Free Ammonia Pretreatment to Improve Bio-hydrogen Production from Anaerobic Dark Fermentation of Microalgae

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ABSTRACT: Microalgae are third generation feedstocks for bio-hydrogen production to achieve a low carbon economy. Nevertheless, the bio-hydrogen production from microalgae is generally low. In this study, an innovative free ammonia (FA i.e. NH_3) pretreatment technology was first demonstrated to improve bio-hydrogen production from the secondary effluent cultivated microalgae during the anaerobic dark fermentation experiments. Scanning Electron Microscope revealed that FA pretreatment disrupted microalgae surface morphology. The soluble chemical oxygen demand (SCOD) release increased from 0.01 g SCOD/g VS microalgae (VS=volatile solids) to 0.05~0.07 g SCOD/g VS microalgae after FA pretreatment of 240~530 mg NH_3-N/L for 1 day, indicating the enhanced microalgae solubilisation. Dark fermentation bio-hydrogen potential experiments showed that bio-hydrogen production from microalgae was substantially improved following FA pretreatment of 240~530 mg NH_3-N/L. The bio-hydrogen production potential and maximum bio-hydrogen production rate increased from 18.2 L H_2/kg VS microalgae and 2.5 L H_2/kg VS microalgae/d to 19.9~22.1 L H_2/kg VS microalgae and 3.1~3.8 L H_2/kg VS microalgae/d, respectively, after FA pretreatment of 240~530 mg NH_3-N/L was implemented on the microalgae for 1 day. This FA technology follows a circular economic model because the required FA is from the FA rich dark fermentation liquid, which is a wastewater treatment waste.

KEYWORDS: *Microalgae; Bio-hydrogen; Free ammonia; Energy; Anaerobic dark fermentation*

INTRODUCTION

The traditional fossil fuels are being consumed at an alarming rate and also cause severe environmental concerns due to the production of greenhouse gases.[1] The alternative energy source is thus urgently needed. Hydrogen has been ubiquitously accepted as an ideal alternative to fossil fuels because its energy yield (i.e. 286 kJ/mol) is higher than hydrocarbon fuels and its combustion product is only water. Nevertheless, hydrogen is currently primarily produced via non-biological approaches such as incomplete oxidation of heavy hydrocarbon compounds and coal gasification, which incurs a high cost. Biological hydrogen production using a sustainable substrate would be a promising option.[2,3]

Wastewater treatment using microalgae has recently attracted intensive attention, which achieves simultaneous wastewater purification and value-added microalgal biomass.[4-6] The microalgae are rich in organic matters and therefore have been considered as third generation feedstocks for bio-hydrogen production through biological anaerobic dark fermentation.[7-9] However, bio-hydrogen production from microalgae is generally low due to the poor biodegradability of microalgae. Therefore, pretreatment technologies have been developed to improve bio-hydrogen production from microalgae.[10-13] For instance, bio-hydrogen production increased from 17.0 L H_2/kg VS microalgae (VS: volatile solids) to 45.5 L H_2/kg VS microalgae after the microalgae were subject to thermo-alkaline pretreatment at 100 °C for 8 h.[11] Yun et al.[12] demonstrated that bio-hydrogen production increased by 22% after the microalgae were ultrasonically pretreated at 20 kHz and 100,000 kJ/kg dry microalgae. Nevertheless, intensive energy and/or chemical inputs are required for these pretreatment technologies.

Recently, our research found that the biodegradability of activated sludge could be improved by free ammonia (FA, NH_3) pretreatment.[14-19] For example, Wei et al.[14] reported that 36% of the FA treated waste activated sludge (at 420 mg NH_3-N/L for 1 day) was degraded in the anaerobic biochemical methane potential experiments, whereas only 30% of the untreated sludge was degraded over the same period. Wang et al. [18] further demonstrated that the degradation of waste activated sludge increased from 23% to 36% following FA pretreatment at 300 mg NH_3−N/L for 1 day during 15 days' aerobic digestion. It was also shown that the degradation of the anaerobically digested sludge (ADS) was improved by 13% over the 8 days post aerobic digestion period after the ADS was subject to FA pretreatment of 440 mg NH_3-N/L for 1 day.[16] The FA pretreatment method was also estimated to be economically favourable because FA is directly attainable from the wastewater treatment plants (WWTPs).[17]

These discoveries led us to raise one question: will FA pretreatment play a role in improving bio-hydrogen production from microalgae by improving microalgae biodegradability? By answering this question, this work aimed to evaluate the effectiveness of FA pretreatment in improving bio-hydrogen production from microalgae for the first time. The effluent of a WWTP secondary settler was first used to cultivate the wastewater born microalgal biomass. Afterwards, 60~530 mg NH_3-N/L was applied to pretreat the cultivated microalgae for 1 day, with 900 mg NH_4^+-N/L pretreatment alone and pH=9.5 pretreatment alone as comparisons. The microalgae surface morphology and solubilisation before and after FA pretreatment were assessed. The bio-hydrogen production from microalgae with and without FA pretreatment was measured during the dark fermentation bio-hydrogen potential experiments. The bio-hydrogen production potential and maximum bio-hydrogen production rate were also estimated to identify the causes for the improved bio-hydrogen production.

