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Strategy of mitigating ammonium-rich waste inhibition on anaerobic digestion by using illuminated bio-zeolite fixed-bed process

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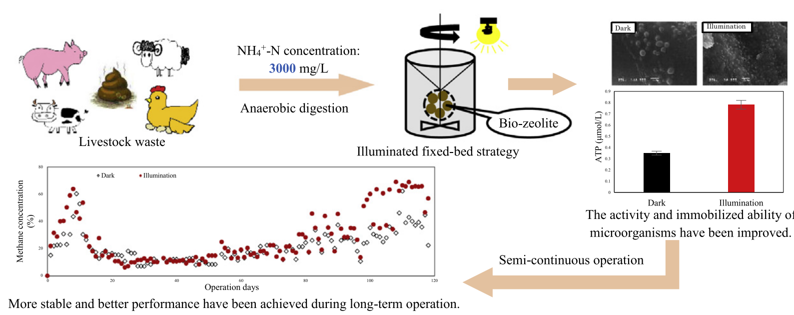
HIGHLIGHTS

- Illumination method was used to mitigate ammonia inhibition in anaerobic digestion.
- Combination of illumination with fixed-bed process become effective.
- The process was well-performed with ammonium-rich substrate in long-term operation.
- The strategy is promising for treating livestock waste practically.

GRAPHICAL ABSTRACT



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ABSTRACT

Intermittent illumination combined with bio-zeolite fixed-bed process was utilized to improve the efficiency of anaerobic digestion with ammonium-rich substrate. The batch experiments were carried out at NH_4^+-N concentration of 2211 mg/L under intermittent illumination and dark (as control) conditions, respectively. The illuminated bioreactor achieved higher methane production (287 mL/g-DOC) and ATP value (0.38 μmol/L) than that under dark condition. Then the bio-zeolite fixed-bed bioreactor (NH_4^+-N concentration: 3000 mg/L) was used to study the additional efficiency on the illuminated ammonium-rich anaerobic digestion process. The result showed that the illuminated fixed-bed bioreactor presented the greatest methane concentration (70%), methane yield (283 mL/g-DOC) and quantity of methanogens comparing with no-bed bioreactor. Furthermore, the illuminated fixed-bed bioreactor achieved better performance during 118-day semi-continuous fermentation. The combination of the intermittent illumination and bio-zeolite fixed-bed strategy contributed to the higher efficiency and stability of the ammonium-rich anaerobic digestion process.

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1. Introduction

Along with the development of world population, increasing amount of meat products had led to the collective feeding industries (Ortner et al., 2014). As a consequence, accumulation of livestock waste constitutes a growing problem, such as pathogenic and contaminated soil, water and air. Anaerobic digestion with high biological transformation is considered to be a promising process to deal with the livestock waste, since both pollution control and energy recovery can be achieved during the process (Siles et al., 2010; Ward et al., 2008). However, anaerobic digestion in which livestock waste serves as the substrate could be largely inhibited by the high concentration of ammonia, which usually results in the decrease of the stable state of methane production rate and the increase in the digestion products, which could contribute to the instability of the anaerobic bioreactor (Calli et al., 2005).

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Generally, ammonia inhibition occurs within the range of 1500–3000 mg NH_4^+-N/L due to the different nature of substrates, inoculums, and environmental conditions, such as, temperature and pH, and acclimation periods (Hejnfelt and Angelidaki, 2009). Moreover, it is reported that the anaerobic digestion processes were inhibited at any pH, when ammonium nitrogen concentration exceeds 3000 mg NH_4^+-N/L (McCarty, 1964). Free (unionized) ammonia levels are considered as the foremost cause of inhibition of methanogenic microflora, due to its high permeability to bacterial cell membrane (Borja et al., 1996). Many studies have aimed at alleviating ammonia inhibition to enhance the efficiency of the anaerobic digestion system using physicochemical and biological methods, such as adding ammonia-adsorption materials, ammonia stripping, struvite precipitation and anaerobic ammoxidation (Wang et al., 2011; Cheung et al., 1997; Kabdasli et al., 2000; Schiweck and Nähle, 1990; Uludag-Demirer et al., 2005; Yang et al., 2009). Conversely, due to the inefficiency, technology problems on operation, and the investment cost, these methods are limited in the practical application of ammonia removal. The study on mitigating ammonia inhibition with optimized method would be of great interest for both industrial applications and academic research.

