

## Sustainable biomethane, biofertilizer and biodiesel system from poultry waste

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### Abstract

In this study we developed and tested a sustainable system that produces high-yield outputs of biomethane, biofertilizer and biodiesel. These were achieved by blending of poultry manure (PM), paper pulp and algae waste sludge in co-digestion producing biomethane, digestate filtrated semi-solid and aqueous, the former as biofertilizer and latter used in algal cultivation to enhance algal biomass for biodiesel production. The varied blending of the substrates resulted in carbon/nitrogen ratios (C/N) of 26, 30, 31, 34 and 37 which were assessed for biomethane. C/N 26 resulted in 1045 mL/L/d (74% biomethane content) which was highest yield comparing to other C/N, C/N 30 achieved in similar (1010 mL/L/d) making the C/N range for optimum biomethane for these substrates ranging between C/N 26 to 30. Pretreatments of the digestate improve the yields of biomethane in C/N 26 and 30 significantly. We assessed all the digestates from each of the C/N 26, 30, 31, 34 and 37 based on nitrogen mineralization and found C/N 26 to 31 as being nutrients-rich. Digestate in algal supplemental indicated glucose depletion linearly depleting, lowest with the nutrients-rich that is C/N 26 to 30. As expected, digestates from C/N 34 and 37 in single-addition failed to yield comparable algal yields then yields from C/N 26, 30 and 31 digestates at 120 h that achieved dry cell weight (DCW) of 7.72, 7.8 and 7.12 g/L respectively. To improve alga biomass yield and enhance cellular lipid content and its final yield, we investigated two-stage supplemental feeding strategy using digestates from C/N 26 and 30. Based on cultivation 'without' digestate that showed growth phases, we added digestate at lag-exponential (0-120 h) and stationary (120-180 h) phases. The supplemental feeding resulted in rapid glucose depletion achieving 9 g/L at 120 and reaching lipid yield 3.77 g/L after 180 h. Conclusively, a circular system using the biowastes discussed or similar in nature can develop and constitute a self-supporting sustainable system opportunities.

**Keywords:** Poultry manure, biomethane, biogas, biofertilizer, *Chlorella vulgaris*, biodiesel.

### Introduction

There is currently a world-wide need to improve the way we handle wastes and to produce energy in a sustainable manner. Taking the waste product of one process and using it as input or fuel for another process is one way to accomplish this; it makes intelligent use of resources, decreases pollution, and broadens their application in sustainable approach. It is the best interest for such approach to demonstrate the feasibility of the application in more sustainable, self supporting manner. The biodigestion of some common waste for biomethane generation is one attractive approach. The two most important factors influencing biomethane production are the C/N ratio of the digester substrates and pre-treatments of the substrates. The workable range for biomethane generation is generally known to be C/N 20/1 and 30/1 (Gene & William, 1986). External pre-treatments of the digester substrate have been shown to enhance biomethane yield (Gunaseelan, 1995). Furthermore, appropriate pre-treatments basically suppress inhibitory substances such as volatile fatty acids and NH<sub>3</sub> and make available the components for efficient digestion, thus enhancing biomethane production (Be'line *et al.*, 2008).

In our recent study (Iyovo *et al.*, 2010) we successfully demonstrated that poultry manure was an important nutrient source for algal biomass

enhancement when we aerobically digested PM and fed the digestates to microalga *Chlorella vulgaris* culture. A more attractive approach was if poultry manure (PM) can be used to produce biomethane. The residue digestate rich in nutrients can be used to enhance algal cultivation (Iyovo *et al.*, 2010). Several studies had revealed that PM a potential biological nutrients source (Amanullah, 2007) and also a mineral-rich waste (Nicholson *et al.*, 1996), when co-digested with a high-carbon source like paper pulp, can perform well in a biogas digester-producing a considerable volume of methane (Cummings, 1995; Fox & Noike, 2004; Abouelenien *et al.*, 2009). Blends of paper pulp waste and algae sludge are also reported to emit considerable biomethane (Yen & Brune, 2007) when co-digested.

After the biomethane gas is collected from the digester for utilization, the digestate can be separated into semi-solid and aqueous. The former as biofertilizer for crop production (Abdel *et al.*, 1995; Kaikake *et al.*, 2009) and the latter as supplemental feed in alga cultivation to enhance algal biomass that will directly govern algal biofuel (Iyovo *et al.*, 2010). Other wastes similar in nature to PM, such as municipal organic waste (Julia, 2009) and human excreta (Heinonen-Tanski & van Wijk-Sijbesma, 2005), have shown promising results as crop fertilizer. Therefore it makes sense that PM digestate

(created as a by-product of PM biodigestion) is also a good candidate for fertilizer.

