Free ammonia pretreatment improves anaerobic methane generation from Algae

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ABSTRACT: Anaerobic methane generation from algae is hindered by the slow and poor algae biodegradability. A novel free ammonia (NH_3 i.e. FA) pretreatment technology was proposed in this work to enhance anaerobic methane generation from algae cultivated using a real secondary effluent. The algae solubilisation was 0.05~0.06 g SCOD/g TCOD (SCOD: soluble chemical oxygen demand; TCOD: total chemical oxygen demand) following FA pretreatment of 240~530 mg NH_3-N/L for 24 h, whereas the solubilisation was only 0.01 g SCOD/g TCOD for the untreated algae. This indicates that FA pretreatment at 240~530 mg NH_3-N/L could substantially enhance algae solubilisation. Biochemical methane potential tests revealed that FA pretreatment on algae at 240~530 mg NH_3-N/L is able to significantly enhance anaerobic methane generation. The hydrolysis rate (k) and biochemical methane potential (P_0) of algae increased from 0.21 d^{-1} and 132 L CH_4/kg TCOD to 0.33~0.50 d^{-1} and 140~154 L CH_4/kg TCOD, respectively, after the algae was pretreated by FA at 240~530 mg NH_3-N/L. Further analysis indicated that FA pretreatment improved k of both quickly and slowly biodegradable substrates, and also increased P_0 of the slowly biodegradable substrate although it negatively affected P_0 of the quickly biodegradable substrate. This FA technology is a closed-loop technology.

Keywords: Algae; Free ammonia; Methane; Energy; Anaerobic digestion; Biodegradability

1 Introduction

One important goal of sewage treatment is to remove nutrients such as phosphorus and nitrogen, which otherwise will cause eutrophication. Consequently, several technologies such as denitrifying biofilters have been established for tertiary treatment of the secondary effluent to enhance nutrients removal (Boelee et al., 2014). However, these technologies are unsustainable because of the intensive resource and/or energy consumption. Alternatively, algae-based sewage treatment systems offer an elegant solution to tertiary treatment of the secondary effluent (Christenson and Sims, 2011; Menger-Krug et al., 2012; Hu et al., 2017; Judd et al., 2017). The algae can grow in sewage by assimilating nutrients and CO_2, thereby removing nutrients and mitigating global warming with a negligible energy consumption. At the same time, the value-added algal biomass will also be produced and can be converted to the valuable biofuel (Chen et al., 2015; Zhou et al., 2019). Therefore, algae-based sewage treatment systems offer the benefits of simultaneous pollutant removal and biofuel generation.

As one type of biofuel generation, anaerobic digestion of algae has now been used to generate methane (Passos et al., 2014; Montingelli et al., 2015; Rodriguez et al., 2015). However, the algae has a low biodegradability, which results in a low methane generation at 0.05~0.31 L CH_4/g VS (VS: volatile solids) (González-Fernández et al., 2011). Therefore, various pretreatment technologies such as mechanical, chemical, microwave, biological and thermal pretreatments have been investigated to improve anaerobic methane generation from algae (Rodriguez et al., 2015; Montingelli et al., 2015; Passos et al., 2014). For instance, Alzate et al. (2012) achieved a 41% increase in methane generation when the algae was pretreated at 170 °C and 6 bar for 15 min. Cho et al. (2013) also demonstrated that the methane generation from algae increased by 15% when the algae was pretreated ultrasonically at 234 MJ/kg VS.

However, these pretreatment technologies are cost intensive due to the requirement of high chemical and/or energy input.

The recent research has shown that free ammonia (FA, NH_3), a chemical that can be directly obtained from the anaerobic digester effluent, is able to improve the biodegradability of both waste activated sludge and primary sludge (Wei et al., 2017a, b; Wang, 2017; Xu et al., 2018; Yang et al., 2018). For instance, it was demonstrated that the anaerobic methane generation from waste activated sludge was improved by approximately 30% after FA pretreatment for 24 h at 420 mg NH_3-N/L (Wei et al., 2017a). Wei et al. (2017b) also demonstrated a 16% increase in anaerobic methane generation from primary sludge under the same FA pretreatment conditions. Yang et al. (2018) showed that sludge production (mass basis) was reduced by 20% after the sludge was first pretreated by FA and then returned to the mainstream reactor for biodegradation. More recently, Wang et al. (2018) reported that 36% of the FA treated waste activated sludge (at 300 mg NH_3−N/L, 24 h) was biodegraded over the 15-day aerobic digestion in comparison to 23% obtained with the original waste activated sludge. It was also demonstrated that the waste activated sludge production (mass basis) in the mainstream reactor decreased after implementing FA pretreatment in the sludge recycling line (Wang et al., 2017).

The above discoveries enabled us to assume that FA pretreatment on algae might be capable of enhancing anaerobic methane generation. To confirm this assumption, this study for the first time assessed the viability of enhancing methane generation from the FA pretreated algae. The sewage born mixed algal culture was first cultivated using the secondary effluent of a local sewage treatment plant. The cultivated algae was then treated using FA at 60~530 mg NH_3-N/L for 24 h, with ammonium pretreatment alone (900 mg NH_4^+-N/L) and with alkaline pretreatment alone (pH=9.5) as references. The algae solubilisation was then assessed. The biochemical methane potential tests were conducted to investigate methane generation from algae with and without FA pretreatment. The model assessment was also adopted to shed light on the reasons behind the enhanced methane generation through predicting both the hydrolysis rate and biochemical methane potential.

2. Materials and methods

2.1. Algae and inoculum sludge

2.1.1. Algae origin and growing media

The algae used for the following biochemical methane potential tests was cultivated using the secondary effluent of a local sewage treatment plant (STP). The sewage born mixed algal culture was employed as the algae inoculum, which was collected from the secondary settler of a biological nutrient removal STP. The secondary effluent of the same plant was used as the growing media. The main characteristics of secondary effluent were: 1.9 mg NH_4^+-N/L, 0.3 mg NO_2^--N/L, 2.5 mg NO_3^--N/L, 2.7 mg PO_4^{3-}-P/L, 59 mg DIC/L (DIC: dissolved inorganic carbon) and pH 7.6.

