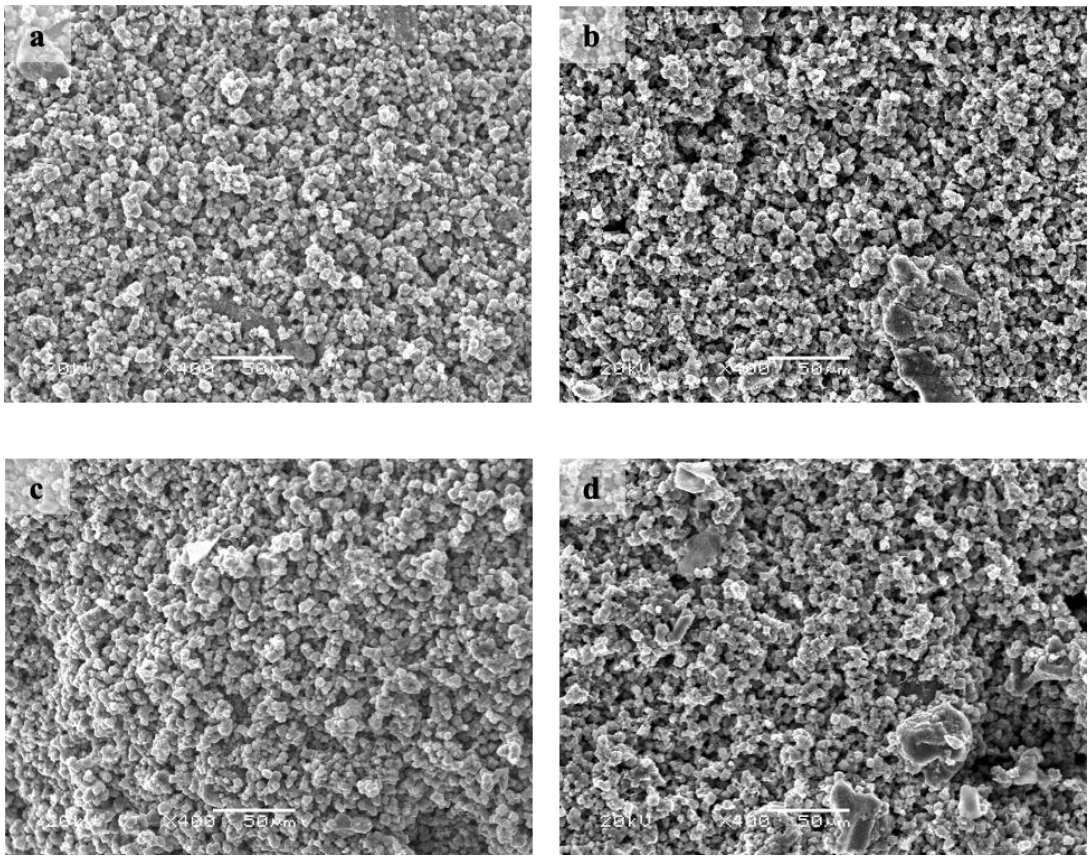


Figure 3.3 Surface morphology of the four adsorbents, (a) UMZ, (b) OMZ, (c) LMZ, (d) OLMZ, magnification, 400×.