Knowing that PM is high in nitrogen and phosphorus content (Nicholson *et al.*, 1996), the use of PM blend digestates in the described sustainable system to produce *Chlorella vulgaris* biomass is unquestionably attractive. It is optimistic that if sufficiently digested to produce high yield of biomethane utilizing PM, which would directly mean that nitrogen mineralization was efficiently done leaving the digestate rich in minerals. Hence, the study investigated the following strategies of supplementary feedings of PM digestates: without supplementation, single-stage supplementation, and two-stage supplementation in the cultivation of micro alga *Chlorella vulgaris* under dark condition. The alga *Chlorella vulgaris* was chosen because it is known for high lipid content and rapid grow (Xu *et al.*, 2006; Chisti, 2007; Bastianoni *et al.*, 2008; Widjaja *et al.*, 2009).

Besides appreciable amounts of nitrogen are needed for alga biomass production for example; a ton of algal biomass could require 45 Kg of nitrogen based on an algae composition of  $CO_{0.48}H_{1.83}N_{0.11}P_{0.01}$  (Grobelaar, 2004). Furthermore, an algal biomass production limit of 263 tons per hectare per year in a bioreactor (Chisti, 2007) would require more than 11 tons of nitrogen per hectare per year, indirectly meaning increasing production output or bioreactor number means needing larger quantities of nitrogen along. Therefore utilizing a source of "free" supplementary nitrogen such as PM digestate is an attractive idea. Sewage sludge have shown to have increased lipid content in *Lipomyces starkeyi* for biodiesel production (Angerbauer *et al.*, 2008). Therefore, similar effect is likely with digestates from anaerobic digestion on microalga *Chlorella vulgaris* cultivation. High PM nitrogen can be used to increase algal biomass then starve the cells by limiting nitrogen supply which would increase cellular lipid. One such strategy is adding carbon source such as glucose at stationary phase (Iyovo *et al.*, 2010) which would use immediate nitrogen to metabolize glucose which in turn starve the cells converting all residue metabolites and glucose to storage biomaterials (Livne & Sukenik, 1992). Accordingly, this study was about blending of poultry manure (PM), paper, and alga sludge waste, in a three-step process producing biomethane, biofertilizer, and alga biomass for biofuel (biodiesel) by feeding the digestate. We measured the results under varying conditions and focus on the effects on resultant digestates on alga cultivation and devised feeding strategies to enhance algal biomass and improve lipid yield to increase biodiesel production.

## Materials and methods

### Biomethane production

Two bench-top anaerobic digesters with working volume of 4.5 L were provided; the digesters were connected to gas collecting tanks. Cow dung as starter culture (source of methanogens) and PM (from *Galus domesticus*) were collected fresh from nearby farm,

Jiangnan University, China. The PM was from a meat producing chicken facility, the sample was collected right after excretion without any sitting time exceeding more than an hour. The feces had been mixed with beddings, feed particles and feathers which have been considered as part of PM. Temperature was kept constant at 30°C for all digestions. Initial algae biomass was provided when microalga *C. vulgaris* was cultured in 8 g/L glucose according to study by Chen and Johns (1991) using Bold's basal media (Bbm) (Watanabe, 1960). The total sum ratio of algal, paper and PM to cow manure as inoculant was fixed at 20:1 and the total of 3 Kg of the substrates in total was maintained. Pretreatment of substrates prior to co-digestion was achieved according to Yen and Brune (2007).

### Low and high dilution for varied nitrogen levels in digester substrate

Two different dilution rates for the PM blends and analyzed their effect upon both biomethane production and algae production. Low dilution: Water added was equivalent to the total weight of the substrates for generation of high nitrogen PM. High dilution: Water was added 1.5 times the weight of total substrates for low nitrogen PM.

### Digestates

After digestion for biomethane production, the digestates were separated into aqueous and semi solids by decantation, filtration and centrifugation processes. The aqueous digestate extract was sterilized in bottles for 15 min at 121°C and stored at 0°C for later use. The remaining semi-solid digestate residues were collected as biofertilizer.

### Alga strain and cell growth conditions

*Chlorella Vulgaris* was donated by Charles University, Czech Republic. Bbm components used were (g/L):  $NaNO_3$  0.25,  $CaCl_2 \cdot 2H_2O$  0.025,  $MgSO_4 \cdot 7H_2O$  0.075,  $K_2HPO_4 \cdot 3H_2O$  0.075,  $KH_2PO_4$  0.175 and NaCl 0.025. Glycine and yeast extract were reduced to 0.1 g/L

Fig. 1. Schematic relation of poultry manure, paper waste and algae sludge for biomethane, biofertilizer and biodiesel via microalga *Chlorella vulgaris* cells.