The combination of microorganism immobilization and ammonia adsorption is considered to be an effective method of mitigating ammonia inhibition during the anaerobic digestion process (Yang et al., 2008; Wang et al., 2011; Zheng et al., 2015). With the high capacity to immobilize microorganisms as a porous surface and ability to tolerant high temperature and acid, the fixed zeolite is useful for improving the methane production of anaerobic digestion with ammonium-rich substrate. Wang et al. developed a fixed zeolite bioreactor which could decrease the startup time and improve the methane production of the anaerobic digestion of ammonium-rich substrate (Wang et al., 2011). In addition, a novel bio-zeolite fixed-bed bioreactor had been developed to mitigate ammonia inhibition, which proved to be favorable in long term practical use with severe ammonium-rich condition (Zheng et al., 2015).

On the other hand, although most conventional anaerobic digesters are operated under dark conditions, the lighted upflow anaerobic sludge blanket method under mesophilic condition have been studied. However, the result showed that methane production was lower than that in continuous dark (Sawayama et al., 1999). In contrast, it has been reported that methane production increased under incandescent lightening in thermophilic anaerobic digestion (Tada and Sawayama, 2004). The methane fermentation process with incandescent lighting resulted that the methane production under continuous illumination was higher than that from dark reactors (Tada et al., 2005). Besides, Yang et al. has investigated the optimal illumination time to improve the methane yield, the efficiency of volatile solid (VS) and removal of dissolved organic carbon (DOC), which has been proved to play an important role in activating methanogens under intermittent illumination condition (Yang et al., 2009). In addition, a system with dual irradiation process was developed to efficiently improve methane production by using activated sludge as the substrate (Yang et al., 2011). However, there are few studies on anaerobic digestion under illumination, especially no research on high NH_4^+-N concentration conditions combining illumination with the fixed-bed process.

This study focused on investigating the effect of intermittent illumination method on ammonia-rich anaerobic digestion. In addition, bio-zeolite fixed-bed reactor with the intermittent illumination process was developed for improving the efficiency of anaerobic digestion on ammonium-rich anaerobic digestion.

2. Materials and methods

2.1. Seed sludge and substrate

The used anaerobic digestion sludge was obtained from a sewage treatment plant in Ibaraki prefecture, Japan. Before the anaerobic digestion experiment, 400 mL digested sludge and 80 mL trace mineral solution were put into 500 mL anaerobic reactor. After one day, 0.5 g glucose was added into the reactor. The cultivation experiment was conducted at 55 °C for two weeks.

In this study, a synthetic medium containing acetate (2.5 g/L), glucose (2.5 g/L), NH_4Cl (200 mg/L), KH_2PO_4 (16 mg/L), yeast extract (300 mg/L) and a trace mineral solution (200 mL/L) was used as substrate. The chemical composition of trace mineral solution as follows: $FeSO_4{\cdot}7H_2O$ (1.11 g/L), $MgSO_4{\cdot}7H_2O$ (24.65 g/L), $CaCl_2$ $2H_2O$ (2.94 g/L), NaCl (23.4 g/L), $MnSO_4{\cdot}4H_2O$ (111 mg/L), $ZnSO_4{\cdot}7H_2O$ (28.8 mg/L), $Co(NO_3)_2{\cdot}6H_2O$ (29.2 mg/L), $CuSO_4{\cdot}5H_2O$ (25.2 mg/L), $Na_2MoO_4{\cdot}2H_2O$ (24.2 mg/L) and H_3BO_3 (31.0 mg/L). In order to obtain a high ammonium condition, a certain amount of NH_4Cl was added additional. The initial pH of the synthetic medium was adjusted to 7.0 by 1 M NaOH. The characteristics of ammonium-rich waste and seed sludge used in this experiment are listed in Table 1.

2.2. Batch anaerobic digestion experiment for no bed and fixed-bed reactors

The batch experiment was conducted at 55 °C. Fermentation bottles (300 mL, SIBATA) with 200 mL working volume were used as bioreactor. Each bioreactor contained 200 mL of synthetic medium including 20% (w/w) digested sludge. The reactors were illuminated by incandescent lamps (LW110V60W, Mitsubishi Oshram, Tokyo, Japan) with light intensity of 6 W/m^2, and the light wavelength ranged from 400 to 780 nm. The bioreactors were illuminated for 60 min per day followed by treatment under dark conditions. And pH was adjusted to 7.0 using 1 M NaOH and 1 M HCl. All the tests were performed in duplicate.