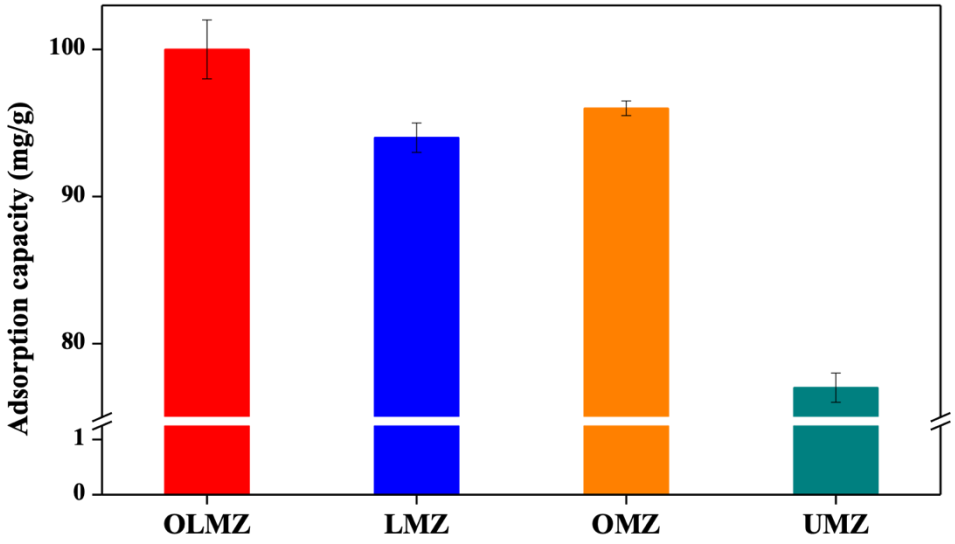


Figure 3.4 The ammonium adsorption capacity of four adsorbents (Initial TAN concentration: 4,000 mg/L, adsorbent dosage: 10 g/L (Error bars designate standard deviations of triplicate experiments).

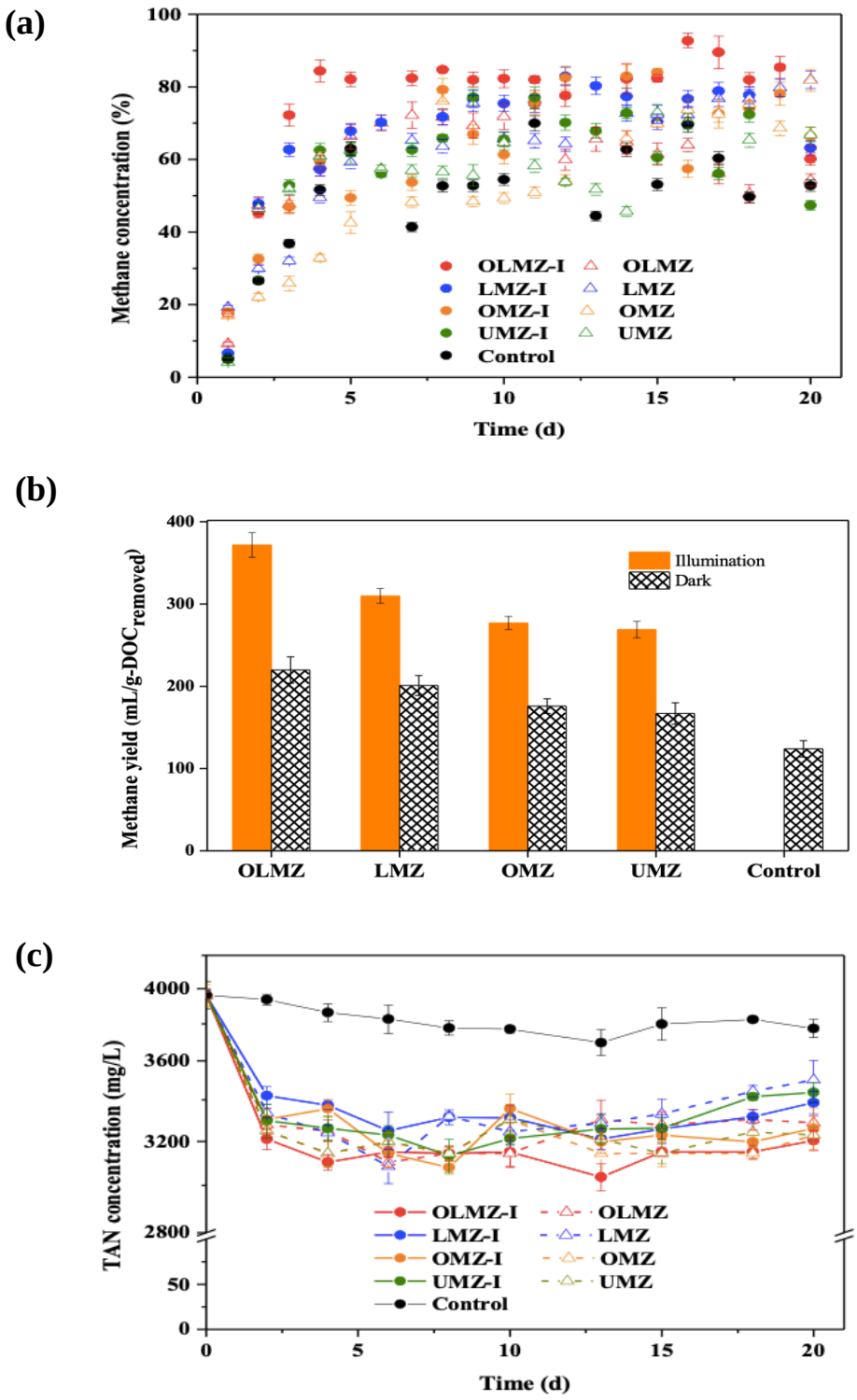
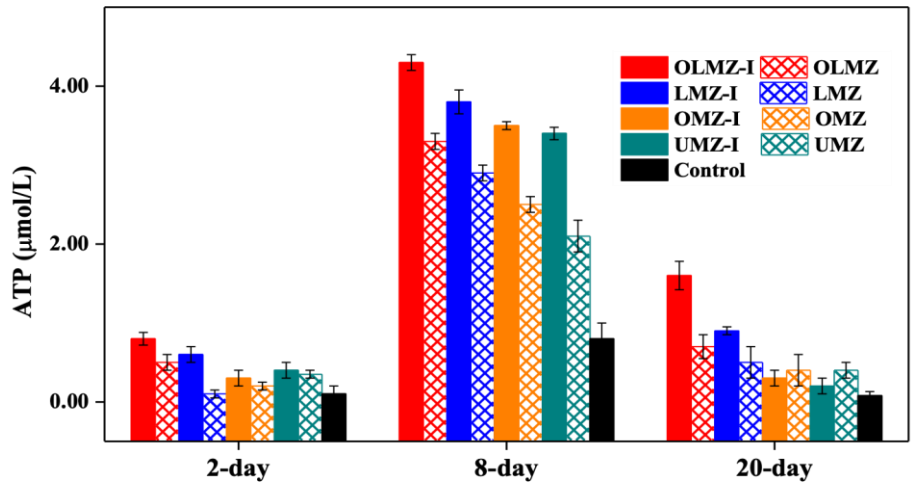