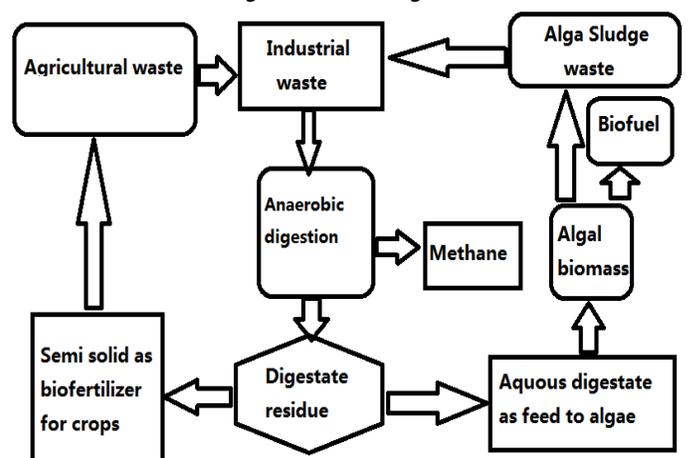
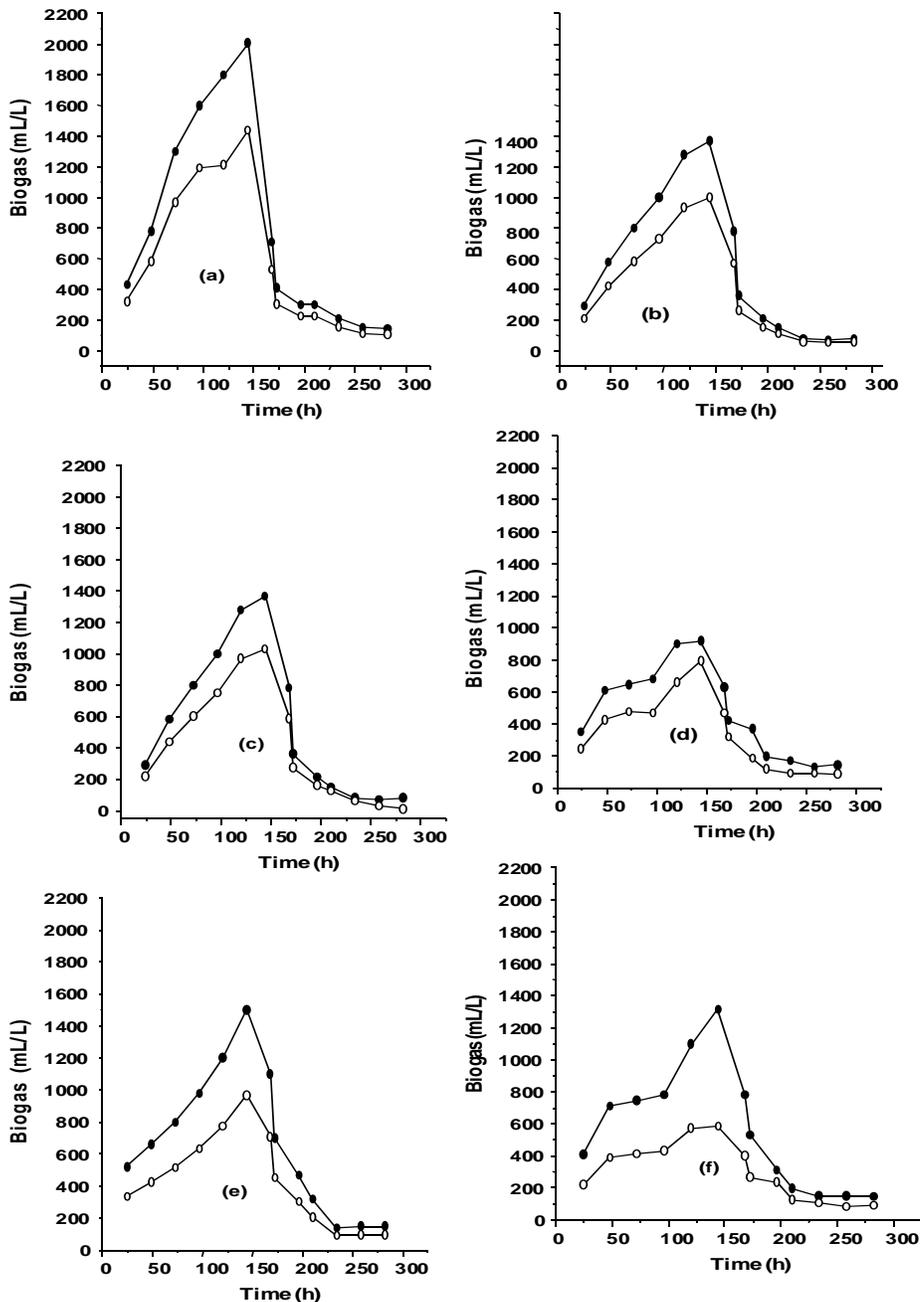


Fig. 2. Biogas emission and their respective biomethane content in various substrate blending dilution rate at 1:1 and without pretreatment. Biogas (●) and biomethane (○) yield according to C/N 26 (a), 30 (b), 31 (c), 33(d), 34 (e) and 37 (f)



each to initiate and promote cell growth. The medium was supplemented with glucose at 10 g/L. Seed culture of the microalga was prepared in a 500 mL flask containing 200 mL medium for 180 h. Shake flask culture experiments were performed in 500 mL flasks, each containing 200 mL medium after inoculating with 10% (v/v) of seed culture (Xu *et al.*, 2006). Temperature, agitation and growth period were fixed at 27°C 150 rpm and 180 h, respectively. It was kept in a reciprocal shaker with in-built fluorescence irradiance at  $35 \mu\text{mol photon m}^{-2}\text{s}^{-1}$ .