MATERIALS AND METHODS

Microalgae and Anaerobically Digested Inoculum Sludge

The wastewater born microalgal biomass that harvested from the secondary settler of an Australian WWTP served as the microalgae inoculum. The effluent of a WWTP secondary settler was adopted to cultivate the microalgae in the beakers for the following dark fermentation bio-hydrogen potential experiments. The key compositions of the secondary settler effluent were: 2.6 mg PO_4^{3-}-P/L, 2.8 mg NO_3^--N/L, 0.2 mg NO_2^--N/L, 1.8 mg NH_4^+-N/L, pH 7.4 and 52 mg dissolved inorganic carbon/L. The cool white fluorescent lamps were used to illuminate the beakers with a 14 h light and 10 h dark cycle. The fluorescent lamps generated an illuminance of 6000 Lux (approximately 80 mmol/m^2/sec) while the lamps were on. The microalgae were cultivated to around 1.4 g TS/L (TS: total solids) and were thereafter thickened for the dark fermentation bio-hydrogen potential experiments as detailed below. The green microalgae were found to be dominant in the cultivated microalgae by microscopic observation. Key characteristics of the thickened microalgae were shown in Table 1.

(Table 1)

The anaerobically digested inoculum sludge was collected from an anaerobic digester with a sludge retention time (SRT) of 20 d treating a mixture of secondary and primary sludges. It was used to biodegrade microalgae as detailed in the dark fermentation bio-hydrogen potential experiments below. Key characteristics of the anaerobically digested inoculum sludge were shown in Table 1. The inoculum sludge was treated by heat at 90 °C for 0.5 h before use to inactivate the H_2 consuming microorganisms.[13]

Pretreatment of Microalgae by FA

The destruction of the microalgae subject to FA, pH 9.5 and 900 mg NH_4^+-N/L pretreatment was explored by a suite of batch experiments, as revealed by their surface morphology and soluble chemical oxygen demand (SCOD) release. Seven conical flasks with microalgae were used for pretreatment, which were constantly mixed and sat in a temperature controlled lab of 22 °C. During FA pretreatment, the total ammonia nitrogen (TAN, i.e. NH_4^+-N+NH_3-N) concentrations were 100, 400, 700 and 900 mg N/L, respectively, in 4 conical flasks by adding various amounts of ammonium chloride solution. The pH was 9.5 ± 0.2 in the FA pretreatment, which was selected based on our previous study.[16] The TAN and pH collectively generated different concentrations of FA ranging from 60 to 530 mg N/L, which were determined by (TAN concentration)×10^{pH}/($e^{6,344/(T+273)}$ +10^{pH}).[20] These FA are acquirable from the dark fermentation liquid and thus enable a closed-loop technology. 900 mg NH_4^+-N/L without pH adjustment was employed for microalgae pretreatment as well, during which pH was around 7.5. Another conical flask was maintained at pH 9.5 in the absence of ammonium addition. The purpose of 900 mg NH_4^+-N/L pretreatment alone and pH 9.5 pretreatment alone was to identify their separate role in improving bio-hydrogen production in the subsequent dark fermentation bio-hydrogen potential experiments. The last conical flask was used as a control without pH control or ammonium addition. These pretreatment experiments sustained for 1 day.

The microalgae surface morphology prior to and after FA pretreatment of 530 mg NH_3-N/L was examined using a Scanning Electron Microscope (SEM). The SCOD concentrations of microalgae prior to and after various pretreatment were also measured.

Dark Fermentation Bio-hydrogen Potential Experiments

Dark fermentation bio-hydrogen potential experiments were employed to evaluate the bio-hydrogen production from microalgae subject to FA, 900 mg NH_4^+-N/L and pH 9.5

pretreatment. A suite of serum vials (60 ml headspace and 100 ml working volume) were used for the bio-hydrogen potential experiments. 50 mL heat treated inoculum sludge and 50 mL microalgae were loaded into every serum vial. Before the serum vials were closed and capped by the rubber stoppers and the aluminium caps, their headspace was sparged with N_2 to generate an anaerobic environment. Afterwards, the serum vials were incubated at 37±1 °C. Two blank serum vials were operated. Both vials contained 50 ml heat treated inoculum sludge and 50 ml tap water. The only difference between the two blanks was that the pH of the tap water was adjusted to 9.5 before adding into one serum vial. Both blanks observed a similar and negligible bio-hydrogen production at the end of the bio-hydrogen potential experiments. The serum vials were mixed through inverting manually once every day. pH was not controlled during the dark fermentation bio-hydrogen potential experiments. The bio-hydrogen potential experiments were conducted in triplicate and sustained for around 20 days until the bio-hydrogen production was negligible.