Figure 3.5 Variations of methane concentration (a), methane yield (b) and TAN concentration (c) of bioreactors with different fixed adsorbents under dark and illumination condition (Error bars designate standard deviations of triplicate experiments).

(a)



(b)

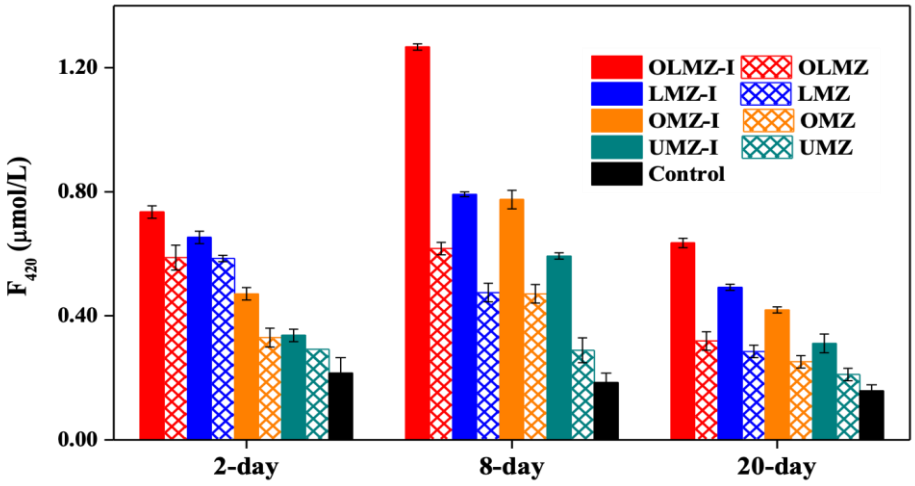


Figure 3.6 ATP values (a) and F_{420} concentration (b) of anaerobes in bioreactors with different fixed adsorbents under dark and illumination condition (Error bars designate standard deviations of triplicate experiments).