Culture broths were centrifuged at 8000 rpm for 10 min and cells were washed twice with distilled water and freeze dried (Xu *et al.*, 2006).

**Supplementation strategy.** Adding digested aqueous PM to improve alga biomass production:

We added the PM blend digestates as supplemental feeding for the algae in two ways.

**Single-stage feeding strategy:** Low nitrogen PM solution supplementation was studied at two amounts: 20 mL/L/d and 40 mL/L/d.

**Two-stage feeding strategy:** High nitrogen PM solution was fed during the “exponential” time interval (0-120 h), and LNPMS was fed during the “stationary” time interval (120-180 h) daily. The amounts added during 0-120 h were 20 mL/L/d followed by 10 mL/L/d from 120-180 h.

#### Analytical procedures

##### Biomethane measurement

Maximum hydraulic retention time (HRT) of 14 d was investigated for biogas and biomethane yields. All digestion were performed at uniform conditions and terminated when no biogas was produced. The total biogas produced was measured by water volume displaced. Biomethane yield was determined according to method outlined by Erguder *et al.* (2001). The volume of biomethane was determined from the total biogas generated daily (mL/L/d), 24 h and also determined as percentage.

##### Digestate analysis

The digestates resulting from the various PM blends were analyzed in terms of their components, using the following methods. Volatile solids (VS) were determined by putting measured samples in an oven at 550°C and determining the ash percentage. Total solids in the samples were measured by evaporating samples at 105°C to constant weight. Total Nitrogen (TN) in semi-solid and solid digestates was determined by the Kjeldahl method. Dissolved Total Nitrogen was determined by diluting a 10 mL sample to 100 mL with distilled water. Samples were injected into the TOC/TN analyzer-LiquiTOC, Elemtar Analysensystem GmbH, and data were obtained from the computer monitor in terms of quantity per liter.  $\text{NH}_3\text{-N}$ ,  $\text{NH}_4\text{-N}$  and uric acid-N were analyzed according to the standard method (MAFF, 1986) Potassium (K) and

Table 1 Biological nutrients in the supernatants (aqueous) as analyzed after digestion ceased

Elements/compounds	Blendings (Carbon/nitrogen)						
	With pre-treatment <sup>p</sup> (C/N 26)	No pre-treatment (C/N 26)	C/N30	C/N31	C/N33	C/N34	C/N37
Total nitrogen (mg/L)	320	400	410	490	540	446	544
Phosphorus (mg/L)	71	68	57	55	36	33	30
Potassium (mg/L)	2590	2580	1003	980	990	845	900
Total carbon (mg/L)	1788	1787	3100	3450	3980	2779	2270
NH <sub>4</sub> -N (mg/L)	110	89	66	55	56	35	38
NH <sub>3</sub> -N	97	102	118	122	231	141	136
Uric acid-N (mg/L) <sup>ad</sup>	18	36	34	39	44	39	47
Uric acid-N (mg/Kg) <sup>l</sup> 379							

<sup>R</sup>Ratio, the designated numbers represent amount of paper, algae and PM blended by mass, respectively. <sup>p</sup>pretreatment, <sup>l</sup>initial & <sup>ad</sup>after digestion.

phosphorus (P) concentrations in the aqueous digestates were analyzed by microwave digestion. A sample of 2 mL was added to 3 mL HNO<sub>3</sub> and was digested using cold microwave technology for 1 h. The solution was transferred to a 50 mL volumetric flask and distilled water was added to the calibration. The potassium (K) concentration was obtained by calibration curves using a Spectr. 220 atomic absorption spectrometer, Varian American at wavelength of 766.5 nm. For phosphorus (P), another 2 mL sample was microwave digested and diluted to 50 mL. A 4 mL was taken and diluted to 2.5 mL ammonium molybdate and sulphuric acid. Stannous chloride and glycerol were used as indicator for the absorbance at 820 nm.

#### Algae cell cultivation

Cell growth performance was monitored by optical density measurements at 540 nm using a UV visible spectrometer (Xu *et al.*, 2006) and by measuring total glucose concentration with a SBA-40C biosensor analyzer (Ding & Tan, 2006). Dry cell weight (DCW) was obtained based on methods by Feng and Johns (1991). Harvested microalga cells were then pulverized and total lipid was extracted by soxhlet extraction method using n-hexane.

#### Results and discussion

Fig. 1 depicts an illustration of the system approach this study investigated. In general, our research indicates that for the PM blends we studied, the most efficient biomethane production resulted from the following conditions; blending to maintain a substrate C/N ratio of 26 or 30, using the dilution ratio of 1:1.5 dilution substrates, and pre-treating the substrate.