2.1.2. Algae cultivation

The sewage born mixed algal culture was cultivated in the glass beakers, which was fed with secondary effluent and was mixed at 100 rpm. The beakers were illuminated from four sides by the cool white fluorescent lamps (20 W each) operated at a 14 h : 10 h light : dark cycle. When the light was on, the lamps provided an average illuminance of 6000 Lux (ca. 80 mmol/m^2/s). The algae was grown to a total solids (TS) concentration of approximately 1.5 g/L and was then concentrated for the biochemical methane potential tests to be described in Section 2.3. More than 97% of the nutrients (nitrogen and phosphorus) in the growing media were removed. Microscopic observation revealed that the cultivated algae culture was dominated by green algae. The main characteristics of the concentrated algae were shown in Table 1.

(Position for Table 1)

2.1.3. Inoculum sludge

The inoculum sludge was harvested from a full-scale mesophilic anaerobic digester treating mixed waste activated sludge and primary sludge. The hydraulic retention time (HRT) of the anaerobic digester was about 15 d. The inoculum sludge was adopted to degrade the algae as described in the following biochemical methane potential tests. Its main characteristics were summarized in Table 1.

2.2. FA pretreatment on algae

The effects of FA, ammonium and alkaline pretreatment on the algae solubilisation were assessed using a series of batch tests, as indicated by the SCOD release. 2.8 L of algae was transferred to seven Erlenmeyer flasks (0.4 L each), which were used as the batch reactors. With regards to FA pretreatment, pH was controlled at 9.5 ± 0.1 using a NaOH solution. Also, different amounts of ammonium solution (NH_4Cl solution) were added to four batch reactors to attain the NH_3-N+NH_4^+-N levels of 100, 400, 700 and 900 mg N/L, respectively, as described in Table 2. These NH_3-N+NH_4^+-N levels were selected because they are directly attainable from the anaerobic digester effluent of the STPs in the engineering application (information from the industry partners). The pH along with the NH_3-N+NH_4^+-N collectively led to FA levels between 60 and 530 mg NH_3-N/L (Table 2). The FA concentrations were calculated using the formula $10^{pH}\times C_{(NH_3\text{-}N+NH_4^+\text{-}N)}/(10^{pH}+K_a\times K_w^{-1})$, where $C_{(NH_3\text{-}N+NH_4^+\text{-}N)}$ represents the NH_3-N+NH_4^+-N concentration, K_a represents the ionization constant for ammonia equilibrium equation and K_w represents the water ionization constant (Wei et al., 2017a). The value of $K_a \times K_w^{-1}$ was calculated through the formula $K_a \times K_w^{-1} = e^{6,344/(T+273)}$ (T is temperature, which was 22 °C in this study).

(Position for Table 2)

In order to determine whether ammonium or alkaline pretreatment alone would increase methane generation, the ammonium pretreatment and alkaline pretreatment were also performed separately. Ammonium pretreatment was carried out at 900 mg NH_4^+-N/L (Table 2). This concentration was selected because this was the largest ammonium concentration employed for the FA pretreatment. No pH control was conducted and a pH value of approximately 7.6 was recorded, causing a low FA level of approximately 16 mg NH_3-N/L. Alkaline pretreatment was carried out at pH 9.5 without addition of external ammonium (Table 2). . Another batch reactor (i.e. control) without ammonium addition or pH control was also operated (Table 2).

All the batch reactors were mixed by the magnetic stirrers at 300 rpm and were located in a temperature controlled room at around 22 °C. All the tests sustained for 24 h.

In all the batch tests, the concentrations of SCOD were measured three times before and after pretreatment. The algae solubilisation was then represented as the released SCOD over the TCOD of algae.

2.3. Anaerobic biochemical methane potential batch tests of algae

Methane generation from algae with FA, ammonium and alkaline pretreatment was assessed using anaerobic biochemical methane potential (BMP) batch tests. The BMP tests were conducted using a series of 160 ml serum vials with a 100 ml working volume. 60 mL inoculum sludge and 40 mL algae were added to each BMP serum vial, resulting in an inoculum sludge to algae percentage of 2.0±0.1 on the VS basis. Before the algae with FA and alkaline pretreatment were added to the BMP serum vial, their pH was adjusted to 7.6 using HCl, which was the pH in the control reactor. The serum vials were then flushed with nitrogen gas for 1 min to secure an anaerobic condition. Then, the serum vials were sealed by a rubber stopper with an aluminium crimp cap and were transferred to an incubator with a temperature of 37±1 °C. Blank containing 60 mL inoculum sludge and 40 mL MilliQ water (i.e. without algae) was also operated. All the tests were performed three times. The BMP tests lasted for 50 days till a negligible amount of biogas generation was observed. The pH was not controlled during the tests. The serum vials were manually shaken for around 30 s before each biogas sampling event (5 mL biogas was taken).

The biogas composition (i.e. CH_4, H_2, CO_2) and generation were measured every day over the first 5 d and then in a 2~5 days' interval. The methane volume was calculated via multiplying the biogas volume by the methane percentage in biogas, and expressed as the value under standard temperature and pressure (25 °C, 1 atm). The methane generation from algae was calculated through subtracting methane generation in the blank serum vial without algae from that in the serum vial with algae. The methane generation was expressed as the methane volume over the TCOD mass of algae (L CH_4/kg TCOD algae).

