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Optimum Photobioreactor Design via Optimizing Cultivation Conditions of *Microcystis aeruginosa.*

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ABSTRACT

Biodiesel production is considered one of the global issues. In recent years, cyanobacterial biodiesel have attracted much attention and investments. The cultivation technique using photobioreactor is mandatory for optimizing the biomass production and oil content also for producing specific products with high purity. In this research, the variance in light sources affects algal growth is recorded through fluorescent, white LED, red LED and blue LED illumination. The result oil of this diversity is analyzed using gas chromatography GC-Mass, while the protein and carbohydrates of the biomass are analyzed in order to enrich the added value. The results revealed that illumination using white LED optimizes the biomass, oil, protein and carbohydrates contents. The carbon chains have the most suitable configuration for biodiesel specifications. Finally, photo-bioreactor unit is designed in capacity of 6.4 L, composite of 8 bubble column each of volume 1L with cavity 20%. This unit produces 31.36 g dry algae per month. It can be repeated several times according to the amount of desired product.

Keywords: Photobioreactor; *Microcystis aeruginosa;* LED; biodiesel; GC-mass; biomass

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INTRODUCTION

Fossil fuels, the major form of energy, are indeed unsustainable because of depleting supplies and carbon dioxide emissions. Egypt Climate Change Report (2012) arranged by Egyptian Environmental Affair Agency stated that Egyptian dependence on fossil fuel is 92% of the energy sector [1]. Nevertheless the energy sector is considered the main source of Greenhouse Gas GHG emissions, the emissions in 2010 is recorded in the range of 275 metric tonne CO_2 equivalent sharing 0.6% of world emissions referred to Industrial Modernization Programme [2].

Renewable fuels are mandatory for environmental and economic sustainability. Cyanobacterial biodiesel seems to be an adorable alternative source of energy [3]. Successful biotechnology based on algae mainly depends on the proper selection of algae with applicable properties for specific culture conditions and products [4]. The fast growing beneficial strains optimized for domestic climatic conditions is necessary to the success of any algal mass culture, particularly for biodiesel. Since fast growth encourages high biomass productivity and decrease cost. High growth rate also decreases contamination risk compared to slower growers in planktonic continuous culture systems [5]. Nevertheless choosing species suited to the biorefinery approach by producing; valuable co-products contribute to both economic success and environmental sustainability [6].

Algal cells consist of lipids, proteins, carbohydrates, vitamins and pigments. Lipids are commonly classified as polar and non-polar lipids. Most polar lipids exist in cell membrane as phospholipids and glycolipids. The non-polar lipids found mainly in the interior part of the cell. These non-polar lipids include triglycerides (TAG) and free fatty acids (FFA) [7]. The major quantities of carbon chains in non-polar lipid have carbon numbers ranging from C_{12} to C_{22}. Shorter chain lengths exist in small quantities. While chains of carbon number less than C_6 are considered free fatty acids. The moderate chain lengths C_{12} to C_{22} are suitable for biodiesel production. These carbon chains may be either saturated or unsaturated [8].

Lipid composition of algae can diverge quantitatively and/or qualitatively by altering culture conditions. Biodiesel production and fatty acid profile of the lipid are so relevant, since biodiesel properties depends upon lipid composition [9]. Irradiance is considered the major stimuli causes changes in fatty acid profile that affect directly the biodiesel properties.

Generally, saturated fatty acids award biodiesel high oxidative stability and higher cetane number. The imperfection of saturated fatty acids is the poor low-temperature properties of them. Biodiesel produced from feed stocks that contain high concentrations of poly unsaturated fatty acids (PUFAs) have good cold-flow properties. However, these fatty acids are vulnerable to oxidation, and tend to be unstable for long time period of storage [10].

The aim of this research is to study the effect of light energy emitted from fluorescent and LEDs on the cultivation process to obtain maximum oil suitable for biodiesel production on bench scale. The optimized data facilitates design a full scale photo-bioreactor.