#### Biomethane production

Fig.1 described the schematic illustration of the whole approach executed and assessed in this study. In the biodigestion, the C/N ratio, pretreatment and dilution rates significantly governed biogas production and its biomethane content. The biomethane yield increased linearly with C/N ratio between 26 and 30 agreeing with other studies (Gene & William 1986; Callaghan *et al.*, 2002; Mshandete *et al.*, 2004; Yen & Brune 2007). For example, C/N 26 (1:3:5) which was blended in order of alga sludge, paper waste and PM achieved remarkable biomethane yield that reached 1049 mL/L/d (74.6%) after

140 h as shown in Fig. 2a, C/N 30 also showed reasonable biomethane in content reaching 1010 mL/L/d (73.4%) than C/N 37 (Fig. 2f) whose biomethane yield was 584.7 mL/L/d (60.1%). Moreover, increased C/N above 30 demonstrated higher biogas but reduced biomethane content. However, it can only be improved by applying dilution and pretreatment (Fig. 3) as discussed subsequently.

#### Dilution & pretreatment

Fig. 3 illustrates the effects of pre-treatment and dilution rate upon biomethane production. We found that, in general, the combination of 1) Pre-treatment and 2) A dilution ratio of 1:1.5 produced the highest level of biomethane. Other significant findings were as follows; the biomethane yield increased when dilution ratio was improved by adding water by mass from 1:1 to 1:1.5 (Fig. 3.1), when both dilutions (1:1 and 1.5:1) were subjected to pretreatments, biomethane content improved as shown in Fig. 3.2. Noticeably, the 1.5:1 dilution achieved higher biomethane output of the biogas mostly around 144 h (day 6), the yield improved higher on pretreatment (1020 mL/L/d, 72%) on average (Fig. 3.1 compared to Fig. 3.2). In comparison, when using low dilution (1:1.5), biomethane content in production improved from 1030 mL/L/d (70%) to 1045 mL/L/d (74%) when pretreatment was added (Fig. 3.3).

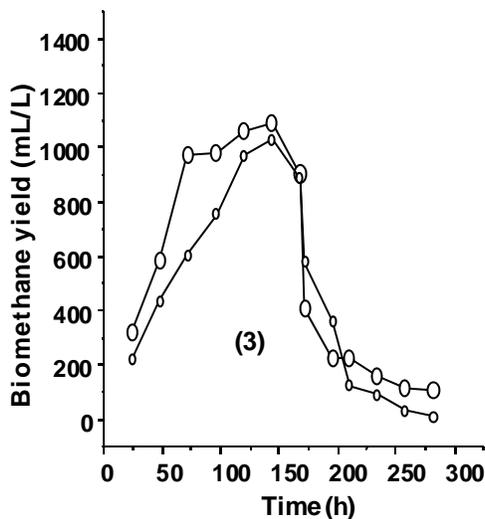
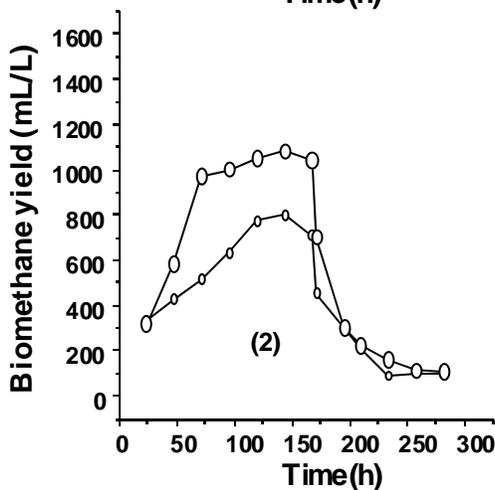
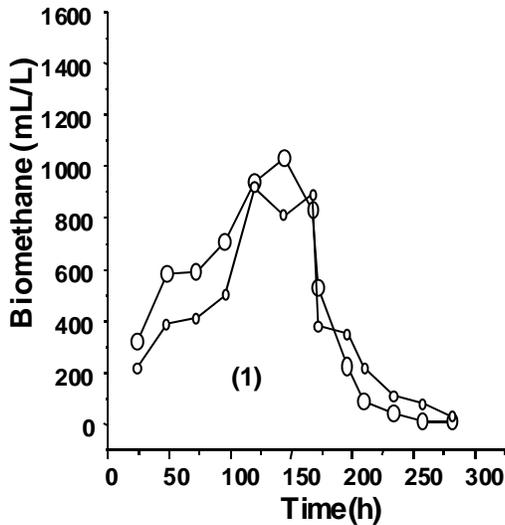
The results of this study indicated that biomethane production from PM blends with the various blend proportions, dilution rates, and pre-treatment options shows a range of biomethane yield values from 584.7 mL/L/d (60.1%) as in Fig. 2f to 1045 mL/L/d (74%) as discussed of Fig. 3.3. This intriguing 14% difference in production shows the importance of making simple low cost applications to enhance biomethane yield. Analysis indicates that the biomethane production from PM blends shows a range of biomethane yields from 60% (Fig. 2f) to 74% (Fig. 3.3). This intriguing 14% difference in production shows the importance of making simple low cost applicable controls to enhance biomethane yield.

#### Assessment of semi-solid digestate as biofertilizer

In this study, we also evaluated the digestate's suitability and value as biofertilizer. Analysis indicates that the PM blends in this study with C/N ratios of 26-30 are the most suitable for biofertilizer. In addition, we

Fig. 3. C/N 26 digestion biomethane comparison in dilution and pretreatment.