2.4. Determining biochemical methane potential and hydrolysis rate of algae

The methane generation kinetics and potential of the algae were reflected through hydrolysis rate (k) and biochemical methane potential (P_0). They were estimated through fitting methane generation data of the BMP tests to a kinetic model (see Equations 1 and 2) using a modified version of software Aquasim 2.1d. Both single substrate kinetic model and two substrate kinetic model were used in this study. The single substrate kinetic model assumes that the algae only comprises a single substrate type, as shown in equation (1) (Wang et al., 2013, 2014):

$$P(t)=P_0 \times (1-e^{-kt}) \qquad (1)$$

where P(t) represents methane generation at time t (L CH_4/kg TCOD algae); P_0 represents biochemical methane potential of algae (L CH_4/kg TCOD algae); t represents time (d).

The two substrate kinetic model considers the algae samples to comprise a slowly biodegradable substrate and a quickly biodegradable substrate, as shown in equation (2) (Wang et al., 2013, 2014).

$$P(t) = P_{0,quick} \times \left(1-e^{-k_{quick}t}\right)+P_{0,slow} \times \left(1-e^{-k_{slow}t}\right) \qquad (2)$$

where $P_{0,quick}$ represents biochemical methane potential of the quickly biodegradable substrate (L CH_4/kg TCOD algae); $P_{0,slow}$ represents biochemical methane potential of the slowly biodegradable substrate (L CH_4/kg TCOD algae); k_{quick} represents hydrolysis rate of the quickly biodegradable substrate (d^{-1}); k_{slow} represents hydrolysis rate of the slowly biodegradable substrate (d^{-1}).

The objective function was expressed as the sum of squared residuals (SSR) between the model estimated data and the measured data (Batstone et al., 2003). The P_0 and k would be determined when the minimized SSR (S_{min}) is achieved. The uncertainty surfaces of P_0 and k were attained by an objective surface searching approach as described in Batstone et al. (2003). The parameter surface is explained by $S_{crit} > S_{min}$ via the F distribution and through assuming the normally distributed residuals.

$$S_{crit}= S_{min}\times(1+ N_p/(N_{data}-N_p)\times F_{\alpha,p, Ndata-Np}) \quad (3)$$

where N_p represents the parameter number (2 in this work, i.e. P_0 and k); N_{data} represents the number of measured data points (17 in this work); $F_{\alpha,p, Ndata-Np}$ represents the F distribution value, which is 3.68 in this work using a 95% confidence limit (i.e. α=0.95).

The algae degradation extent (Y) was calculated using P_0 according to equation (4):

$$Y=P_0/380 \quad (4)$$

where 380 represents theoretical biochemical methane potential of algae at standard conditions (1 atm, 25 °C) (L CH_4/kg TCOD algae).

2.5. Analytical methods and statistical analysis

The disposable millipore filter units (pore size: 0.22 µm) were employed to filter the algae samples for analysing the concentrations of PO_4^{3-}-P, NH_4^+-N, NO_2^--N, NO_3^--N, DIC and SCOD. The PO_4^{3-}-P, NH_4^+-N, NO_2^--N, NO_3^--N, TS, VS, TCOD and SCOD concentrations were determined according to the standard method (APHA, 2005). DIC was determined by the standard method using a total carbon analyser (APHA, 2005). The protein was measured by the Lowry-Folin method with BSA as the standard and the carbohydrate was measured by the phenol-sulfuric method with glucose as the standard (Lowry et al., 1951; Herbert et al., 1971).

A manometer was employed to determine the pressure and biogas volume at the start of each sampling campaign. The produced biogas volume was calculated according to the increased pressure in the headspace of the BMP serum vials and expressed at standard conditions (25 °C, 1 atm). Biogas composition (i.e. CO_2, CH_4 and N_2) was determined using a gas chromatograph

(SHIMADZU GC-2014) equipped with a flame ionization detector (FID) and a thermal conductivity detector (TCD).

The significance of the results were assessed by a variance analysis using the software of SPSS. The $p>0.05$ and $p<0.05$ were regarded as statistically insignificant and statistically significant, respectively.

3. Results

3.1. Effect of FA pretreatment on algae solubilisation

Figure 1 showed the algae solubilisation after FA (60~530 mg NH_3-N/L), ammonium (900 mg NH_4^+-N/L) and alkaline (pH=9.5) pretreatment for 24 h. On the whole, FA pretreatment enhanced algae solubilisation, as indicated by the much higher ($p<0.05$) SCOD release than that in the control reactor. For instance, SCOD release was only approximately 0.01 g SCOD/g TCOD from the algae without any pretreatment. In contrast, SCOD increased by approximately 0.05~0.06 g SCOD/g TCOD from the FA-treated algae at the FA concentrations of 240~530 mg NH_3-N/L, causing an up to 6 times higher algae solubilisation in comparison to the control. The highest algae solubilisation was observed when the FA levels were at 420 and 530 mg NH_3-N/L, where SCOD increased by 0.06 g SCOD/g TCOD. Alkaline pretreatment at pH 9.5 and FA pretreatment at 60 mg NH_3-N/L had a similar effect on algae solubilisation. They also led to a higher algae solubilisation (i.e. 0.04 g SCOD/g TCOD) in comparison to the control but was not as effective as the FA pretreatment at 240~530 mg NH_3-N/L (i.e. 0.05~0.06 g SCOD/g TCOD). In contrast, ammonium pretreatment at 900 mg NH_4^+-N/L did not significantly contribute to ($p>0.05$) the SCOD release (~0.01 g SCOD/g TCOD) compared with the control. The higher algae solubilisation indicates that the algal cell wall was destroyed with the intracellular materials released.

