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Sustainable Bioenergy Bioprocessing: Biomethane Production, Digestate as Biofertilizer and as Supplemental Feed in Algae Cultivation to Promote Algae Biofuel Commercialization

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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 to get semi-solid and aqueous, the former as biofertilizer and latter was 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 to range between C/N 26 to 30. In comparison, C/N 31 to 37 achieved lower biomethane yields indicating. 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. We filtered the digestate and used in algal supplemental feed and also found that glucose depletion was linearly depleted (as sufficiently used in cell growth) 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. Based on this study, it is conceivable that a circular system using the biowastes discussed or those of the similar nature can develop and constitute a self-supporting sustainable system from waste treatment, biogas to algal biofuel opportunities. The simple approach taken in algal cultivation under the condition studied further showed that microalgae biofuel can be easily promoted and commercialized as a revenue generating back-yard entity for housewhole. The way-forward for microalgae biofuel is to attract and make more population as a fun art.

Keywords: Poultry manure; Biomethane; Biogas; Biofertilizer; *Chlorella vulgaris*; Alga biodiese

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 (carbon/nitrogen) 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 and 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_3 and make available the components for efficient digestion, thus enhancing biomethane production (Vedrenne et al., 2008).

In our recent study (Iyovo et al., 2010), we successfully demonstrated that poultry many waste was an important nutrient source for algal biomass enhancement when we aerobically digested PM and feed 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

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considerable volume of methane (Cummings 1995; Fox and Noike 2004; Abouelenien et al., 2009). Blends of paper pulp waste and algae sludge are also reported to emit considerable biomethane (Yen and 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 Magid 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 (Martínez-Blanco, 2009) and human exreta (Heinonen-Tanski and 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 $CO_{0.48}H_{1.83}N_{0.11}P_{0.01}$ (Grobbelaar, 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 *Limpomyces 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 and 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. vulagris* was cultured in 8 g/L glucose according to study by Feng Chen (Chen and Johns, 1991) using Bold's basal media (Bbm) (A.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 (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 minutes 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 (grams per litre): $NaNO_3$ 0.25, $CaCl_2.2H_2O$ 0.025, $MgSO_4.7H_2O$ 0.075, $K_2HPO_4.3H_2O$ 0.075, KH_2PO_4 0.175, and NaCl 0.025.

Glycine and yeast extract were reduced to 0.1 g/L each to initiate and promote cell growth. The medium was supplemented with glucose at 10g/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 μmolphoton $m^{-2}s^{-1}$. 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.

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

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/day followed by 10 ml/L/day from 120-180 h.

Analytical procedures

Biomethane measurement

Maximum hydraulic retention time (HRT) of 14 days 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 and Tezel et al (Erguder et al., 2001). The volume of biomethane was determined from the total biogas generated daily (ml/L/day), 24 hourly 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 were 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/TNb analyzer-LiquiTOC, Elemtar Analysensystem GmbH, and data were obtained from the computer monitor in terms of quantity per liter. NH3-N, NH4-N and uric acid-N were analyzed according to the standard method (MAFF 1986) Potassium (K) and phosphorus (P) concentrations in the aqueous digestates were analyzed by microwave digestion. A sample of 2.0 ml was added to a 3.0ml HNO3 and cold microwave digested for 1.0 h. The solution was transferred to a 50 ml volumetric flask and distilled water 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 4.0 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 and Tan, 2006)

Dry cell weight (DCW) was obtained based on methods by Feng and Johns (Chen and Johns,1991). Harvested microalga cells were then pulverized and total lipid extracted by soxhlet extraction method using n-hexane.

Results and Discussion

Figure 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

The Figure 1 depicts 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 and William, 1986; Callaghan et al., 2002; Mshandete et al., 2004; Yen and 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/day (74.6%) after 140 h as shown in Figure 2a, C/N 30 also showed reasonable biomethane in content reaching 1010 ml/L/day (73.4%) than C/N 37 (Figure 2f) whose biomethane yield was 584.7 ml/L/day (60.1%). Moreover, increased C/N above 30 demonstrated higher biogas but reduced biomethane content, however was only improved by applying dilution and pretreatment (Figure 3) as discussed in the following.