The gas production and composition were measured periodically over the bio-hydrogen potential experiments period. The cumulative bio-hydrogen volume over the organic dry weight of the microalgae (i.e. L H_2/kg VS microalgae) was used to represent bio-hydrogen production.

Calculation of Maximum Bio-hydrogen Production Rate and Bio-hydrogen Production Potential

The maximum bio-hydrogen production rate (R_{max}) and the bio-hydrogen production potential (P_{max}) of microalgae were predicted by fitting the cumulative bio-hydrogen production results to a modified Gompertz Equation (Eq. 1) using OriginPro 8.0.[11] They reflect the bio-hydrogen production kinetics and potential of the microalgae, respectively. The R_{max} and P_{max} were

obtained while the sum of squared residuals between the measured bio-hydrogen production data and the equation predicted data was minimal.

$$P(t)=P_{max}\times\exp\{-\exp[R_{max}\times e\times(L-t)/P_{max}+1]\} \quad (1)$$

where P(t) (L H_2/kg VS microalgae) is cumulative bio-hydrogen production from microalgae at time t; P_{max} (L H_2/kg VS microalgae) is bio-hydrogen production potential of microalgae; R_{max} (L H_2/kg VS microalgae/d) is the maximum bio-hydrogen production rate of microalgae; e is the base of the Natural Logarithms, which is equal to 2.718; L (d) is the bio-hydrogen production lag time; t (d) is dark fermentation bio-hydrogen production experiments time.

Analytical Procedures and Statistical Analysis

The concentrations of VS, TS, SCOD, TCOD, NO_3^--N, NO_2^--N, NH_4^+-N, dissolved inorganic carbon and PO_4^{3-}-P were measured in accordance with the standard methods.[21] The carbohydrate and protein were determined by the phenol-sulfuric approach with glucose as the standard and by the Lowry-Folin approach with Bovine Serum Albumin as the standard, respectively.[22,23]

SEM images were taken to assess the difference between the microalgae surface morphologies prior to and after FA pretreatment at 530 mg NH_3-N/L.[24] Succinctly, the microalgae suspension was fixed by 2.5% glutaraldehyde for 2 h and then washed by the PBS buffer. Afterwards, the microalgae were dehydrated in a series of ethanol solutions and then were located in a freeze-drying machine for critical point drying. The dried samples were then mounted on conductive adhesive tapes on a sample holder followed by gold sputter coating. Then, the SEM (SEM, S4700, Hitachi) was used to observe the microalgae surface morphology.

The produced gas volume in the dark fermentation bio-hydrogen potential experiments was measured by a manometer based on the headspace gas pressure of the serum vials. The bio-hydrogen ratio in gas was determined using a gas chromatograph. The bio-hydrogen production in the serum vials was determined through multiplying the bio-hydrogen ratio in gas by the gas volume. The bio-hydrogen production from microalgae was determined as the difference between the bio-hydrogen production in the serum vial with microalgae and that in the blank without microalgae.

Independent samples *t*-test was used to evaluate significant differences. A value of $p > 0.05$ was considered insignificant, whereas a value of $p < 0.05$ was considered significant.

RESULTS AND DISCUSSION

How Does FA Pretreatment Affect Microalgae Surface Morphology and Solubilisation

Figure 1 shows the microalgae surface morphology before (Figure 1A) and after (Figure 1B) FA pretreatment at 530 mg NH_3-N/L. The microalgae surface was tight and smooth before pretreatment (Figure 1A). Nevertheless, the microalgae surface became shrank and some microalgae cells collapsed after FA pretreatment at 530 mg NH_3-N/L for 1 day (Figure 1B). This demonstrated that FA pretreatment could cause microalgae surface defection and damage the microalgae.

Similarly, the SCOD increased by 0.04~0.07 g SCOD/g VS microalgae after FA pretreatment at 60~530 mg NH_3-N/L (Figure 1C). Nevertheless, the SCOD only increased by 0.04, 0.01 and 0.01 g SCOD/g VS microalgae in the cases of pH 9.5 pretreatment, 900 mg NH_4^+-N/L pretreatment and control, respectively (Figure 1C). This demonstrated that FA pretreatment could enhance microalgae solubilisation.