For the batch of no bed experiment, ammonium-rich synthetic medium was used as the substrate at ammonium nitrogen concentrations of 2211 mg/L. The control test was performed by using a synthetic medium without high NH_4^+-N concentration.

For the fixed-bed experiment, the zeolite A-3 obtained from Wako Pure Chemical Industries, Ltd, was used as the adsorbent in this study. Porous nylon (PN: (H-[HN(CH_2)$_X$CO]-OH), T&T, Kainan, Japan) was used as the bed material (Wang et al., 2011). Prior to experiment, it has been washed with distilled water to remove any non-adhesive impurities, and then dried in an oven at 105 °C for 12 h. In the fixed-bed bioreactor (Bed), 2 g zeolite A-3 was fixed in the bedding materials suspended in the bioreactor. The control test was performed in the bioreactors without zeolite and bedding materials.

2.3. Semi-continuous experiment

Two fixed-bed bioreactors consisting of 250 mL Duran bottle (SIBATA) with 200 mL working volume vessel containing PN and

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

Parameters	Digested sludge
Chemical oxygen demand (COD, mg/L)	6500
Total nitrogen (TN, mg/L)	5489
Total solid (TS, mg/L)	13,292
Volatile solid (VS, mg/L)	9500
Ammonium nitrogen (NH_4^+-N, mg/L)	1547
pH	7.1

2 g zeolite as the fixed bed was used. 200 mL of synthetic medium including 20% (w/w) digested sludge was added to each reactor. The composition of the reactors was similar to that of the previous batch test. One of the bioreactors was illuminated for 60 min/day, and the other one was set as the control without illumination. The initial NH_4^+-N concentration was 3000 mg/L and the pH was adjusted to 7.0 using 1 M NaOH and 1 M HCl. After start-up, the bioreactors were fed with synthetic medium at different organic loading rate (OLR) and hydraulic retention time (HRT) as listed in Table 3. The temperature was maintained at 55 °C until day 118. All the experiments were conducted in duplicate.

2.4. Analytical methods

The analysis of total solid content (TS), volatile solid content (VS) were determined according to standard methods (APHA) (Eaton et al., 2005). The pH, biogas production and composition were detected and recorded everyday. The composition of the biogas was analyzed by gas chromatography (GC-8A, SHIMAZU, Japan), using a machine equipped with a thermal conductivity detector (80 °C) and a Porapak Q column (60 °C). Nitrogen was used as the carrier gas. Every alternate day, 2 mL of sampled digestate was centrifuged to precipitate the microbes. The supernatant was used to measure the dissolved organic carbon (DOC) with a TOC analyzer (TOC-5000A, Shimadzu, Kyoto, Japan). In this study, ATP concentration was tested on the liquid sample which was taken out from the bioreactors at the end of the experiment. The activity of the microorganisms was indicated by the adenosine triphosphate (ATP) concentration by using a Bac Titer-GLoTM Microbial Cell Viability Assay (Promega, USA). The concentration of ammonium nitrogen was determined according to Nessler‘s reagent colorimetric method of the China State Environment Protection Administration (GB 7479-87) (Wang et al., 2011). The cellular morphologies present on the bedding material and zeolite were observed using a Scanning Electron Microscope (SEM, DS-720, Topcon, Tokyo, Japan).

3. Results and discussion

3.1. Effect on mitigating ammonia inhibition with intermittent illumination process

Table 2 showed the methane concentration, methane yield and chemical oxygen demand (COD) removal under different conditions. It was shown that the methane content of illuminated bioreactor (92.1%) was improved significantly than that of the dark condition (62.0%) under ammonium-rich condition, which was extremely close to the illuminated bioreactor without inhibition (93.2%). Methane concentration of biogas is one of the crucial parameters for anaerobic digestion process monitoring, which presents the steady balance of methane and carbon dioxide generated by the methanogenesis and acetogenesis (Park and Li, 2012). Higher methane concentration leads to a more stable anaerobic digestion process. The superior methane concentration of the illuminated bioreactors indicated that intermittent illumination could mitigate the ammonia inhibition occurred during the anaerobic digestion.