- (1) High [○] and low dilution [○], (2) High dilution [○] and high dilution with pretreatment [○], (3) Low dilution [○] and low dilution with pretreatment [○].



found that mineralization (the break-down into useful plant absorbable nutrients) can be further increased by pre-treating the substrates before digestion. Table 1 shows a breakdown of the digester substrate components. Analysis indicates that as volatile solids were increased by means of blending, more nitrogen was mineralized and retained. These results are similar to those reported in another study (Kirchmann & Witter, 1988). Clearly, it means that as more carbon was available, nitrogen was more mineralized. For the PM blend with C/N ratio of 26, the amount of uric acid before and after digestion was 36 mg/L and 18 mg/L, respectively (starting from an initial 394 mg/Kg). This indicates that decomposition was efficient in this treatment. In addition, the PM blend with C/N 26,  $\text{NH}_4^+$  increased from 89 to 110 mg/L on pre-treatment.

*Algae production results Alga biomass production with and without digestate supplementation:* When the algae was cultivated without digestate supplementation (Fig. 4A), results showed that at 120 h for DCW, glucose, and lipid were 5.88 g/L, 1.7 g/L and 1.9 g/L, respectively. In comparison, in single-stage supplementation (batch cultivation), we added 20 mL/L supplement from each of the digestates (C/N 26, 30, 31, 34 & 37) and measured the DCW and lipid concentration as compared with the control (Fig. 4). In general, results showed that single-stage supplementation increased alga yield. The following were noticeable; supplementation with C/N 26 and 30 digestates (Fig. 4B-C) resulted improved in DCW yields of 7.72 and 7.8 g/L at 120 h, respectively. The supplementation with C/N 31, 34 and 37 digestates (Fig. 4D-F) also resulted in an increase in DCW yields: 7.12, 6.7 and 6.5 g/L, respectively. However, these yields, although better than with no supplementation, were lower than C/N 26 and 30 digestates supplementation.

Glucose supplementation was performed using 20 mL/L addition from each of the digestates (C/N 26, 30, 31, 34 and 37) in glucose dosage of 10, 15 and 20 g/L as shown in Fig. 5G-I. Residual glucose analysis showed glucose depletion reflecting nutritional value and most possibly the biodigestion efficiency among digestate from C/N 26, 30 and 31. The differences in the level of residual glucose at different glucose dosage showed that C/N 26-30 was richer agreeing with biomethane yields discussed.

*Algae Production with two-stage supplementation:* Based on the results from 'without' supplementation and 'with' supplementation, we investigated methods for feeding PM digestates to the alga in a two-stage manner, using the C/N 26 and C/N 30 digestates only. In addition, with single digestate supplementation as shown in Fig. 6X comparing to two-stage feeding strategies (Fig. 6Y-Z), the yield of DCW and glucose residual were 7.5 g/L and, 0.6 g/L at 120 h respectively, while lipid rose to 4 g/L after 174 h after. In one method, after adding C/N 26 digestate in varying doses during both phases of alga growth: the initial, or "exponential" stage (0-120 h), and the "stationary" phase (120-180 h). After 120 h, this method