(Figure 1)

How Does FA Pretreatment Affect Dark Fermentation Bio-hydrogen Production from Microalgae

Figure 2A shows the cumulative bio-hydrogen production from microalgae with various pretreatment. 900 mg NH_4^+-N/L pretreatment had a similar ($p>0.05$) bio-hydrogen production to the control, with approximately 18 L H_2/kg VS microalgae produced in the 20 days' dark fermentation period. This demonstrated that bio-hydrogen production was not affected by 900 mg NH_4^+-N/L pretreatment. The cumulative bio-hydrogen productions in the cases of pH 9.5 pretreatment and FA pretreatment at 60 mg NH_3-N/L were comparable (both at approximately 19 L H_2/kg VS microalgae after 20 days' fermentation) and slightly higher ($p<0.05$) than control. In contrast, FA pretreatment at 240~530 mg NH_3-N/L obtained a much higher ($p<0.05$) bio-hydrogen production during the whole dark fermentation period with 20~22 L H_2/kg VS microalgae produced at the end of the bio-hydrogen production experiments. Also, the cumulative bio-hydrogen production increased with the increased FA concentrations. The above collectively indicated FA pretreatment at 240~530 mg NH_3-N/L is efficient in improving bio-hydrogen production from microalgae and the improved bio-hydrogen production is mainly due to FA per se other than pH 9.5 pretreatment or ammonium pretreatment alone.

(Figure 2)

How Does FA Pretreatment Affect Maximum Bio-hydrogen Production Rate and Bio-hydrogen Production Potential of Microalgae

The cumulative bio-hydrogen production simulated by the modified Gompertz Equation was shown in Figure 2B, which demonstrates that the modified Gompertz Equation well captured (R^2>0.97 in all cases) all the cumulative bio-hydrogen production data. The estimated bio-hydrogen production lag time (L) of microalgae was similar in all cases (L<0.1 d, data not shown). The calculated maximum bio-hydrogen production rate (R_{max}), bio-hydrogen production potential (H_{max}) in all cases were shown in Table 2. Table 2 indicates that FA pretreatment at 60~530 mg NH_3-N/L significantly improved ($p<0.05$) the microalgae R_{max} from 2.5 L H_2/kg VS microalgae/d to 2.9~3.8 L H_2/kg VS microalgae/d. This indicated that FA pretretament is effective in improving maximum bio-hydrogen production rate of microalgae. In contrast, pH 9.5 pretreatment only improved ($p<0.05$) the microalgae R_{max} from 2.5 L H_2/kg VS microalgae/d to 2.9 L H_2/kg VS microalgae/d and 900 mg NH_4^+-N/L pretreatment did not play a role ($p>0.05$) in the microalgae R_{max}.

(Table 2)

In terms of bio-hydrogen production potential (H_{max}), the microalgae with FA pretreatment at 60 mg NH_3-N/L, pH 9.5 pretreatment and 900 mg NH_4^+-N/L pretreatment had a similar ($p>0.05$) H_{max} (approximately 18.5 L H_2/kg VS microalgae) to the microalgae without pretreatment (i.e. control). In comparison, FA pretreatment at 240~530 mg NH_3-N/L increased ($p<0.05$) the microalgae H_{max} from 18.2 L H_2/kg VS microalgae to 19.9~22.1 L H_2/kg VS microalgae. This revealed that FA pretretament at 240~530 mg NH_3-N/L is effective in improving the bio-hydrogen production potential of microalgae.

Table 2 also demonstrates that both R_{max} and H_{max} increased with the increased FA concentrations. This reveals that the bio-hydrogen production might be further improved if the FA concentration was increased. This will require further verification in the future experiments.

A Green Technology to Improve Bio-hydrogen Production from Microalgae

The concept of the proposed FA pretreatment technology to improve bio-hydrogen production from microalgae is shown in Figure 3. The secondary effluent cultivated microalgae are loaded into an FA pretreatment reactor, where the microalgae are mixed with the FA rich dark fermentation liquid. The microalgae surface morphology defection and microalgae solubilisation would occur in the FA pretreatment reactor. Thereafter, the FA pretreated microalgae are loaded into the anaerobic dark fermentation reactor to attain the improved bio-hydrogen production. If the dark fermentation liquid does not contain adequate FA, the FA concentration can also be increased by dosing a slight amount of base for pH elevation. The beauty of this technology lies in the utilisation of the FA rich dark fermentation liquid, which is a waste in the WWTPs. This technology is therefore 'green' and follows a circular economic model of recycling the waste as a highly valuable microalgae pretreatment agent. The FA technology is also promoting a paradigm shift in the WWTPs away from removing nutrients from wastewater only to a more holistic approach that utilises technology to polish secondary effluent for value added microalgae production and maximise clean energy recovery concurrently.

(Figure 3)

Practical Implications

Our world is facing the challenge of increasing energy requirement. Although fossil fuels continue to be significant feedstocks, the desire for sustainable feedstocks is becoming stronger. Microalgae have been considered as promising feedstocks for energy production. As bio-hydrogen is a desirable clean energy carrier for achieving a low carbon economy, bio-hydrogen production from microalgae has attracted extensive attention.[7-9] Unfortunately, the bio-hydrogen production from dark fermentation of microalgae is low. This is the first study to reveal that the improved bio-hydrogen production from microalgae can be achieved with FA pretreatment at 240~530 mg NH_3-N/L, which contributed to an increased maximum bio-hydrogen production rate and a higher bio-hydrogen production potential of microalgae. The microalgae surface morphology defection and enhanced microalgae solubilization by FA pretreatment might lead to the improved bio-hydrogen production. The findings open a new door for the decision makers and engineers to maximise clean energy production, thereby establishing a more sustainable 'bio-hydrogen society'