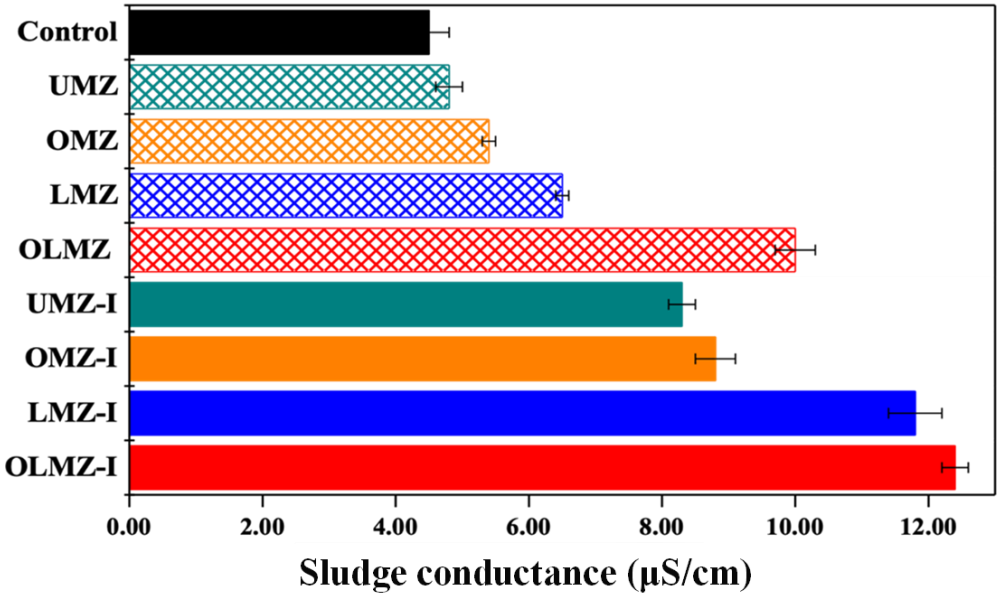


Figure 3.7 Conductance of sludge attached in the bioreactors with different fixed adsorbents under dark and illumination condition (Error bars designate standard deviations of triplicate experiments).

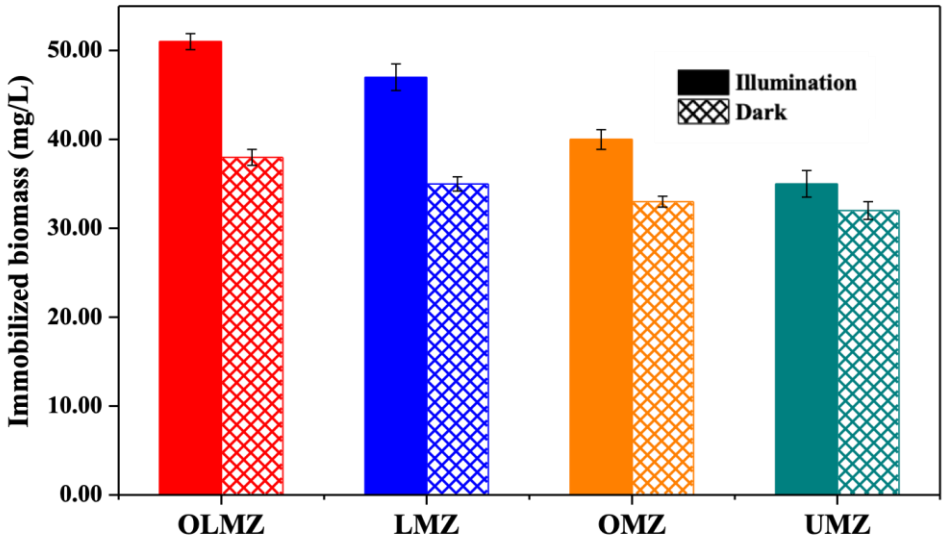


Figure 3.8 Biomass quantity immobilized on the surface of fixed adsorbents system in different bioreactors under dark and illuminated condition (Error bars designate standard deviations of triplicate experiments).