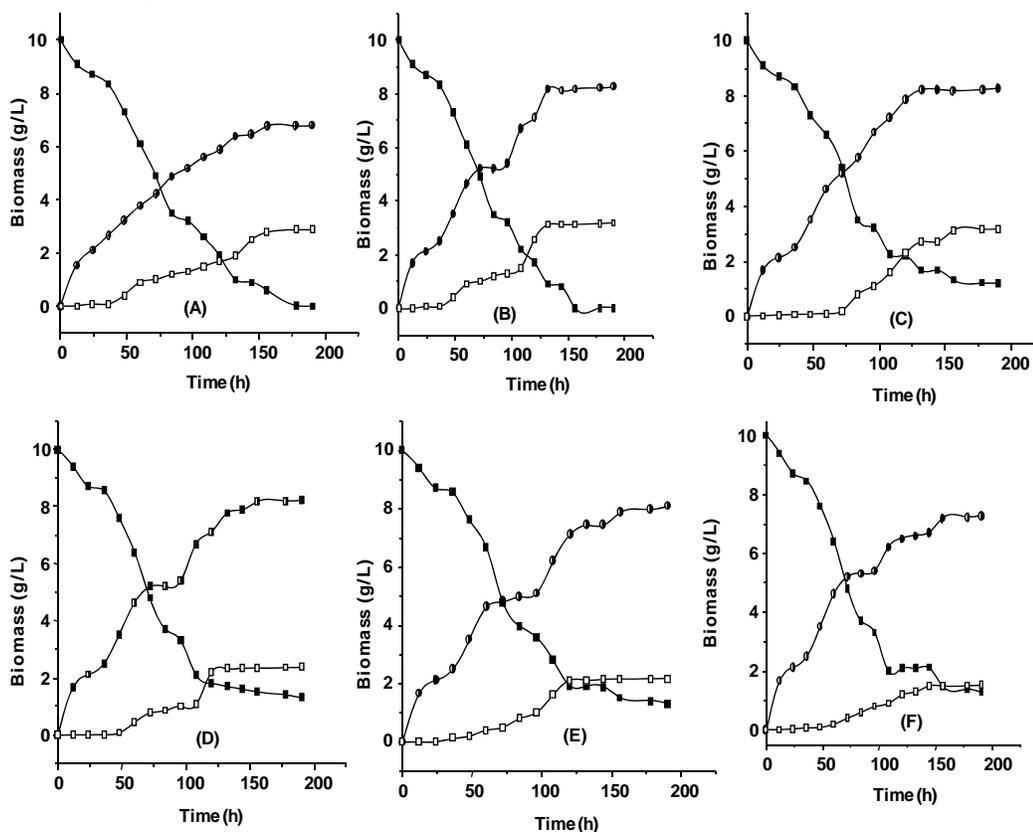
of two-stage feeding yielded 9 g/L (Fig. 6Y) lipid yield of 3.77 g/L at 180 h, compared to 8.22 g/L with single-stage feeding (Fig. 4b) with lower lipid yield. Thus, when a PM blend of C/N 26 is used for supplemental feed, two-stage feeding is superior to single-stage feeding. In the second method, we also tested a more complex method of two-stage supplemental feeding. Based on the fact that in algae cultivation, it's generally understood that high nitrogen levels improve cell growth and low nitrogen levels promote lipid yield (Meng *et al.*, 2009; Widjaja *et al.*, 2009) Therefore, we added a high nitrogen PM digestate (C/N 30) to encourage rapid cell concentration during the initial-exponential stages (0-120 h) and a low nitrogen PM digestate (C/N 26) to maintain cellular activities in the stationary stage by quantities prescribed in the methods. We then measured the effects on biomass and lipid yield (Fig. 6Z). Results showed DCW of 8.6 g/L at 120 h, and lipid yield 3 g/L after 165 h agreed that two-stage feeding was feasible, although smaller differences (comparing Fig. 6X-Z). Clearly, in respective to lipid yield, the first method of feeding (Fig. 6Y) and the second method (Fig. 6Z) indicated that lipid yield in first method reached 3 g/L after 180 h while the second method at 165 h. To sum, the results agreed that two-stage feeding of efficiently biodigested digestates as demonstrated in this study could enhance lipid content and thus directly improve

algal biodiesel production. Moreover, we also noticed rapid depletion of residual glucose after stationary phase (during which 3 mL/L/d digestate was fed), this was assumed to utilize in the biosynthesis of lipids (Livne & Sukenik, 1992) by using it to produce ATP increasing lipid content and yield during station phase (120-180 h). Therefore, the addition of the low PM in the stationary phase not only enhanced cell concentration but improved lipid yield. There are noticeable differences between algae cultivation in the control group, the single-stage PM digestate feeding group, and two-stage PM digestate feeding group (Fig. 4A, Fig. 6X-Z). Algal biomass enhancement and production were generally increased with supplemental feeding of PM blend digestates, more specifically, the increasing lipid yield after glucose and nutrients are being used up noticeable during stationary phase. The highest increases in biomass were occurring with two-stage supplementation which this study was optimistic that two-way digestate feeding will improve algal cultivation.

### Conclusion

The biowastes discussed in this study are feasible for integrated production of biomethane, biofertilizer and algal biodiesel via microalga *Chlorella vulgaris*. As expected, C/N 26-30 positively emitted high yield biomethane from the substrates enlisted and in turn

Fig. 4. Applying varied different digestate in single digestate addition cultivation. DCW ( $\square$ ), residual glucose ( $\blacksquare$ ) and lipid ( $\square$ ): (A) Bbm-without digestate supplementation, and varying digestates; (B) C/N 26, (C) C/N 30, (D) C/N 31, (E) C/N 34 and (F) C/N 37.



produced a nutrient rich digestate feasible for use as biofertilizer and for optimizing biodiesel production from microalga *Chlorella vulgaris*. This biomethane yield, plus the measured enhancement of biofuel algae production (via increased cell concentration and high lipid yield) positively proved the concept that bio-processing of the wastes discussed can supply energy within a sustainable system while promoting a benign environment. Many small-scale farms, individuals and groups seek to become more independent of fuel crisis, sustainable in resources management and intelligent use of waste and scarce resources can develop sustainable bioenergy processing in the manner studied in this work.

Fig.5 Glucose supplementation in batch cultivation (■, 10 g/L, ▣, 15 g/L, ■, 20 g/L) with varied digestates (G) CN 26, (H) CN 30 and (I) CN 31 using uniform quantity (40 mL/L) from each in supplemental feeding (single addition).