Some pretreatment technologies have already been proposed in the past to improve the bio-hydrogen production from microalgae, such as ultrasonic pretreatment, microwave pretreatment and chemical pretreatment.[10-13] Nevertheless, these pretreatment technologies are expensive because they consume large amounts of energy or chemicals. In comparison, the FA pretreatment technology only requires a chemical (i.e. FA) that is a by-product of wastewater/sludge treatment. Therefore, only negligible chemical and energy input are needed. It should be pointed out that the bio-hydrogen production efficiencies of this FA pretreatment technology and the other pretreatment technologies cannot be directly compared because the microalgae characteristics play a paramount role in the bio-hydrogen production. In order to make the comparison reasonable, the same microalgae subject to different pretreatment technologies should be used as the feedstocks for bio-hydrogen production experiments. Also,

this study only aims to demonstrate the feasibility of improving bio-hydrogen production from microalgae with FA pretreatment and the FA pretreatment technology is only in its infancy for the time being. Therefore, the comprehensive optimization experiments are required to identify the optimal FA pretreatment conditions. In addition, continuous tests still need to be done to better evaluate this technology in the future.

CONCLUSIONS

This study evaluated the effectiveness of FA pretreatment in improving bio-hydrogen production from microalgae via dark fermentation bio-hydrogen potential experiments. FA pretreatment on microalgae is capable of disrupting microalgae surface morphology and enhancing microalgae solubilisation, thereby causing an improved bio-hydrogen production from microalgae during anaerobic dark fermentation. Further analysis revealed that FA pretreatment increased both the bio-hydrogen production potential and maximum bio-hydrogen production rate of microalgae. This FA technology follows a circular economic model since the required FA is from the FA rich dark fermentation liquid, which is a wastewater treatment waste.

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Notes

The authors declare no competing financial interest.

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SUPPORTING INFORMATION

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Table 2. Estimated maximum bio-hydrogen production rate (R_{max}) and bio-hydrogen production potential (H_{max}) of microalgae during dark fermentation at varying FA concentrations (with standard errors).

Figure 1. SEM images (A and B) of microalgae before and after FA pretreatment at 530 mg NH_3-N/L for 1 day, and SCOD release (C) from microalgae after various pretreatment. Error bars represent standard errors.

Figure 2. Cumulative bio-hydrogen production from microalgae with various pretreatment. A: measured cumulative bio-hydrogen production; B: cumulative bio-hydrogen production with simulation curves based on the modified Gompertz Equation. Error bars represent standard errors.

Figure 3. Schematic diagram of the green, closed-loop free ammonia pretreatment technology to improve bio-hydrogen production from microalgae during anaerobic dark fermentation in a wastewater treatment plant.

Table 1. Key characteristics of microalgae and anaerobically digested inoculum sludge (with standard errors of triplicate tests).

Parameter	Microalgae	Anaerobically digested inoculum sludge
Total solids (TS) (g/L)	18.9 ± 0.3	25.5 ± 0.3
Volatile solids (VS) (g/L)	14.6 ± 0.1	19.9 ± 0.2
Soluble chemical oxygen demand (SCOD) (g/L)	0.6 ± 0.1	0.8 ± 0.2
Total chemical oxygen demand (TCOD) (g/L)	21.6 ± 0.1	25.6 ± 0.3
pH	7.5 ± 0.1	7.8 ± 0.1
Protein (% VS)	49 ± 7	Not Determined
Carbohydrate (% VS)	15 ± 6	Not Determined
pH	7.5 ± 0.1	7.8 ± 0.1

Table 2. Estimated maximum bio-hydrogen production rate (R_{max}) and bio-hydrogen production potential (H_{max}) of microalgae during dark fermentation at varying FA concentrations (with standard errors).

Pretreatment condition	R_{max} (L H_2/kg VS microalgae/d)	H_{max} (L H_2/kg VS microalgae)
Control	2.5 ± 0.1	18.2 ± 0.3
NH_4^+=900 mg N/L	2.6 ± 0.1	18.6 ± 0.3
pH=9.5	2.9 ± 0.1	18.7 ± 0.5
FA=60 mg N/L	2.9 ± 0.1	18.6 ± 0.5
FA=240 mg N/L	3.1 ± 0.3	19.9 ± 0.4
FA=420 mg N/L	3.4 ± 0.3	21.4 ± 0.5
FA=530 mg N/L	3.8 ± 0.4	22.1 ± 0.6