(Position for Figure 1)

3.2. Effect of FA pretreatment on methane generation from algae

The methane generation over the entire 50 days' BMP tests was shown in Figure 2. Generally, FA pretreatment for 24 h at 240~530 mg NH_3-N/L achieved more ($p<0.05$) anaerobic methane generation compared to the control over the entire period. When the FA concentration was 530 mg NH_3-N/L, the highest methane generation from algae was obtained. The algae with alkaline pretreatment at pH 9.5 had a comparable ($p>0.05$) methane generation to that with FA pretreatment at 60 mg NH_3-N/L, and only produced a slightly larger ($p<0.05$) amount of methane than control. This is consistent with the results of Cho et al. (2013), who also reported that alkaline pretreatment (pH=9.0) would slightly increase methane production. Additionally, the methane generation from the algae with ammonium pretreatment at 900 mg NH_4^+-N/L was comparable ($p>0.05$) with that from the control, revealing ammonium pretreatment did not contribute to the increased methane generation. These collectively suggested that FA pretreatment at 240~530 mg NH_3-N/L is capable of improving methane generation from algae and it is primarily FA itself instead of ammonium or pH alone that plays a role in the enhanced methane generation.

(Position for Figure 2)

3.3. Hydrolysis rate and biochemical methane potential estimation

Both single substrate and two substrate models were employed to estimate hydrolysis rate (k) and biochemical methane potential (P_0).

Single substrate model

The simulated methane generation profiles using the single substrate model were shown in Figure 3A, which reveals that the methane generation data were satisfactorily captured by the model. The estimated P_0 and k of algae with FA, ammonium and alkaline pretreatment are summarized in Table 3. In general, FA pretreatment at 240~530 mg NH_3-N/L increased ($p<0.05$) the algae hydrolysis rate (k) by 55~140% (from 0.21 d^{-1} to 0.33~0.50 d^{-1}) compared to the control, with the highest hydrolysis rate achieved at 530 mg NH_3-N/L. Similarly, the methane potential from algae (i.e. P_0) was also enhanced ($p<0.05$) while the FA levels were between 240 and 530 mg NH_3-N/L, where P_0 increased by 6~17% (from 132 L CH_4/kg TCOD to 140~154 L CH_4/kg TCOD) compared with the algae without pretreatment. The algae degradation extent (Y) also increased ($p<0.05$) from 0.35 to 0.37~0.41 d^{-1} accordingly after the algae was pretreated by 240~530 mg NH_3-N/L (see Table 3). The confidence regions of k and P_0 were also plotted in Figure 4. Compared to the algae without pretreatment, the general moving trend of the confidence regions at FA concentrations of 240~530 mg NH_3-N/L was towards the right and upward, indicating both k and P_0 of algae were improved by FA pretreatment.

(Position for Figure 3)

(Position for Table 3)

(Position for Figure 4)

Ammonium pretreatment at 900 mg NH_4^+-N/L did not significantly affect ($p>0.05$) k and P_0 compared with the control. Also, alkaline pretreatment at pH 9.5 increased k by 14% (from 0.21 d^{-1} to 0.24 d^{-1}) and slightly increased P_0 by 3% (from 132 L CH_4/kg TCOD to 136 L CH_4/kg TCOD). The increases in P_0 and k by pH 9.5 pretreatment were similar to those by FA pretreatment at 60 NH_3-N/L, but were significantly lower compared with those by FA pretreatment at 240~530 mg NH_3-N/L. These collectively revealed that it is FA (at 240~530 mg NH_3-N/L) that plays a dominant role in the increased k and P_0.

Two substrate model

The simulated methane generation profiles using the two substrate model were shown in Figure 3B, which revealed that the model well captured the methane generation data. This result implied that the algae was composed of both quickly biodegradable substrate and slowly biodegradable substrate. The estimated values of k_{quick}, $P_{0,quick}$, and k_{slow}, $P_{0,slow}$ were shown in Table 4. In general, k_{quick} substantially increased ($p<0.05$) after FA pretreatment at 240~530 mg NH_3-N/L. For example, k_{quick} increased from 0.23 d^{-1} to 0.74~1.54 d^{-1} after implementing FA pretreatment with the highest k_{quick} achieved at the highest tested FA concentration (i.e. 530 mg NH_3-N/L). In contrast, $P_{0,quick}$ decreased ($p<0.05$) following FA pretreatment at 240~530 mg NH_3-N/L. For instance, $P_{0,quick}$ decreased from 121 L CH_4/kg TCOD to 74~88 L CH_4/kg TCOD after implementing FA pretreatment 240~530 mg NH_3-N/L. In terms of slowly biodegradable substrate, FA pretreatment at 240~530 mg NH_3-N/L increased both k_{slow} and $P_{0,slow}$ from 0.05 d^{-1} and 15 L CH_4/kg TCOD to 0.12~0.14 d^{-1} and 69~82 L CH_4/kg TCOD, respectively. These results indicated that FA pretreatment at 240~530 mg NH_3-N/L negatively affected the biochemical methane potential of the quickly biodegradable substrate. But it improved the hydrolysis rate of both quickly biodegradable substrate and slowly biodegradable substrate, and also increased the biochemical methane potential of the slowly biodegradable substrate.

(Position for Table 4)

In contrast, ammonium pretreatment at 900 mg NH_4^+-N/L did not play a role ($p>0.05$) in k_{quick}, $P_{0,quick}$, and k_{slow}, $P_{0,slow}$. Alkaline pretreatment at pH 9.5 and FA pretreatment at 60 mg NH_3-N/L had a similar effect on k_{quick}, $P_{0,quick}$, and k_{slow}, $P_{0,slow}$. They increased k_{quick}, k_{slow} and $P_{0,slow}$ from 0.23 d^{-1}, 0.05 d^{-1} and 15 L CH_4/kg TCOD to approximately 0.40 d^{-1}, 0.11 d^{-1} and 60 L CH_4/kg TCOD, respectively. However, $P_{0,quick}$ was reduced from 121 L CH_4/kg TCOD to about 80 L CH_4/kg TCOD after pH 9.5 pretreatment and 60 mg NH_3-N/L pretreatment. This revealed that alkaline pretreatment at pH 9.5 played a similar role in the quickly and slowly biodegradable substrates to the FA pretreatment at 60 mg NH_3-N/L, but to a much less extent compared with the FA pretreatment at 240~530 mg NH_3-N/L.