Dilution and Pre-Treatment

Figure 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 (Figure 3.1), when both dilutions (1:1 and 1.5:1) were subjected to pretreatments, biomethane content improved as shown in (Figure 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/day, 72%) on average (Figure 3.1 compared to Figure 3.2). In comparison, when using low dilution (1:1.5), biomethane content in production improved from 1030 ml/L/day (70%) to 1045 ml/L/day (74%) when pretreatment was added (Figure 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/day (60.1 %) as in Figure 2 f to 1045 ml/L/day (74%) as discussed of (Figure 3.3). This intriguing 14% difference in production

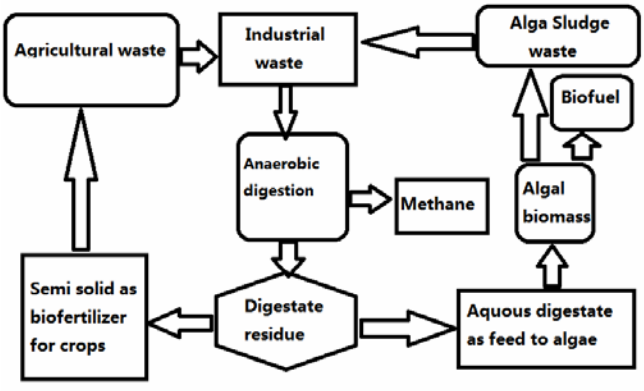

Figure 1: Schematic relation of poultry manure, paper waste and algae sludge for biomethane, biofertilizer and biodiesel via microalga *Chlorella vulgaris* cells.