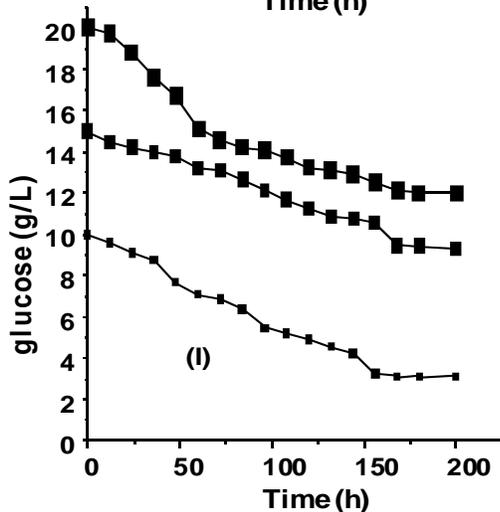
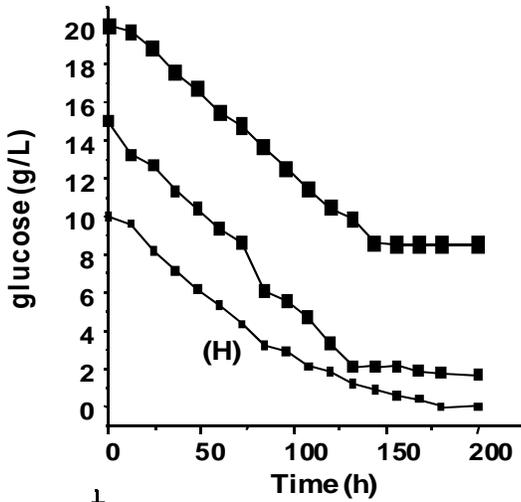
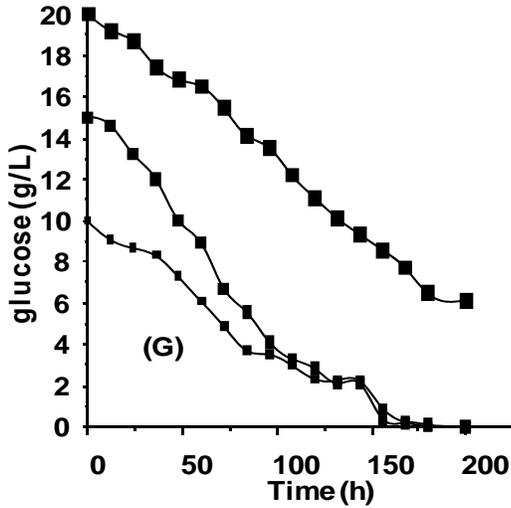
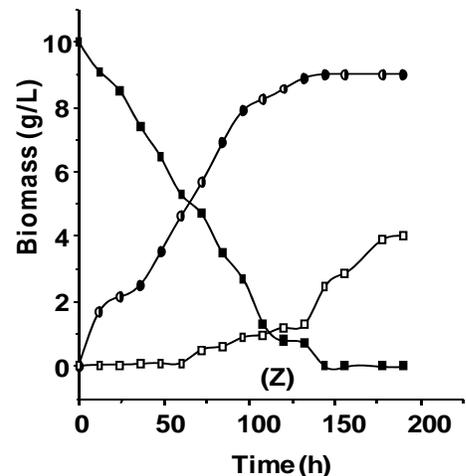
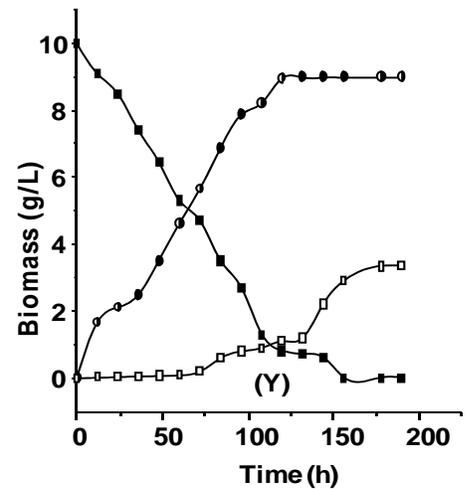
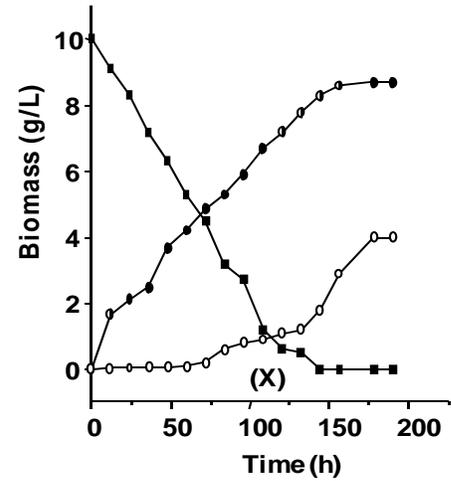


Fig. 6. Single (X) 20 ml/L digestate supplementation from C/N 26 and two stage feeding (Y-Z) Two-stage feeding. (Y) Addition of high (C/N 30) and low (C/N 26) nitrogen digestates at 0-120 h (initial-exponential) and 120-180 h (stationary phase), respectively. (Z) Addition of only C/N 26 digestate at 12 hourly (0-120 h) by 12 ml/L and 3 ml/L at 0-120 and 120-180, respectively.



### Acknowledgments

The study acknowledged the support in grant from the key program of National natural science foundation of China (No. 20836003).

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