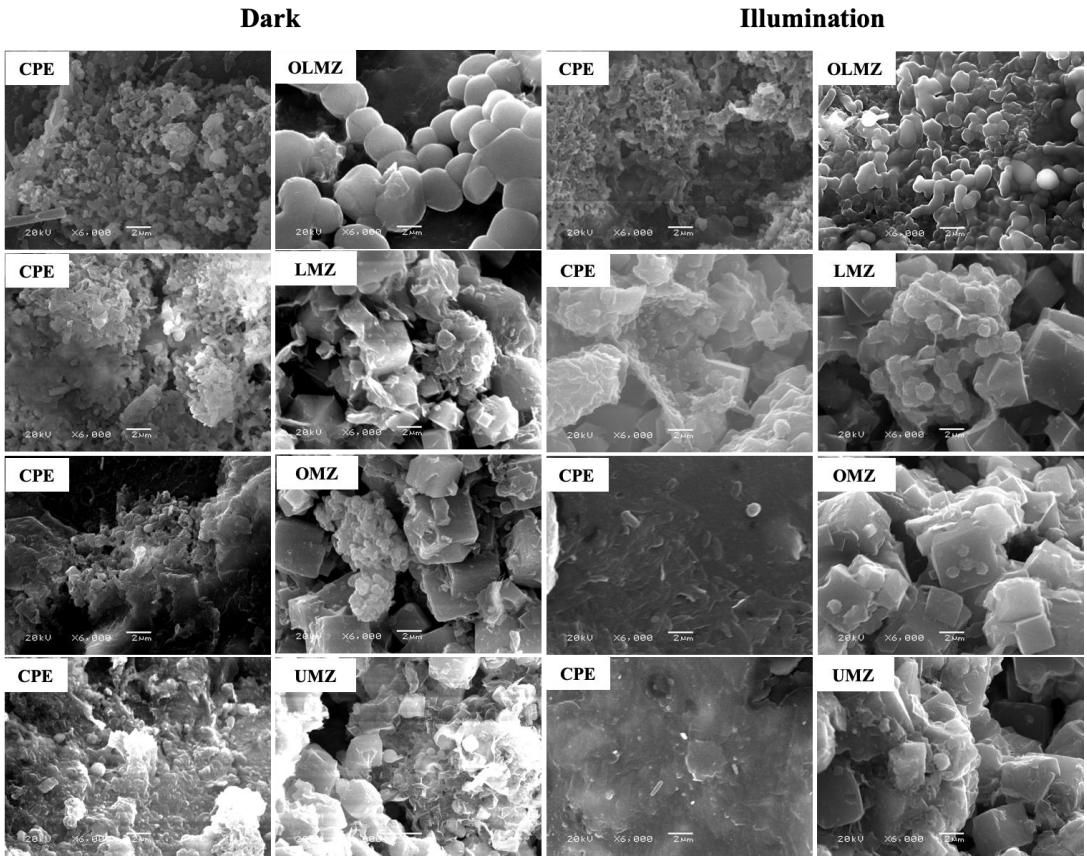


Figure 3.9 SEM photographs of the microbes immobilized in four CPE fixed adsorbents bioreactors under dark or illumination condition, magnification, 6,000×.