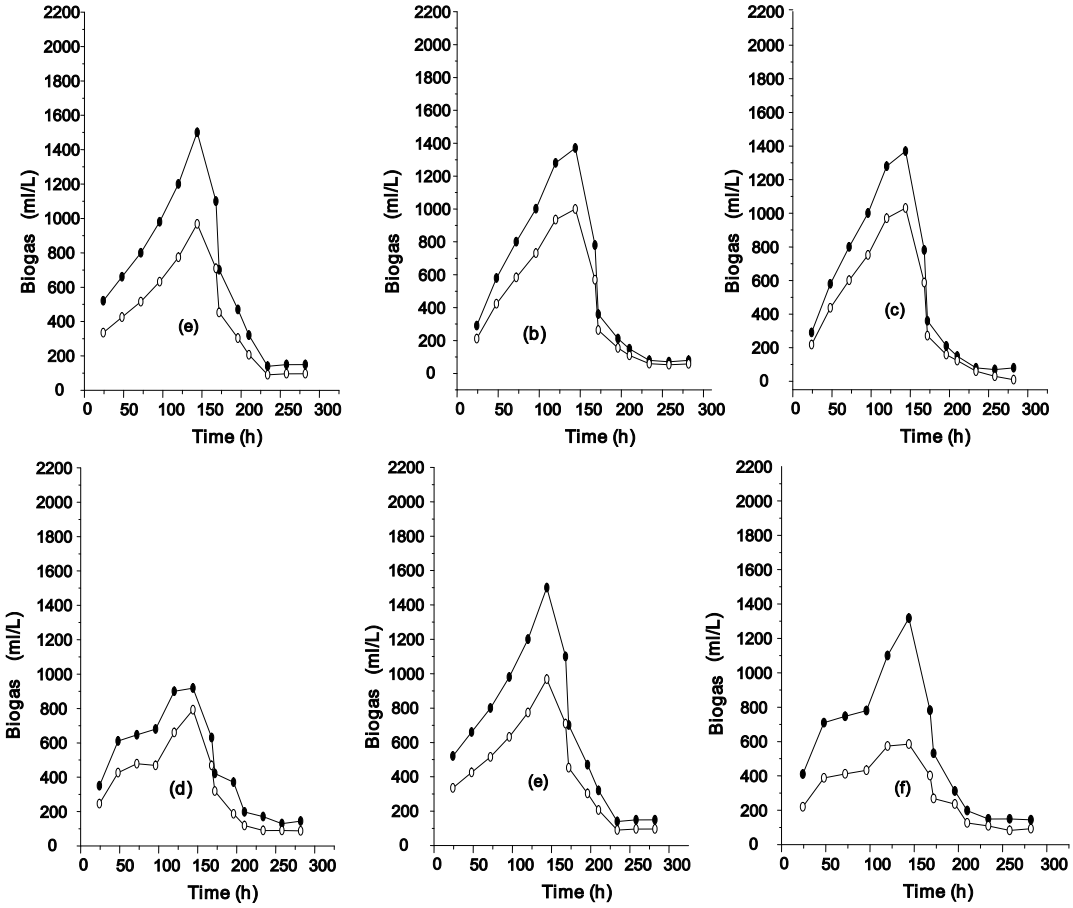

Figure 2: Biogas emission and their respective biomethane content in various substrate blending dilution rate at 1:1 and without pretreatment. Biogas (•) and biomethane (○) yield according to C/N 26 (a), 30 (b), 31 (c), 33(d), 34 (e) and 37 (f).

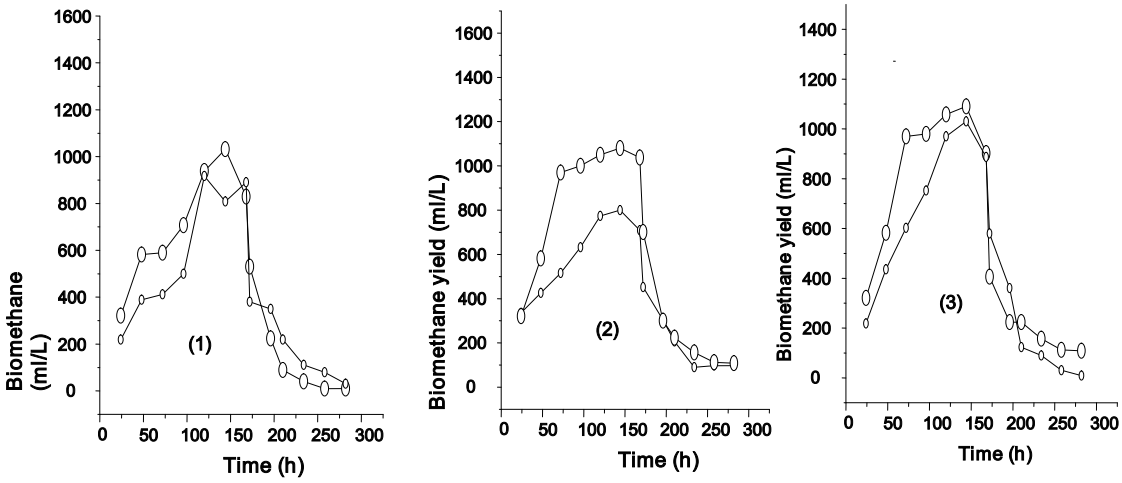

Figure 3: C/N 26 digestion biomethane comparison in dilution and pretreatment. (1) High [◡] and low dilution [○], (2) High dilution [○] and high dilution with pretreatment [◯], (3) Low dilution [○] and low dilution with pretreatment [◯].

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% (Figure 2f) to 74% (Figure 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 dig estate's suitability and value as biofertilizer. Analysis indicates that the PM blends in this

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study with C/N ratios of 26-30 are the most suitable for biofertilizer.