MATERIALS AND METHOD

Subculture (inoculum preparation)

Cyanobacteria are considered a promising oil source of biodiesel. Moreover that the extraction process itself is easier than other categories according to cells structure. The selected blue green algal strain is *Microcystis aeruginosa.* The inoculum is prepared at controlled conditions; temperature 20±2°C, irradiance 1500 lx (20.3 µmol m^{-2} s^{-1}) of white cool fluorescent, light duration 24 hr for 7 days and inoculum concentration about 25 µg chlorophyll/l (2 mg biomass/l). The initial minerals of input medium (modified BG11) contain 1094.3 mg NO_3^-/l and 21.8 mg PO_4^{-3}/l. This controlled culture is the inoculum of each run of all experiments.

System Preparation

Air lift bubble column is prepared as illustrated in Figure 1. The cultivation conditions applied for this set of experiments are: Photon flux density PFD 81 µmol m^{-2} s^{-1} and initial inoculum concentration 67 mg/l. The batch period of each duplicated runs is 15 days. The light used for cultivation is white cool fluorescent lamps or light emitting diodes LED white, red and blue fixed parallel to PBR columns. This system is isolated for more accurate light intensity measuring.

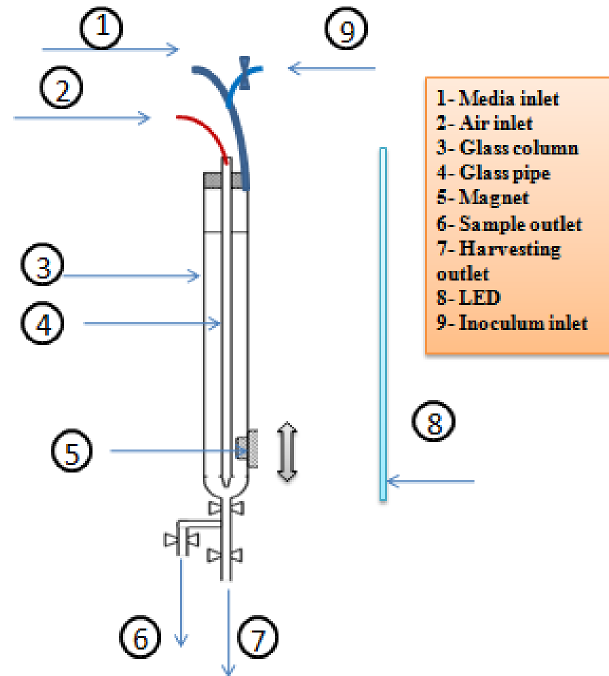

Figure 1: simplified sketch of designed photobioreactor

In this system mixing is applied by air bubbling that is 1.0 vvm (volume of air per volume of culture in one minute). Air is also considered as a secondary source of carbon since it contains traces of carbon dioxide 0.03% of air volume, while the main source of carbon is obtained from the culture medium. Air is delivered to the column's bottom by silicon tubes connected to glass tubes with internal diameter 1 mm. Mixing with air insure proper distribution of nutrients, avoid settling and agglomeration of cells and provide a portion of CO_2 necessary for photosynthesis. Each column was designed with a top gap to allow gas exchange and to prevent oxygen accumulation. This gap occupied 20% of the column's volume. The columns and internal pipes were sterilized using 70% ethanol.

The term µmol m^{-2} s^{-1} is based on photon numbers in a certain wave band incident per unit time on a unit area divided by Avogadro number [11]. Algae and plants can be photosynthetically active between 380-750 nm. The photon flux density (PFD) in µmol m^{-2} s^{-1} of fluorescent lamps is calculated as an average value of several points along the PBR column, while LED ribbon has a number of small lamps arranged along it. The distance between LED ribbon and PBR column and the number of lamps should be calculated to insure that all studied cases are exposed to the same factors.