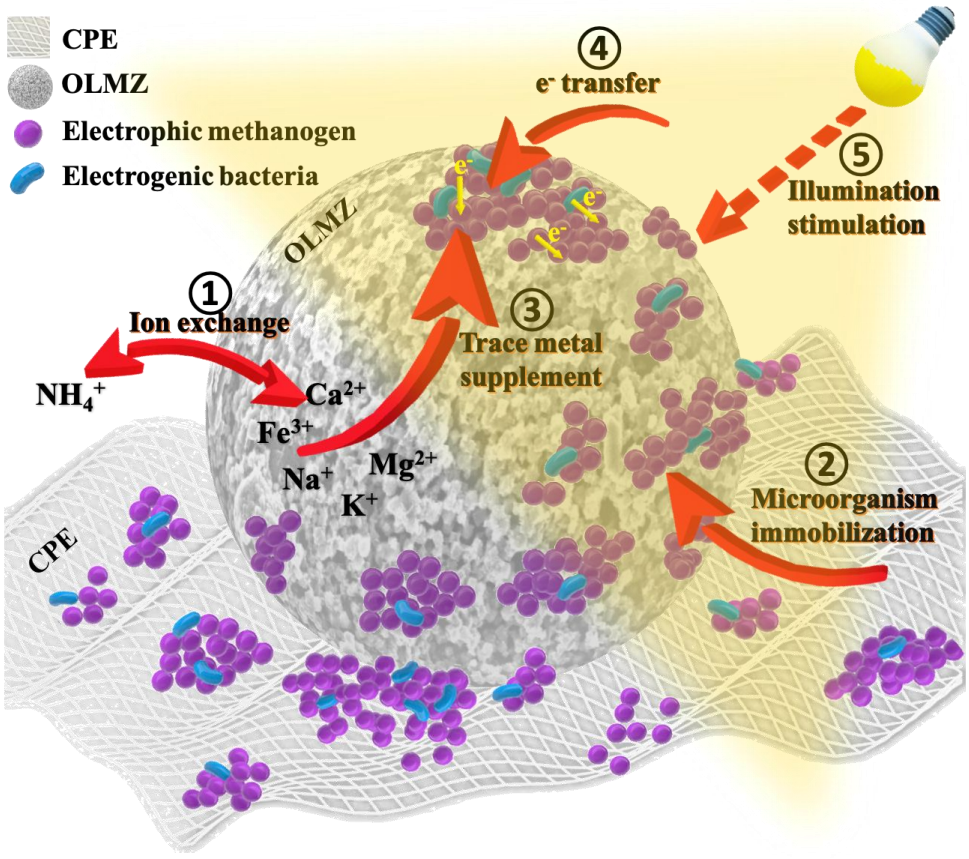


Figure 3.10 Proposed mechanism of integrating OLMZ fixed bioreactor and intermittent illumination for high efficient ammonium-rich anaerobic digestion.

Chapter 4 Conclusions

In this study, a novel CPE fixed oyster shell and lignite modified zeolite (OLMZ) fixed bioreactor incorporated with intermittent light illumination process was developed for anaerobic digestion of ammonium-rich livestock wastes. Compared to traditional ammonia removal strategies, several benefits make the illuminated CPE fixed OLMZ bioprocess a better performance and promising strategy for practical use. Such as high ammonium absorb efficiency and readily for microorganism immobilization. The major conclusion could be drawn from the previous chapters as follows:

4.1 Investigation of optimal material for the bed material fixed zeolite system on mitigating ammonia inhibition during anaerobic digestion

Three typical polymer materials were investigated to develop the high efficient bedding material fixed zeolite bioreactor in anaerobic digestion of extremely high concentration ammonium swine wastes, including porous nylon, chlorinated polyethylene and polymer foaming sponge. After that, the CPE fixed zeolite system was studied in the semi-continuous anaerobic digestion process at high-ammonium concentration to investigate the long-term practical applicability. The following conclusions were obtained:

(1) CPE is the best material for zeolite fixing in development of high efficiency bed material fixed zeolite bioreactor in the anaerobic digestion treating extremely high concentration ammonia swine wastes.

(2) The synergy of ammonia adsorption and immobilized microorganisms by CPE

fixed zeolite system contributed greatly to the mitigation of ammonia inhibition and enhancement of anaerobic digestion efficiency.

(3) The CPE fixed zeolite bioreactor obtained good and stable performance at high ammonium concentration is a promising bioreactor for long-term practical use of anaerobic digestion ammonium-rich livestock wastes.

4.2 Establishment of illuminated CPE fixed modified zeolite bioprocess on restrain ammonia inhibition of anaerobic digestion

To develop an effective anaerobic digestion bioprocess for high efficient ammonium-rich livestock waste fermentation, zeolite optimization was carried out by integrating the oyster shell and lignite as modification materials for enhancing the efficiency and economy of CPE fixed zeolite bioreactor. In addition, the feasibility and possible benefits of combining the CPE fixed optimized zeolite bioreactor with intermittent illumination for high efficient ammonium-rich anaerobic digestion have been investigated. Following conclusions were obtained from this chapter:

(1) OLMZ illustrated advanced ammonium adsorption capacity and abundant metal cations content, effectively mitigated the negative impacts of ammonia and improved the microorganism growth.