4. Discussion

Our world requires new sustainable feedstocks to reduce our reliance on fossil fuels to ensure a sustainable economy. Algae is a promising alternative feedstock for sustainable biofuels production. Anaerobic digestion of algae to generate methane is a common way of producing biofuels. However, the methane generation from anaerobic digestion of algae is limited and pretreatment of algae before anaerobic digestion is required. This study for the first time demonstrated that FA pretreatment at 240~530 mg NH_3-N/L on algae is effective in enhancing methane generation in the anaerobic digester. Both the increased biochemical methane potential and hydrolysis rate of the algae contributed to the increased methane generation. The increased biochemical methane potential reveals that some non-biodegradable substrate in algae was transformed into the biodegradable ones. The higher algae solubilization after FA pretreatment might be the reason for the enhanced methane generation. Two substrate model further indicated that the improved methane generation was due to the improved hydrolysis rate of both quickly biodegradable substrate and slowly biodegradable substrate, and also due to the increased biochemical methane potential of the slowly biodegradable substrate.

Figure 5 demonstrates a closed-loop concept in an STP based on the proposed FA pretreatment technology to enhance methane generation from algae. The algae harvested from the algae cultivation pond that fed with secondary effluent is added to an FA pretreatment unit. In the FA pretreatment unit, the algae is treated by the FA containing anaerobic digester effluent to improve the algae biodegradability. It should be noted that the FA required in this technology is a by-product of sewage treatment and is attainable from the anaerobic digester effluent, which contains an FA level of 30~560 mg NH_3-N/L (information from industry partners). If the desirable FA concentration is not enough in the FA pretreatment unit, a small amount of alkali can be dosed to increase the pH and thus the FA level. After that, the FA treated algae is transferred to the anaerobic digester to achieve enhanced methane generation. It should be noted that the addition of the FA treated algae to the anaerobic digester will increase the ammonium concentration in the anaerobic digester to some extent. However, this would not negatively affect the performance of the anaerobic digester, as demonstrated in this study. The methane is then converted to power and heat in the combined heat and power (CHP) unit. The by-product CO_2 is added to the algae cultivation pond to facilitate the algae growth. This closed-loop FA technology achieves enhanced methane generation and further purified sewage simultaneously utilizing a by-product of sewage treatment with a negligible chemical or energy input. This will transform the STPs from a 'linear economy' operating mode into a 'circular economy' operating mode.

(Position for Figure 5)

While generating more energy with FA pretreatment, this FA technology could also create a further environmental payoff. The CO_2 emission is estimated to reduce by 410 kg CO_2/tonne

TCOD algae based on the associated CO_2 emission from energy generation (i.e. 0.29 kg CO_2/MJ) (personal communication with industry partners). Therefore, FA pretreatment on algae also delivers an environmental benefit.

In this study, the cumulative methane generation from the un-pretreated algae at the end of the methane potential test was about 160 L CH_4/kg TCOD algae, which is consistent with the commonly reported results (40~280 L CH_4/kg TCOD algae) (Alzate et al., 2012; González-Fernández et al., 2011). Due to the relatively low methane production from the un-pretreated algae, algae pretreatment to enhance methane generation has been a hot topic and plenty of pretreatment technologies have been proposed (Rodriguez et al., 2015; Montingelli et al., 2015; Passos et al., 2014), including mechanical, chemical, microwave, biological and thermal pretreatments. For example, methane generation was increased by 58% after the algae was pre-treated by microwave at 110 MJ/kg VS (Passos et al., 2014). Unfortunately, these technologies incur large chemical and/or energy consumptions and thus are cost intensive. In contrast, the FA technology is economically favourable because it is relying on a by-product of sewage treatment (i.e. anaerobic digester effluent) and therefore requires negligible chemical/energy input. However, it might be difficult to compare the efficiencies of different pretreatment technologies only based on the published literatures. This is due to the fact that the efficiencies will also rely on the algae characteristics and the comparisons can only be valid when different pretreatment technologies are applied to the same algae.

It should be pointed out that this is only a proof-of-concept work to validate the feasibility of enhancing methane generation from algae using FA pretreatment. Therefore, this FA technology was not optimized in this study. Technology optimization definitely needs to be conducted in the future to determine the optimal FA level together with the pH and ammonium levels. In addition, the detailed mechanism study was not conducted in this study. This is because the scope of the mechanism study will be too large and therefore will need a separate comprehensive study that cannot be accommodated in this initial proof-of-concept study. The detailed mechanism study will be carried out in the future. Also, the methane production from co-digestion of the FA pre-treated algae and sewage sludge should also be conducted in the future.

5. Conclusions

This study assessed the viability of enhancing methane generation from the FA pre-treated algae cultivated using the secondary effluent through anaerobic biochemical methane potential tests. FA pretreatment on algae at 240~530 mg NH_3-N/L is able to enhance algae solubilisation and improve anaerobic methane generation from algae. The enhanced methane generation is attributed to the increased algal biochemical methane potential and hydrolysis rate caused by FA pretreatment. Further analysis indicated that FA pretreatment improved the hydrolysis rate of both quickly and slowly biodegradable substrates, and also increased biochemical methane potential of the slowly biodegradable substrate but it negatively affected biochemical methane potential of the quickly biodegradable substrate. This FA pretreatment technology is a closed-loop technology. This technology can also significantly decrease CO_2 emission. Therefore, this FA technology would potentially reduce society's fossil resource dependency and carbon footprint simultaneously.

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List of Figures and tables

Table 1. Main characteristics of algae and inoculum sludge (with standard errors).