Photons at different wave lengths have different energies, so the number of photons and the energy per photon at each wave length are essential to calculate total energy affects photosynthesis. Measurement of the photon flux density of light sources with narrow spectra using quantum sensors often causes significant under or over estimation at certain wave length [12].

A photon has a quanta of energy E_p which is defined by Berthhold (2016) [13]:

$$E_p = h \times f = h \times \left(\frac{c}{\lambda}\right)$$

Where: h = Planck's constant = 6.63×10^{-34} Js
f = frequency 1/s
c = Speed of light = 2.998×10^{8} m/s
λ = wave length m

The number of photons N_p can be calculated by

$$N_p = E/ E_p = E \times (\lambda \times 10^{-9}) / (h \times c)$$

Where: E = irradiance W/m^2

The photon flux PFD can be determined by:

$$PFD = N_p / N_A$$

Where: N_A = Avogadro's number = 6.022×10^{23} mol^{-1}

$$PFD = E \times \lambda \times 10^{-9}/ (6.63 \times 10^{-34} \times 2.998 \times 10^{8} \times 6.022 \times 10^{23} \times 10^{-6})$$
$$= E \times \lambda \times 0.836 \times 10^{-2} \ \mu mol\ m^{-2}\ s^{-1}$$

The energy per µ mol photon is different according to the variance of the light sources applied. In this work, the calculated energy per µ mol photon is 0.44, 0.22, 0.259, 0.19 for white cool fluorescent lamp of 36 W and 25 lm, white LED of 0.5 Wand 24 lm, blue LED of 0.5 W and 6.5 lm and red LED of 0.5 W respectively. Thus; number of LEDs is distributed along the light ribbon each in specific distances to ensure equal photon flux density for all studied cases. For 70 cm illuminated length 36 white LED lamps are fixed in the ribbon with distance 1.9 cm in between. While 42 blue LED lamps are fixed with distance 1.6 cm in between. Finally, 31 red LED lamps are fixed along the ribbon in distance 2.2 cm in between.

Culture follow-up and cell analyses

The cultivation process and growth curve were monitored by several measurements which are:

Nitrate concentration in nutrients:

This reaction is based on cadmium reduction using NitraVer5 Nitrate Reagent Powder Pillow that is manufactured by HACH Company U.S.A. The test results were measured at 500 nm

Phosphate concentration in nutrients:

This reaction is based on Ascorbic acid using PhosVer3 Phosphate Reagent powder pillow that is manufactured by HACH Company U.S.A. The test results were measured at 880 nm

pH:

Measuring pH that shouldn't exceed 11using pH meter daily

Microscopic investigation:

It is a daily check to insure healthy culture cells free of contaminates.

Chlorophyll-a content:

Chlorophyll a is extracted by methanol and measured at 630, 647, 664 nm and calculated using the following equation

$$C = (11.85R664 - 1.54R647 - 0.08R630) \times \frac{M}{S} \times 1000$$

Where; C = Chlorophyll concentration (µg/l). R664, R647, and R630 represent the absorbance of the extracted samples at 664, 647, and 630 nm respectively. M= Methanol volume. S= Sample volume

Chlorophyll-a can be used as an algal biomass indicator. It represents 1.5% of the organic matter of algae; therefore algal biomass (mg/l) can be estimated by multiplying the chlorophyll-a (µg/l) content by a factor of 67 $\times 10^{-3}$ [14]. However chlorophyll evaluation as a measure for algal biomass is better than optical density, since the measure of turbidity should not be used beyond early stationary phase due to possibility of bacterial growth [15].

Biomass productivity:

Cellular dry weight (CDW) is measured after overnight drying at 80°C, and the biomass productivity is calculated as [16].

$$CDW = \frac{CDW_L - CDW_E}{t_L - t_E}$$

Where; CDW_E represents the dry weight (g /L) at days of early cultivation phase (from beginning) (t_E), CDW_L represents the dry weight (g/l) at days of end of stationary phase (t_L).
Oil extraction:

Oil was extracted using hexane and iso-propanol in ratio 3:2 according to a method applied by Halim et al. (2011) [17]. A modification is applied to previous method during this work by soaking the mixture for four hours after homogenizing; then the extraction process was applied at 40°C for 2 hours.