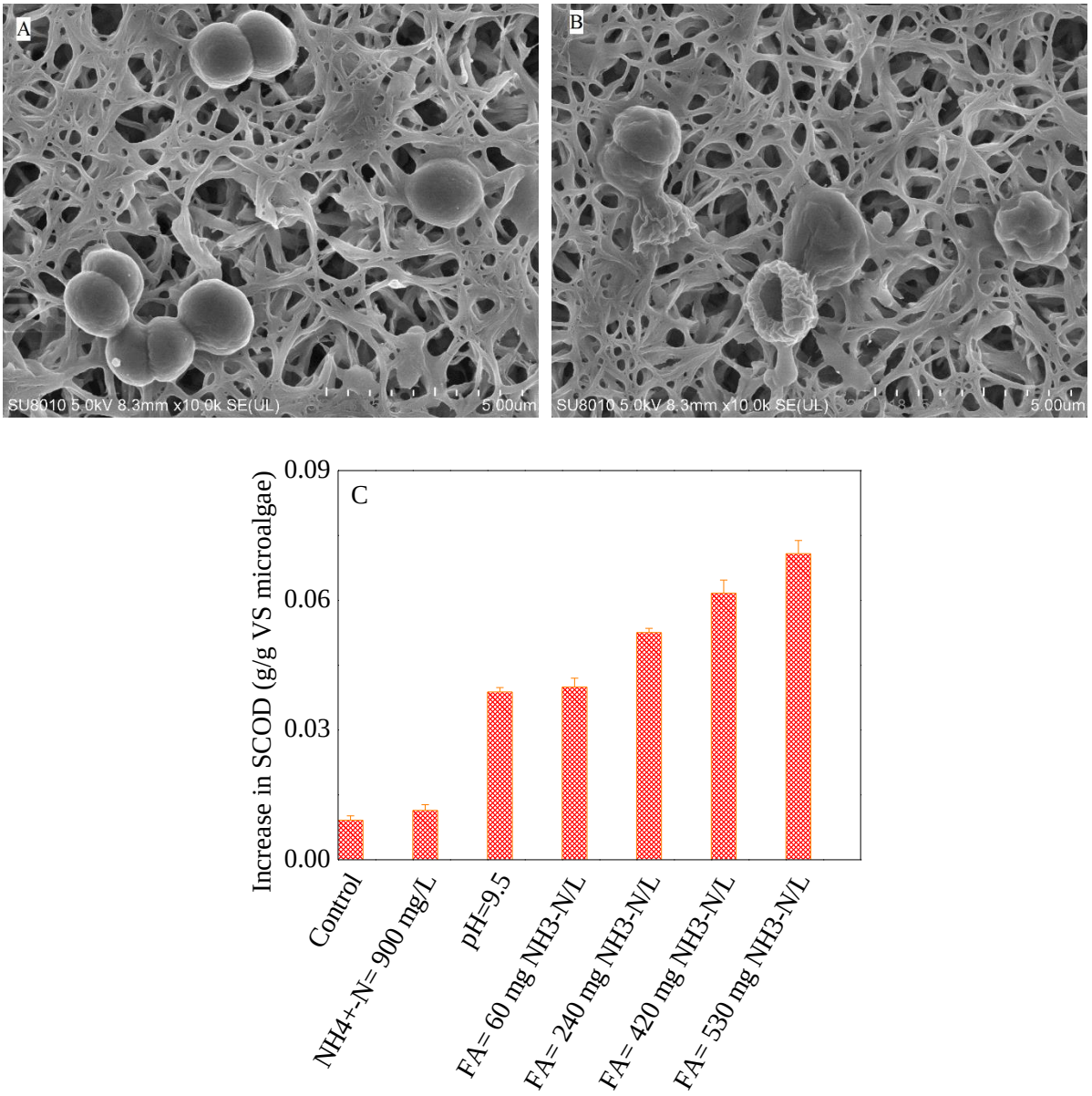

Figure 1. SEM images (A and B) of microalgae before and after FA pretreatment at 530 mg NH_3-N/L for 1 day, and SCOD release (C) from microalgae after various pretreatment. Error bars represent standard errors.