A methane yield of 287 mL/g-DOC with COD removal of 64.0% was observed from the illuminated bioreactor with high ammonium nitrogen concentration substrate, which is nearly 30% higher than those of the dark bioreactor (227 mL/g-DOC) (Table 2). Sάnchze et al. studied pig waste treatment by using upflow anaerobic digestion sludge bed reactor (UASB) with initial NH_4^+-N concentration less than 650 mg/L and the COD removal efficiency rate achieved 40% after operation (Sάnchze et al., 1995). In this present work, only by exposing the bioreactor to light at optimal time without setting up typical equipment can accelerate the methane production and COD removal efficiency rate considerably. In the previous study, it has been reported that an appropriate illumination time could be effective in improving COD degradation, as well as contribute to greater methane production (Yang et al., 2009). The result achieved by the ammonium-rich group proved for the first time that even under high ammonium nitrogen concentration condition, optimal illumination could improve the performance of anaerobic digestion process.

3.2. Microorganism activity under illumination process

As shown in Fig. 1, it was clear that the adenosine triphosphate (ATP) concentration of illuminated bioreactors was higher than that of the bioreactors under dark condition. The ATP concentration of the illuminated bioreactor with ammonium-rich substrate (N2211) achieved 0.38 μmol/L, which was 18% higher than that of the bioreactors under dark condition. The higher ATP value of the illuminated bioreactors demonstrated higher microorganism activity than that of dark conditions. The activity of the microorganisms in the bioreactor is a crucial parameter which influences the performance of anaerobic digestion process (Alshameri et al., 2014). ATP could reflect the activity of microorganism efficiency of anaerobic digestion process, which is considered as an indicator of metabolically active cells and a sign of microbial density (Chu et al., 2003; Chung and Neethling, 1988). The better biogas production by the short illumination time of the digester could be explained by the improved activity of the methanogens under an optimum illumination time such as 60 min/day, during which enough activated methanogens were supplied to the illuminated reactor (Yang et al., 2009). This result corresponded with the reactor performance in the Table 2. Although most methanogens is suitable for dark condition, the CH_3-S-CoM reductase which catalyzes the CH_3-S-CoM to form CH_4 only under the presence of light. In the terminal step of methanogenesis, CH_3-S-CoM is reductively demethylated with N-(7-mercapto-heptanoyl)-l'threonine O^3-phosphate (HS-HTP) as electron donor according to the following equation (Ellermann et al., 1988): CH_3-S-CoM + HS-HTP → CH_4 (↑) + CH_3-S-S-HTP. It has been reported that the inactive CH_3-S-CoM reductase which is extracted from *Methanobacterium thermoautotrophicus* delta H, catalyzing CH_3-S-CoM to form CH_4 at the final step of methanogenesis, could be activated only by exposure to light. Its photoactivation in methanogens by illumination could be the reason for enhanced methane production (Yang et al., 2009). As a consequence, illumination strategy proved to be useful for improving activity of methanogens leading to the

Table 2
Reactor parameters of batch experiments under different conditions.

	Initial NH_4^+-N concentration (mg/L)	CH_4 (%)	COD removal (%)	Methane yield (mL/g-DOC)
Control	234	71.26	72.4	492
Control (Illumination)	234	93.19	83.6	588
N2211	2211	62.01	44.7	227
N2211 (Illumination)	2211	92.12	64.0	287

Table 3
The methane yield, C/N ratio under different hydraulic retention time (HRT) and organic loading rate (OLR) in the semi-continuous anaerobic digestion of ammonium-rich substrate.

Operation periods (d)	HRT (d)	OLR (kg-VS/m^3/d)	Methane yield (mL/g-DOC$_{removal}$)		C/N	
			Dark	Illumination	Dark	Illumination
0–49	40	0.125	242	282	0.18	0.53
50–77	40	0.188	108	210	0.60	0.75
78–97	20	0.375	271	496	0.83	0.93
98–107	10	0.750	213	426	0.80	0.90
108–118	5	1.500	154	356	0.78	0.88

Average of stable state for each phase is shown in the table.

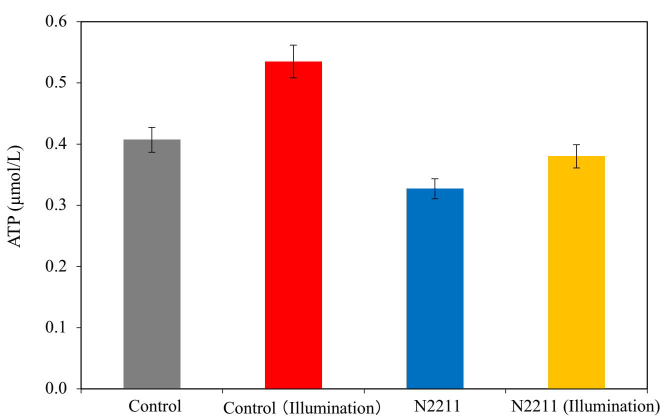

Fig. 1. ATP value in the bioreactors under different conditions during thermophilic anaerobic digestion.