In addition, we 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 and 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, 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 (Figure 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 and 37) and measured the DCW and lipid concentration as compared with the control (Figure 4). In general, results showed that single-stage supplementation increased alga yield. The following were noticeable; supplementation with C/N 26 and 30 digestates (Figure 4 B-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 (Figure 4 D-F) also resulted in an increase in DCW yields: 7.12, 6.7 and 6.5 g/L, respectively. However, these yields, although

Elements/compounds	Blendings (Carbon/nitrogen)						
	With pre-treatment [P](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_4-N (mg/L)	110	89	66	55	56	35	38
NH_3-N	97	102	118	122	231	141	136
Uric acid-N (mg/L)[ad]	18	36	34	39	44	39	47
Uric acid-N (mg/Kg)[I] 379							

[R]ratio, the designated numbers represent amount of paper, algae and PM blended by mass, respectively. [P]pretreatment, [I]initial and [ad]after digestion

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

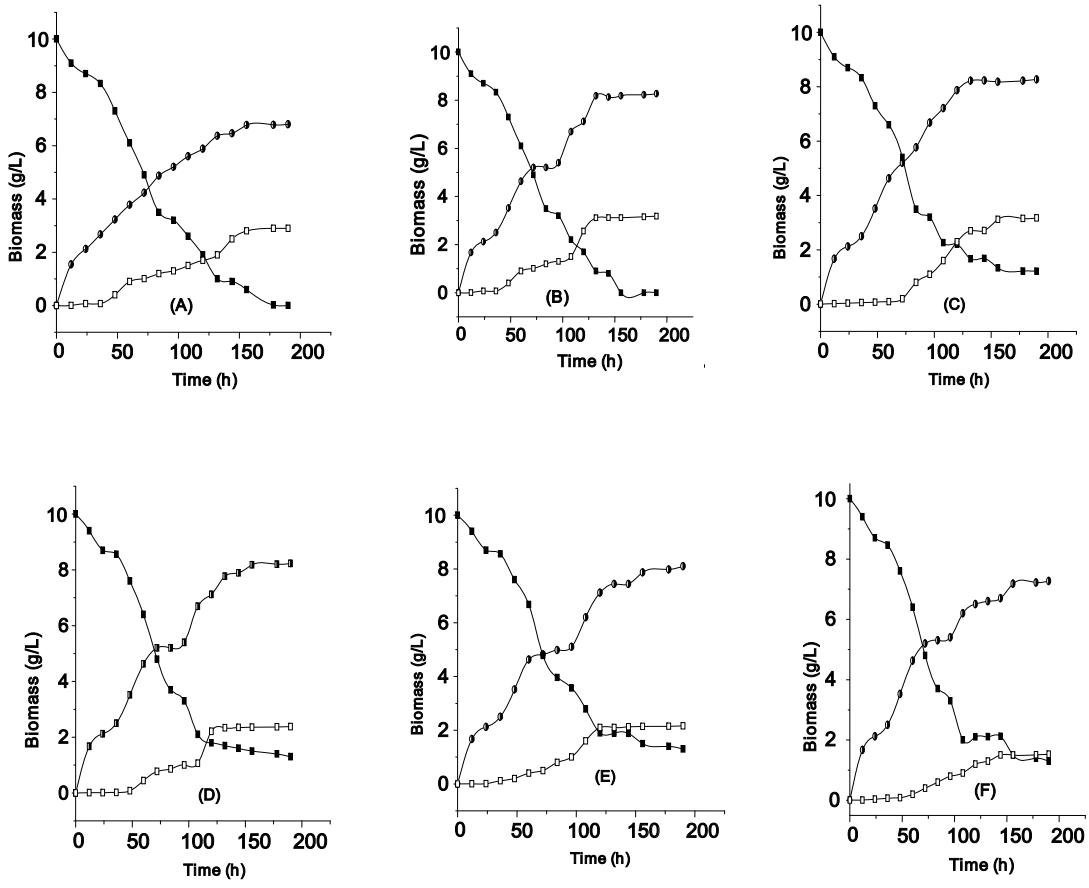


Figure 4: Applying varied different digestate in a single digestate addition cultivation. DCW (◑), residual glucose (■) and lipid (□): (A) Bbm-without digestate supplementation, and varying digestates; (B) C/N 26, (C) C/N 30, (D) C/N 31, (E) C/N 34 and (F) C/N 37.

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 Figure 5 G-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.