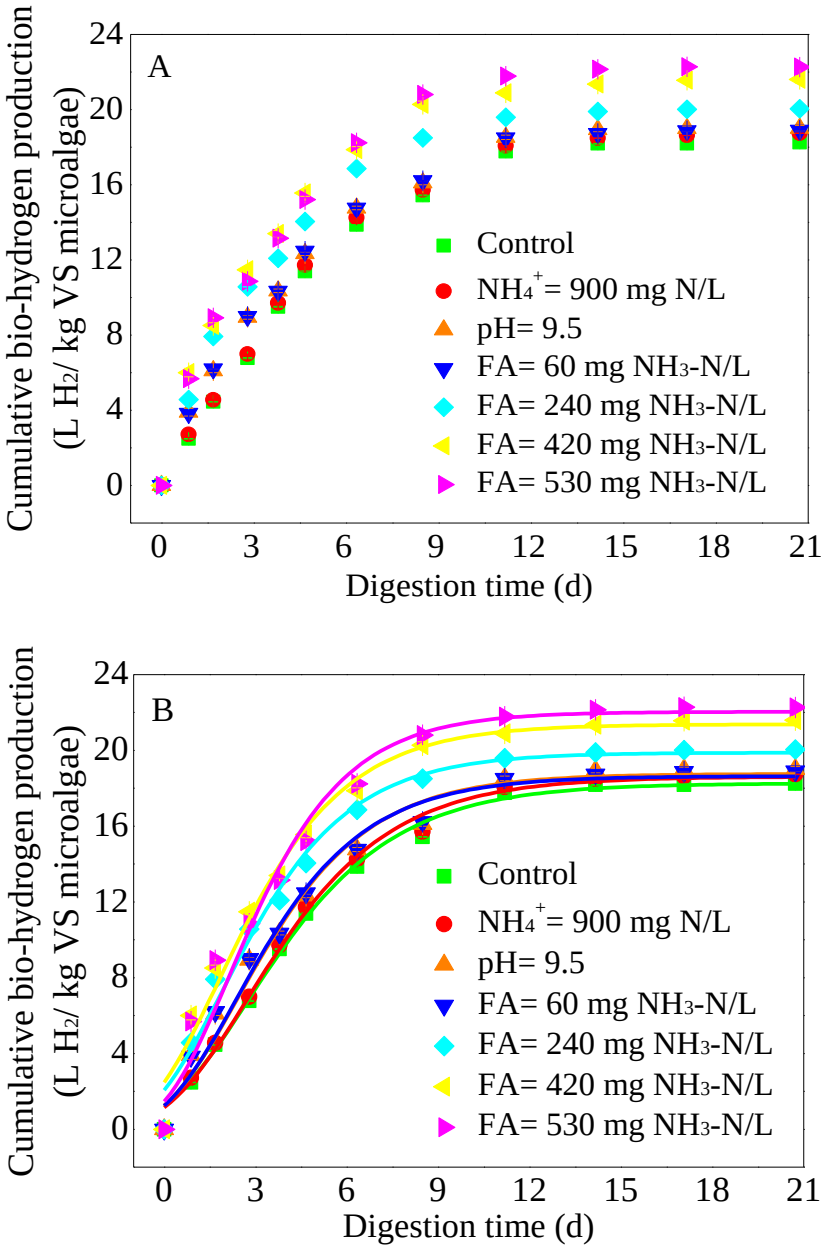

Figure 2. Cumulative bio-hydrogen production from microalgae with various pretreatment. A: measured cumulative bio-hydrogen production; B: cumulative bio-hydrogen production with simulation curves based on the modified Gompertz Equation. Error bars represent standard errors.