(2) Light stimulation enhanced the activity of methanogens for bio-methane conversion.

(3) Synergy of ammonia removal, metal ions supplement, microorganism immobilization, electrical communication and methanogens light activation by illuminated CPE fixed OLMZ bioprocess contributed to the improved anaerobic

digestion efficiency.

(4) The novel CPE fixed OLMZ bioreactor coupled with intermittent light stimulation bioprocess strategy shows great potential for pollution control and resources recovery for improving the bio-methane conversion of ammonium-rich waste.

(5) The two modification materials used in this study, oyster shell is a waste for aquaculture industry need to be treated and lignite with low combustion value. Hence, this strategy also provides a promising way for recycling of oyster shell and efficient utilization of lignite.

5.3 Future research

In this study, a novel high efficiency illuminated CPE fixed OLMZ bioprocess was developed. The innovative strategy exhibited great potentiality for bio-methane recovery from ammonium-rich waste. Synergy of ammonia removal, microorganism immobilization, metal ions supplement, electrical communication between methanogenic communities and photo activation on methanogens by this novel bioprocess contributed to the improved anaerobic digestion efficiency. This present study provides a valuable platform for improving the biogas production efficiency and advance anaerobic digestion engineering.

Firstly, in considering of the subsequent practical application, a pilot-scale continuous experiments with realistic substrate livestock wastes are necessary to confirm whether this novel illuminated CPE fixed zeolite bioreactor is still suitable for long-term practical fermentation.

Meanwhile, in order to exactly elucidate the synergistic effects of the illuminated CPE fixed OLMZ and illustrate the responses of methanogens, microbial community analysis should be performed in the future anaerobic digestion experiment.

In addition, after the anaerobic digestion operation, the OLMZ saturated with ammonium and immobilized large quantity of microorganisms is hard to be reused for ammonia removal in the new round operation. However, as the OLZM is mainly composed of zeolite and doped with oyster shell and lignite. Being taken form the digester at the end of fermentation, the ammonium saturated OLMZ could be used as an ideal N-fertilizer due to the abundant ammonium content. In the future study, evaluation experiments should be done to evaluate the potential and efficiency of ammonium saturated OLMZ as a soil fertilizer.

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[1] **H.Y. Zheng,** A. Sharma, Q. Ma, C. Zhang, T. Hiranuma, Y. Chen, G. Chen, Y.N. Yang.: Development of an oyster shell and lignite modified zeolite (OLMZ) fixed bioreactor coupled with intermittent light stimulation for high efficient ammonia-rich anaerobic digestion process, Chemical Engineering Journal. 398 (2020) 125637. (IF: 10.652)

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[3] C.Y. Zhao, N. Zhang, **H.Y. Zheng**, Q. Zhu, M. Utsumi, Y.N. Yang.: Effective and long-term continuous bio-hydrogen production by optimizing fixed-bed material in the bioreactor. Process Biochemistry, 83 (2019) 55–63. (IF: 2.952)

[4] N. Zhang. **H.Y. Zheng**, X.H. Hu, Q. Zhu, M.S. Stanislaus, S.Y. Li, C.Y. Zhao. Q.H. Wang, Y.N. Yang.: Enhanced bio-methane production from ammonium-rich waste using eggshell-and lignite-modified zeolite (ELMZ) as a bio-adsorbent during anaerobic digestion. Process Biochemistry, 81 (2019) 148–155. (IF: 2.952)

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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, Chemical Engineering Journal. 280 (2015) 106–114. (IF: 10.652)

[7] D.W. Li, **H.Y. Zheng**, Q. Wang, X. Wang, W. Jiang, Z.Y. Zhang. Y.N. Yang.: A novel double-cylindrical-shell photoreactor immobilized with monolayer TiO_2-coated silica gel beads for photocatalytic degradation of rhodamine B and methyl orange in aqueous solution, Separation and Purification Technology. 123 (2014) 130–138. (IF: 5.774)

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