Parameter	Algae	Inoculum sludge
Total solids (TS) (g/L)	20.6 ± 0.2	26.8 ± 0.2
Volatile solids (VS) (g/L)	15.4 ± 0.2	20.9 ± 0.2
Total chemical oxygen demand (TCOD) (g/L)	21.9 ± 0.2	27.1 ± 0.3
Soluble chemical oxygen demand (SCOD) (g/L)	1.0 ± 0.1	0.8 ± 0.1
Protein (% VS)	42 ± 5	Not Determined
Carbohydrate (% VS)	14 ± 6	Not Determined
pH	7.6 ± 0.1	7.9 ± 0.1

Table 2. Pretreatment conditions adopted in this study[a].

Pretreatment	NH_4^++NH_3 (mg N/L)	pH	FA (mg NH_3-N/L)
Control	2[b,c]	7.6[c]	0.04
FA	100	9.5	60
	400	9.5	240
	700	9.5	420
	900	9.5	530
Alkaline	2[c]	9.5	1
Ammonium	900[b]	7.6[c]	16

[a]Temperature was 22 °C in all tests

[b]At pH 7.6, more than 98% of the (NH_4^++NH_3)-N will exist in the form of NH_4^+-N. Therefore, the ammonium concentrations in the cases of control and ammonium pretreatment were about 2 and 900 mg NH_4^+-N/L.

[c]2 mg (NH_4^++NH_3)-N/L and pH 7.6 were the (NH_4^++NH_3)-N concentration and pH value in the raw algae.

Table 3. Estimated hydrolysis rate (k), biochemical methane potential (P_0) and degradation extent (Y) of algae at different FA concentrations using a single substrate model (with standard errors).

Pretreatment	k (d^{-1})	P_0 (L CH_4/kg TCOD algae)	Y
Control	0.21 ± 0.01	132 ± 1	0.35 ± 0.01
FA 60	0.25 ± 0.01	135 ± 1	0.36 ± 0.01
FA 240	0.33 ± 0.02	140 ± 2	0.37 ± 0.01
FA 420	0.40 ± 0.04	149 ± 3	0.39 ± 0.01
FA 530	0.50 ± 0.05	154 ± 3	0.41 ± 0.01
pH 9.5	0.24 ± 0.01	136 ± 1	0.36 ± 0.01
NH_4^+-N 900	0.21 ± 0.01	135 ± 1	0.36 ± 0.01

Table 4. Determined k_{quick}, $P_{0,quick}$, Y_{quick} and k_{slow}, $P_{0,slow}$, Y_{slow}, $P_{0,total}$ at different FA concentrations using a two-substrate model (with standard errors).

Pre-treatment / Parameters	Control	pH 9.5	NH_4^+-N 900	FA 60	FA 240	FA 420	FA 530
k_{quick} (d^{-1})	0.23±0.04	0.39±0.06	0.23±0.03	0.41±0.07	0.74±0.03	1.40±0.14	1.54±0.06
$P_{0,quick}$ (L CH_4/kg TCOD algae)	121 ± 26	81 ± 16	124 ± 20	78 ± 19	79 ± 3	74 ± 4	88 ± 2
Y_{quick}	0.32±0.07	0.21±0.04	0.33±0.05	0.21±0.05	0.21±0.01	0.19±0.01	0.23±0.01
k_{slow} (d^{-1})	0.05±0.13	0.11±0.02	0.03±0.10	0.12±0.03	0.12±0.01	0.14±0.01	0.14±0.01
$P_{0,slow}$ (L CH_4/kg TCOD algae)	15 ± 18	59 ± 15	17 ± 10	61 ± 18	69 ± 2	82 ± 4	74 ± 2
Y_{slow}	0.04±0.05	0.16±0.04	0.04±0.03	0.16±0.05	0.18±0.01	0.22±0.01	0.19±0.01
$P_{0,total}$ (L CH_4/kg TCOD algae)	136 ± 32	140 ± 22	141 ± 22	139 ± 26	148 ± 4	156 ± 6	162 ± 3

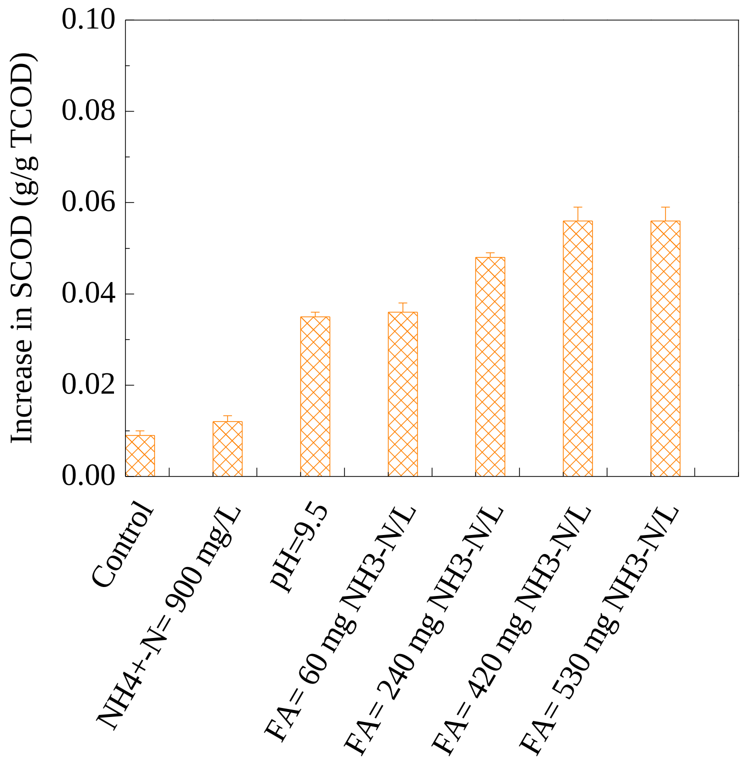


Figure 1. Increase in SCOD after 24 h algae pretreatment using FA (60~530 mg NH_3-N/L), alkaline (pH=9.5) and ammonium (900 mg NH_4^+-N/L). Error bars represent standard errors.