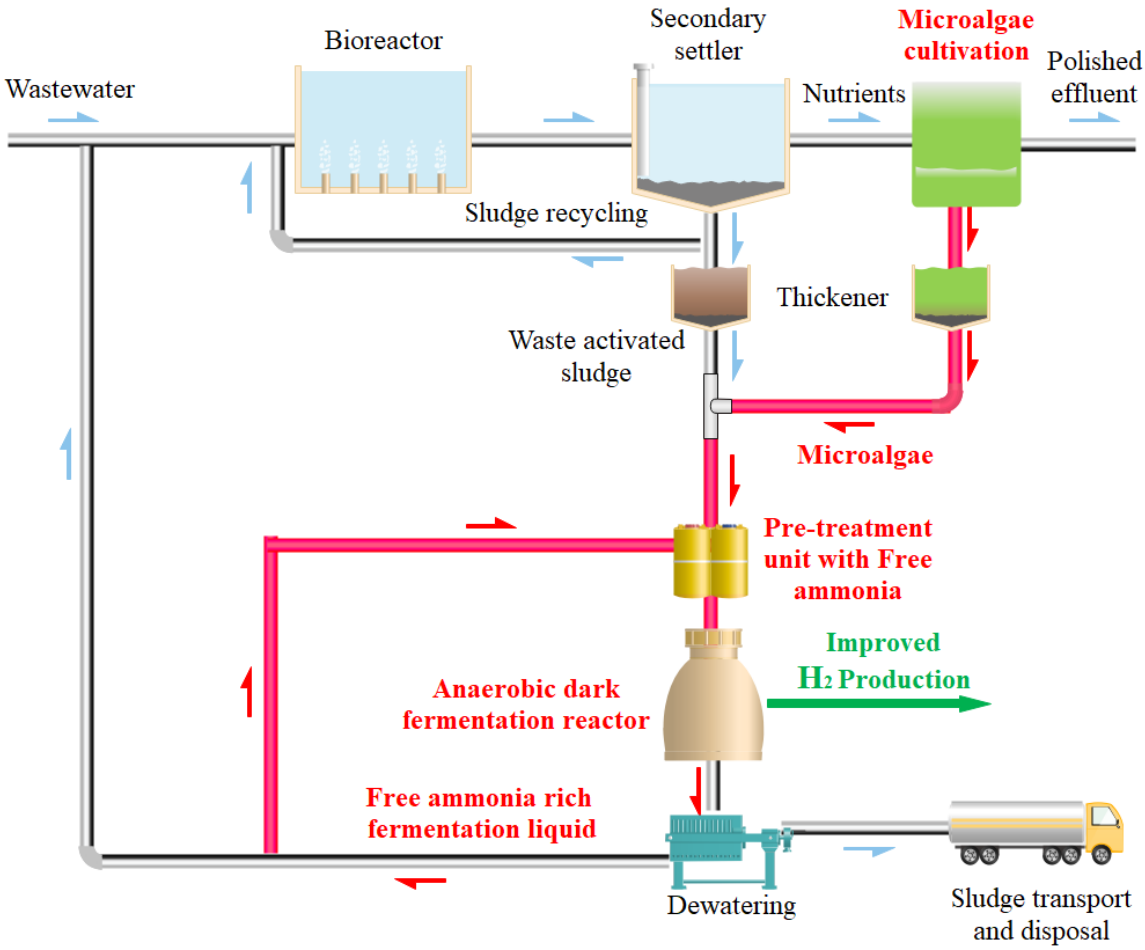

Figure 3. Schematic diagram of the green, closed-loop free ammonia pretreatment technology to improve bio-hydrogen production from microalgae during anaerobic dark fermentation in a wastewater treatment plant.

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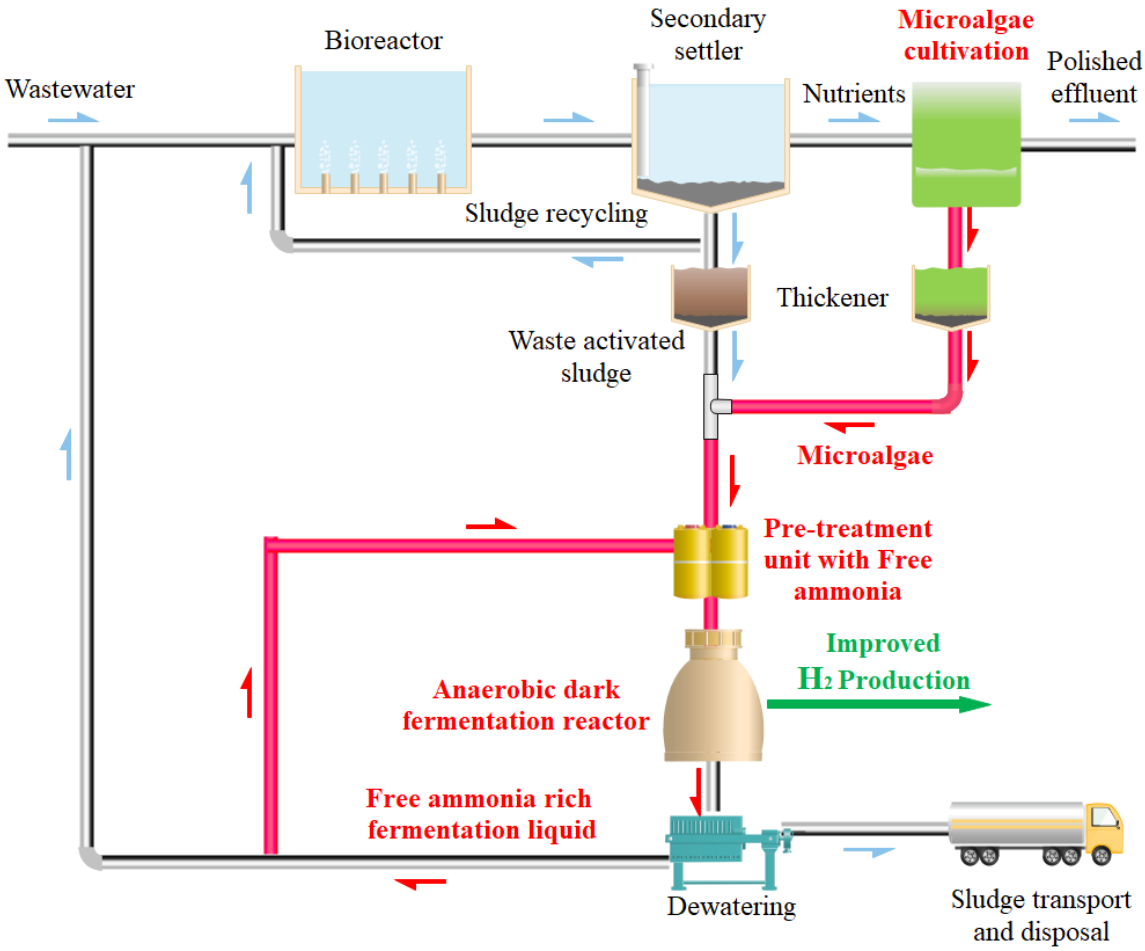

Synopsis: An innovative free ammonia technology was proposed to improve bio-hydrogen production from anaerobic dark fermentation of microalgae