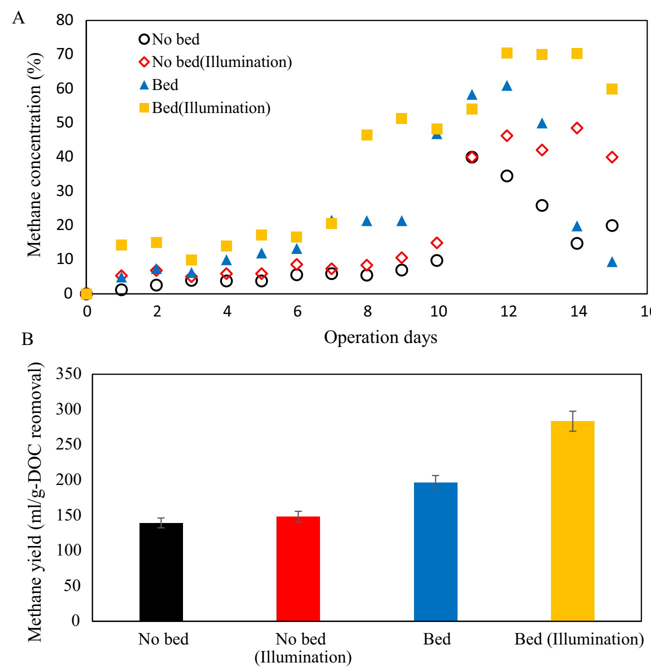

Fig. 2. Methane concentration (A) and Methane yield (B) in the bioreactor with ammonium concentration of 3000 mg/L under thermophilic condition (55 °C).

enhancement on methane production even under ammonium-rich condition.

3.3. Performance with illuminated fixed-bed process

To further improve the efficiency of the illuminated anaerobic digestion, the bio-zeolite fixed-bed anaerobic digestion reactor was carried out into the process. The methane concentration which are shown in Fig. 2A designated that the illuminated bio-zeolite fixed-bed bioreactor achieved the best performance with methane concentration of 70% at the end of the experiment and reached to a stable stage after day 12, while bioreactor under dark condition achieved the peak around day 10 and then began to decrease. The illuminated fixed-bed bioreactor provides more stable and better performance on aerobic digestion with high ammonium nitrogen concentration. According to Fig. 2A, the addition of fixed-bed shortened the start-up period effectively. Resch et al. and Zheng et al. reported that the addition of fixed-bed lead to the reduction of NH_4^+-N concentration and could effectively reduce the start-up time and enhance the methane production (Resch et al., 2009; Zheng et al., 2015). It could be indicated that the illumination strategy with fixed-bed bioreactor could further provide a high efficiency of the anaerobic digestion process.

Furthermore, Fig. 2B shows the methane yields in the bioreactors. The fixed zeolite bioreactor which was conducted under the intermittent illumination condition attained the highest methane yield, of about 283 mL/g-DOC$_{removal}$, which is more than 2 folds of the dark condition bioreactor without fixed-bed (139 mL/g-DOC$_{removal}$). Corresponding to the accumulated methane production showed the same order as Bed (Illumination) > Bed > No bed (Illumination) > No bed. This result also supports that the strategy of combining the illumination with bio-zeolite fixed-bed reactor gave a better performance during the ammonium-rich anaerobic digestion experiment.

Fig. S1 shows the SEM photographs of the bedding material (PN) and zeolite with microbes under both intermittent and dark conditions. From the SEM photographs, it can be easily observed that the quantity of microbes attached on both the bedding material (Fig. S1.B) and zeolite (Fig. S1.D) under intermittent illumination condition is much more than that under dark condition (Fig. S1. A, C). In addition, the SEM images revealed that under intermittent illumination condition, the biofilm (as the arrows indicated in Fig. S1) was formed after two weeks of incubation, which usually require 3 weeks or even longer to take shape (Uemura and Harada, 1993). This is probably due to the intermittent illumination that excited the methanogens, and the intermittent illumination not only activated the microorganisms, but improved the multiplication ability of the microorganisms as well. The microorganisms under the intermittent illumination condition is more active and have high ability to immobilize on the fixed-bed compare to dark condition. Therefore, the bioreactor combining both bio-zeolite fixed-bed and the intermittent illumination condition showed high tolerant to the high-concentration ammonium environment. The illuminated bio-zeolite fixed-bed bioreactor could partially remove ammonia, which is regarded as a main inhibitor to microorganisms, improved the methane production and could provide a favorable environment for the adherence of microorganisms (Zheng et al., 2015; Yang et al., 2004).