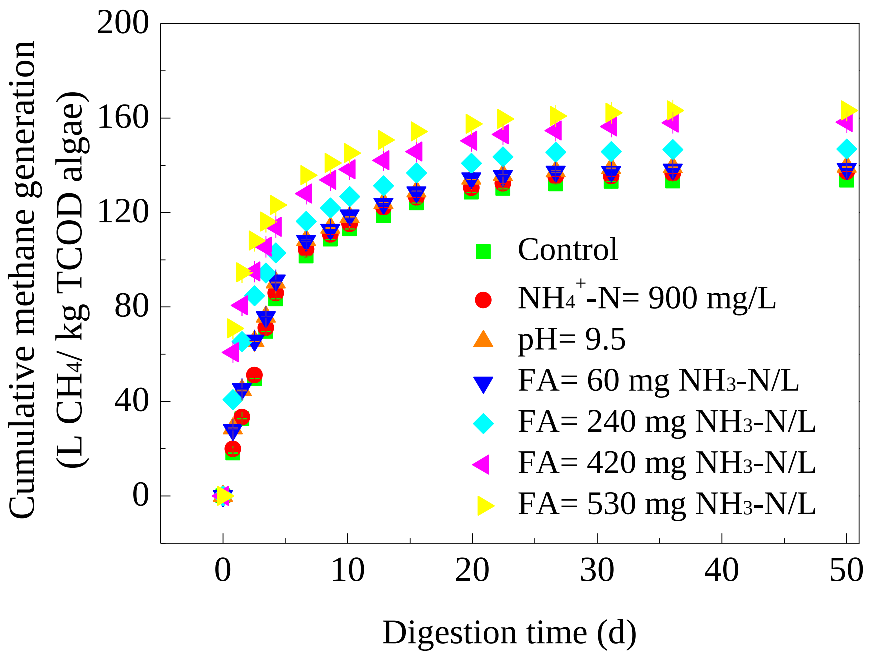
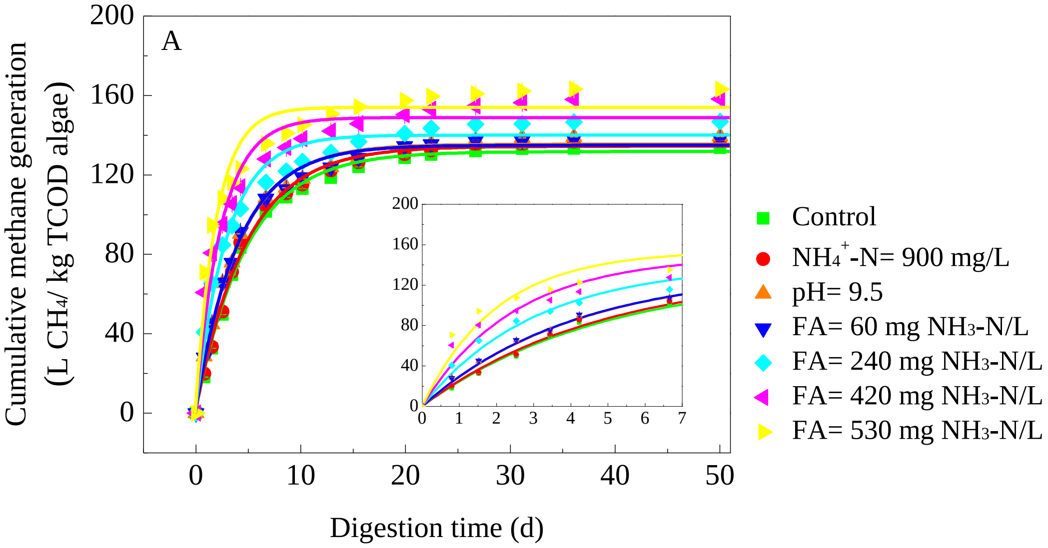


Figure 2. Cumulative methane generation from algae with FA (60~530 mg NH_3-N/L), alkaline (pH=9.5) and ammonium (900 mg NH_4^+-N/L) pretreatment. Error bars represent standard errors.



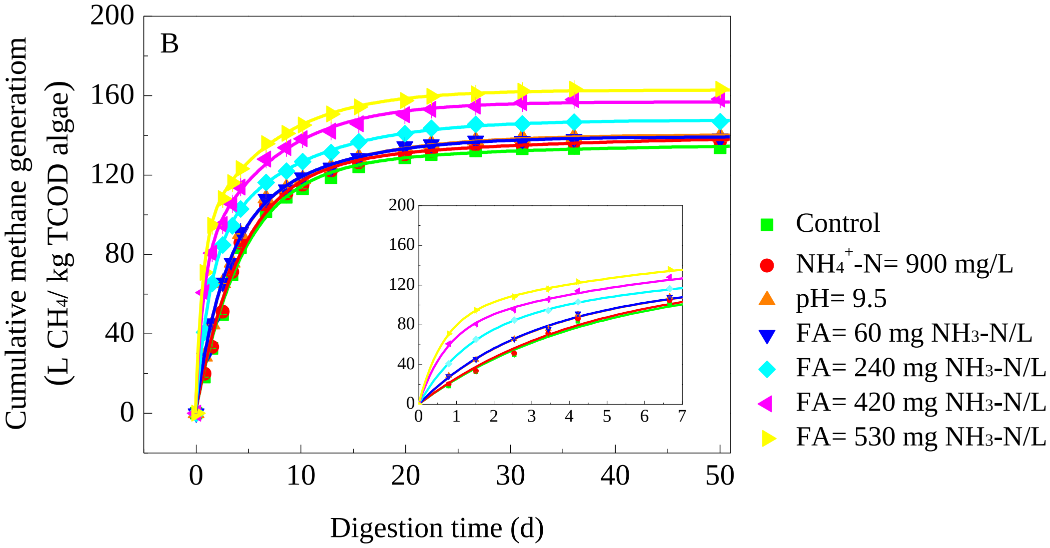


Figure 3. Measured and simulated biochemical methane generation curves by model fit (A): using a single substrate model; (B): using a two substrate model (symbols represent experimental measurements and lines represent model fit. Insets in Fig. 3 show the enlargement of the first 8 days. Error bars represent standard errors.