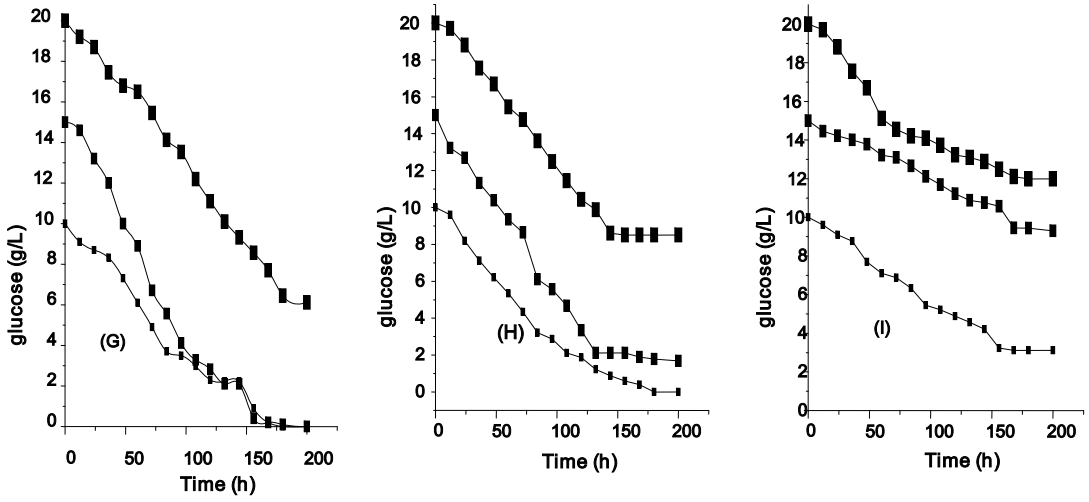
Algae Production with Two-Stage Supplementation: Based on the results from 'without' supplementation and 'with' supplementation, we investigated methods for feeding PM digestates to the alga in a two-stage manner, using the C/N 26 and C/N 30 digestates only. In addition, with single digestate supplementation as shown in Figure 6 X comparing to two-stage feeding strategies (Figure 6 Y-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 (120-180h). After 120 h, this method of two-stage feeding yielded 9 g/L (Figure 6Y) lipid yield of 3.77 g/L at 180 h, compared to 8.22 g/L with single-stage feeding (Figure 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 (Figure 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 Figure 6 X-Z). Clearly, in respective to lipid yield, the first method of feeding (Figure 6Y) and the second method



Figurer 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).

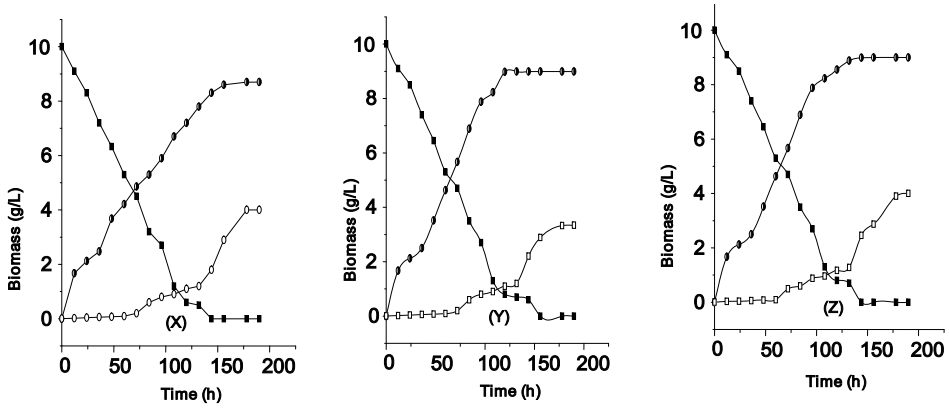


Figure 6: Single (X) 20 ml/L digestate supplementation from C/N 26 and two stage feeding (Y-Z) Two-stage feeding. (Y) Addition of high (C/N 30) and low (C/N 26) nitrogen digestates at 0-120 h (initial-exponential) and 120-180 h (stationary phase), respectively. (Z) Addition of only C/N 26 digestate at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

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(Figure 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 and 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 (Figure 4 A, Figure 6 X-Z). Algal biomass enhancement and production was 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 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 seeking 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.

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