3.4. Semi-continuous anaerobic digestion of ammonium-rich substrate by using the intermittent illuminated process

During semi-continuous operation period, the bio-zeolite fixed-bed bioreactor was used to investigate the long-term practical effectiveness of intermittent illumination in anaerobic digestion process. The result of methane concentration and methane yield are shown in Fig. 3(A) and Table 3. As shown in Fig. 3(A), the fixed-bed bioreactor was beginning to produce methane at the second day and the methane concentration was continuously increase until the 8th day. In this period, the illuminated bioreactor showed apparently higher methane concentration and shorter start-up period than that of the dark bioreactor. After day 50, when the organic loading rate (OLR) was increased to 0.375 g-VS/m^3/d at the hydraulic retention time (HRT) of 20 days, the methane concentration of illuminated bioreactor increased and obviously higher than that of the dark bioreactor. Further increasing the OLR to 1.500 g-VS/m^3/d at the HRT of 5 days, the illuminated bioreactor showed a stable methane concentration and reached to around 70%, while that of the dark bioreactor remained below 40%. The illuminated bio-zeolite fixed-bed bioreactor obtained higher and more stable methane concentration with long-term operation, even at short HRT and high NH_4^+-N concentration condition.

The average methane yield in the bioreactors during different HRTs is shown in Table 3. The illuminated bioreactor showed higher methane yield during the whole semi-continuous operation. After the HRT reached to 20 days, the average methane yield of illuminated bioreactor achieved to the peak of 496 mL/g-$DOC_{removal}$, which was 1.8 times of the dark condition. With the shortening of the HRT, illuminated bioreactor still showed a higher average methane yield of 426 mL/g-$DOC_{removal}$ at the HRT of 10 days and 356 mL/g- $DOC_{removal}$ at the HRT of 5 days, which was 2.1 and 2.3 times of the dark bioreactor, respectively. This result indicated that the illumination strategy provided a more stable and sustainable system for the long-term operation during the anaerobic digestion with ammonium-rich substrate.

Fig. 3(B) shows the variation of NH_4^+-N concentration in the two bioreactors during the 118-day anaerobic digestion. At the start of the fermentation experiment, the NH_4^+-N concentration showed a decreasing tendency, and then decreased in both the illuminated and dark bioreactors after 10 days, while the methane concentration reached to the first peak around 70% as shown in Fig. 3(A). The result showed positive effect on the ammonia absorption at the start period of anaerobic digestion. The same trend was observed by Zheng et al. and Yang et al., which indicated that the bio-zeolite fixed-bed system effectively absorbed the ammonia at the beginning period of the fermentation process (Zheng et al., 2015; Yang et al., 2004). The NH_4^+-N concentration increased sharply after day 31, and reached to the peak of 3913 mg/L at day 54. After that with the addition of organic carbon, the NH_4^+-N concentration began to decrease. This result may probably result from the consumption of ammonium nitrogen by the multiplication of microorganisms. Ammonium nitrogen is regarded as an essential nutrient for bacterial growth, which can be consumed by the microorganisms during the anaerobic digestion process (Sung and Liul, 2003). As a consequence, the multiplied microorganisms consumed the ammonium nitrogen, leading to the decrease in ammonium concentration. After that, despite the same kind of bedding material and zeolite was provided in different bioreactors, the illuminated bioreactor showed an apparently lower NH_4^+-N concentration than that of the dark bioreactor. The lowest NH_4^+-N concentration observed was 2101 mg/L in the illuminated bioreactor when the HRT decreased to 20 days achieving the highest