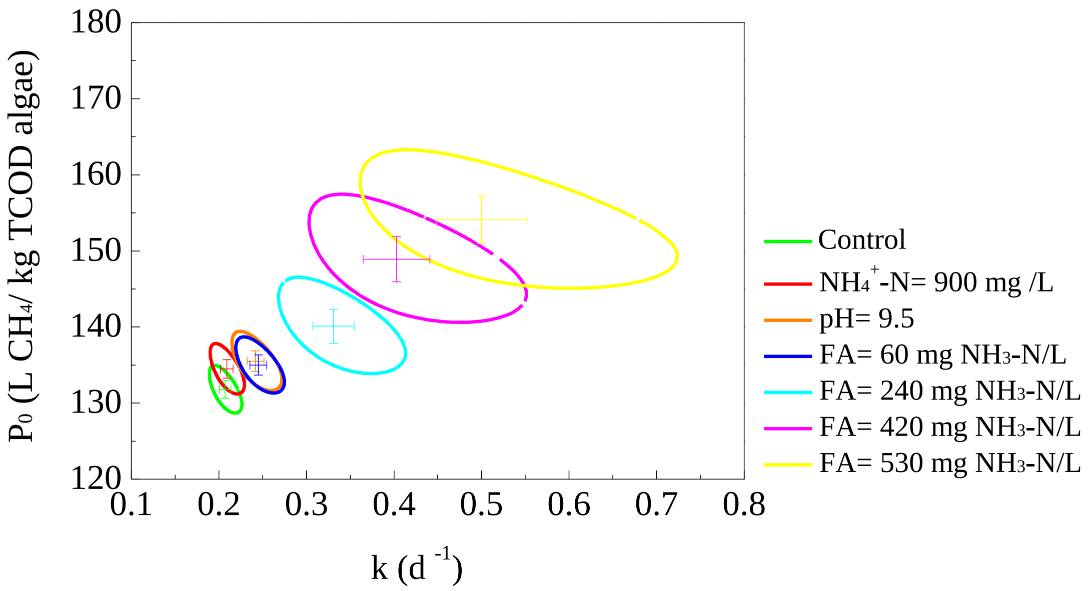


Figure 4. 95% confidence regions for hydrolysis rate (k) and biochemical methane potential (P_0) in various pretreatment systems. Error bars represent standard errors.

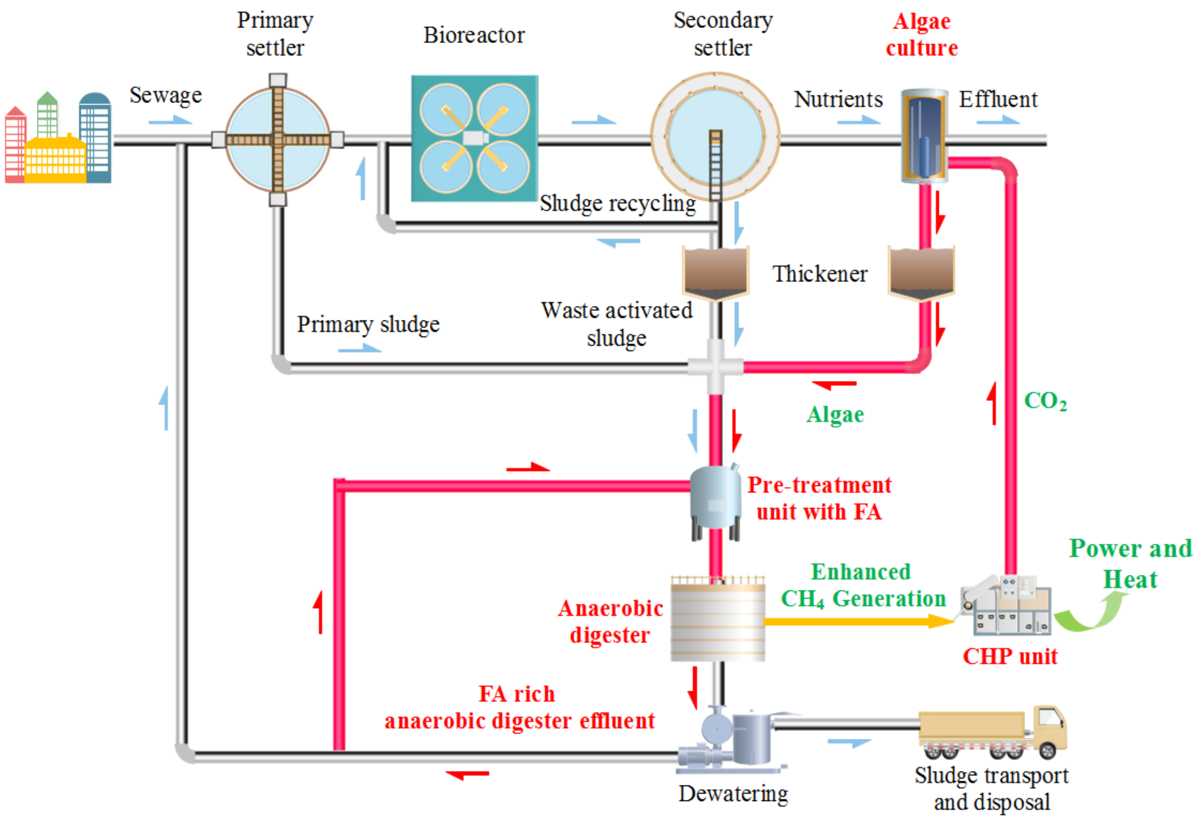


Figure 5. A "closed-loop" concept in an STP based on the proposed FA pretreatment technology to enhance methane generation from algae.