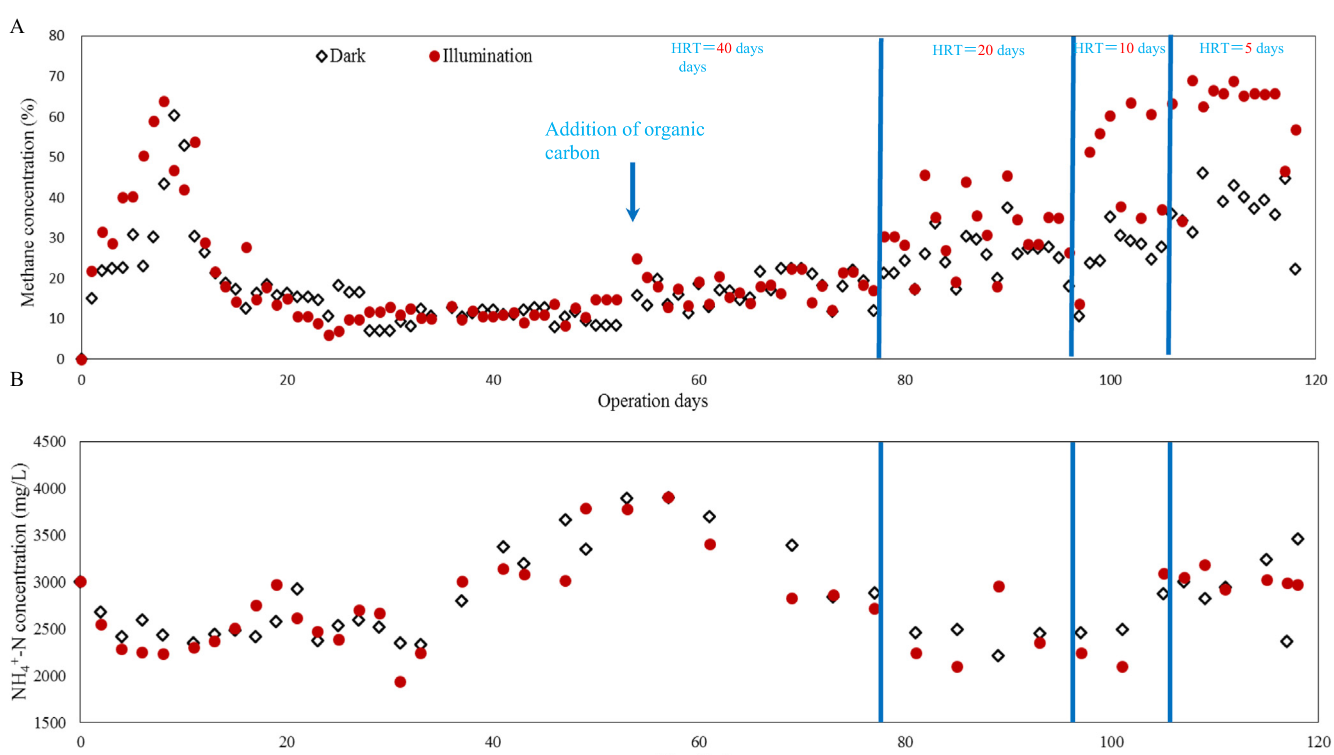

Fig. 3. (A) The variation of daily methane concentration and (B) the variation of ammonium nitrogen concentration during 118-day semi-continuous anaerobic digestion of ammonia-rich substrate.

methane yield of 496 mL/g-$DOC_{removal}$ (Table 3). This result indicated that the illumination strategy with the fix-bed system successfully provide a much easier condition for the microorganisms to tolerate the severe high ammonium condition during the semi-continuous anaerobic digestion with ammonia-rich substrate.

3.5. Immobilized microbes on the illuminated semi-continuous anaerobic digestion process

The result of biomass quantity immobilized on the fixed bed and ATP value shown as in Fig. 4 strongly supported consequence of NH_4^+-N concentration variation. The illuminated bioreactor showed 1.4 folds biomass quantity of the dark bioreactor indicating that the illuminated bioreactor immobilized more microorganisms corresponding to a better performance of anaerobic digestion (Fig. 4A). ATP concentration (Fig. 4B) of the illuminated bioreactor is 2.2 times higher than that of dark condition. This result revealed that the illuminated condition had a positive effect on microorganism multiplication leading to lower NH_4^+-N concentration and higher methane yield.

Fig. S2 shows the SEM photographs of the bedding material and zeolite with microbes under both intermittent light and dark conditions. From the SEM photographs, it can be easily observed that under the illuminated condition, more microorganisms immobilized on the both bedding material and zeolite (Fig. S2.B, D). This result is corresponding to the result of ATP concentration and biomass quantity that the illuminated bioreactor presented a better activity of microorganisms and higher tolerance to the ammonium-rich condition resulting in a better performance during the semi-continuous anaerobic digestion process (Fig. 4).

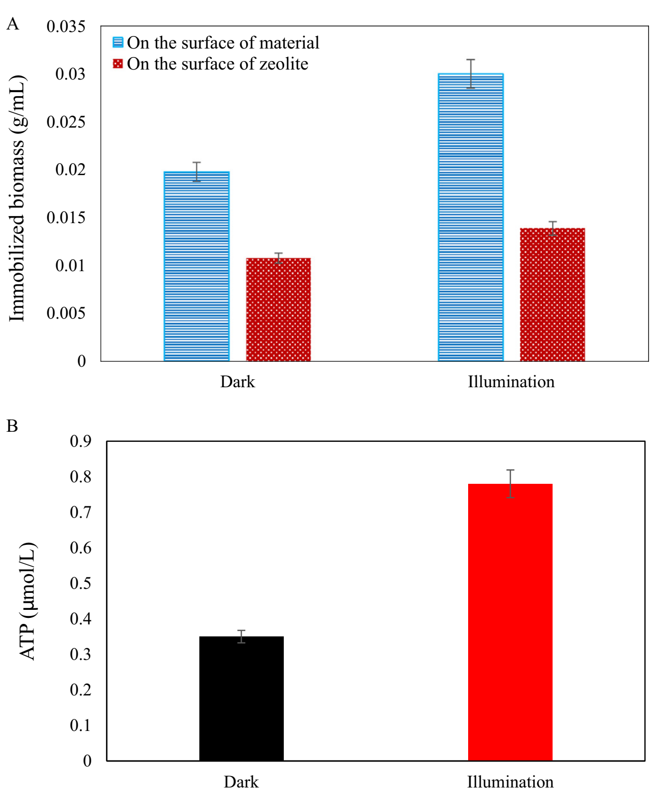

Fig. 4. (A) Biomass immobilized on the surface of zeolite and bed materials in different bioreactors and (B) ATP values of anaerobic microbes attached on the zeolite in bioreactors after 118-day semi-continuous anaerobic digestion of ammonia-rich substrate.

The variation of the C/N ratio during the 118-day semi-continuous experiment is shown in Table 3. Through the operation, the illuminated bioreactor showed higher C/N ratio than the dark condition. The illuminated bioreactor also gave a lower ammonium nitrogen concentration, which cause a higher ATP value and more microbes immobilized on the fixed-bed (Fig. 4). The highest C/N ratio was observed when the HRT was 20 days in the illuminated bioreactor, meanwhile the highest methane yield of 496 mL/g-$DOC_{removal}$ was also achieved in the same reactor. It has been commonly considered that for a better digestion, the C/N ratio should be about 25–30, as a result that the bacteria use the carbon 25–30 times higher than nitrogen (Krishna and Kalamdhad, 2014). The C/N ratio resulted in an imbalance between the rates of hydrolysis, acidogenesis and methanogenesis, and the solubilized organic matter was not converted into CH_4 completely (Aboudi et al., 2015). J. Zhang et al. reported that with decrease of HRT and increased organic loading may cause the incomplete hydrolysis of organic matters and decrease the acidogenesis and methanogenesis, as a consequence of decrease on methane yield (Zhang et al., 2015). However, in this study, the excess nitrogen leading to the ammonia inhibition resulted in the low C/N ratio below 1. The illuminated fixed-bed bioreactor remained a stable and well-performed stage even under severe C/N ratio condition, meanwhile the bio-zeolite fixed-bed served as a suitable carrier for microbes, which have a high tolerance to the toxic environments, produced positive impact on ammonia adsorption, drive to the balance of the methane production process (Yang et al., 2008).

Therefore, the intermittent illumination strategy contributes to the tolerance of anaerobes to high ammonium nitrogen concentration in the fixed-bed bioreactor, initiating to the effective enhancement on high methane concentration and shortening of start-up period. The illuminated bioreactor during 118-day semi-continuous anaerobic digestion clearly indicated that the intermittent illumination strategy is an appropriate and promising option for mitigating ammonia inhibition during the anaerobic digestion with ammonia-rich substrate.

4. Conclusions

In this study, intermittent illumination strategy was explored to alleviate ammonia inhibition during anaerobic digestion with ammonium-rich substrate. The illuminated bio-zeolite fixed-bed process has been proved to be promising for mitigating ammonia inhibition for the first time. The illumination contributes to better performance by improving the activity of microorganisms. Moreover, the illuminated bio-zeolite fixed-bed enhanced the ammonia adsorption and immobilized microbe quantity efficiently. The semi-continuous operation verified the practical application of the illumination strategy. This work could provide a unique viewpoint for mitigating ammonia inhibition by developing an eco-friendly, low cost, easy operation system using sunlight in the recent future.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.biortech.2016.09.053.

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