Gas chromatography analysis (GC-Mass):

Gas chromatography–mass spectrometry (GC-MS) is used as an analytical method that gathers the aspects of gas chromatography, and mass spectrometry to identify oil content. In this practical work, the extracted oil from microalgae is more viscous than petroleum oil. TAG and free fatty acids (FFA) are not volatile that is conflict with the function of GC-Ms analysis. Therefore; it is preferred to convert TAG and FFA into fatty acid methyl esters (FAMEs) prior to analysis to reduce the molecular weight and increase their volatility [18].

Total protein content:

Total protein content is determined by Khjledal method and then multiplied with a factor 6.25 to give the total protein content by digesting [19]

Total carbohydrate content:

Total carbohydrates content is measured by hydrolysis and measured at 485nm [20].

RESULTS AND DISCUSSION

The selection of the most preferred light system is based on the biomass yield, the oil content and the suitability of oil for biodiesel production. The growth curves of four duplicated runs are plotted in Figure 2. The growth curves were fitted by Boltzmann Model using Origin 85E Software with adjusted R^2 0.96, 0.89, 0.99, 0.98 for white LED, red LED, blue LED and fluorescent respectively.

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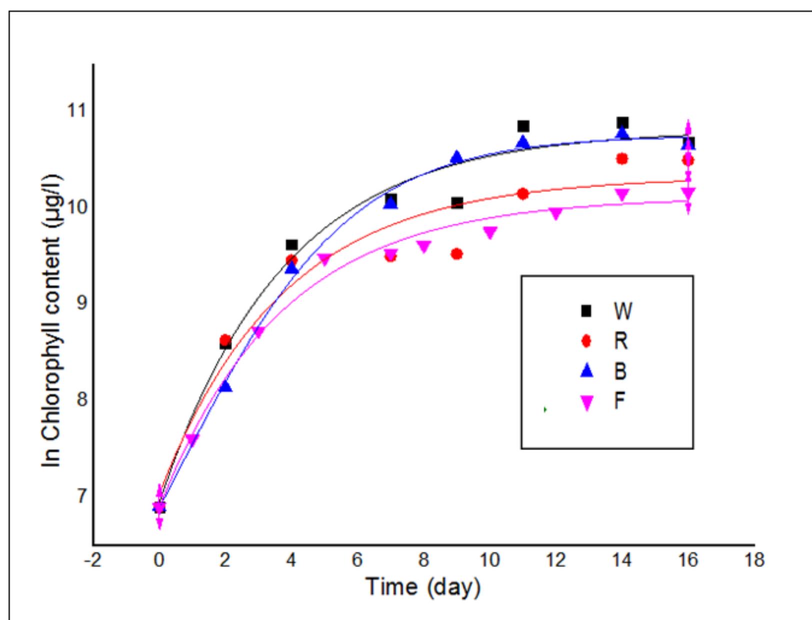

Figure 2: Cultures of *Microcystis aeruginosa*, using white cool fluorescent, red LED, blue LED, and white LED

Carbon chain composition is evaluated using gas chromatography analysis. The percentages of carbon chains are summarized in Table 1.

Table 1: Fatty acid composition (wt.%) of extracted oil

Fatty acid	S*	F*	W*	B*	R*
Lauric C_{12-0}	4.5	4.6	10.2	5.4	10.5
Myristic C_{14-0}	1.8	4.9	4.0	6.5	3.9
Palmitic C_{16-0}	30.4	31.0	33.6	36.0	33.1
Palmitoleic C_{16-1}	7.5	2.1	1.7	9.2	4.9
Stearic C_{18-0}	23.3	20.3	15.0	16.0	16.1
Oleic C_{18-1}	17.2	21.3	23.9	19.5	22.3
Linoleic C_{18-2}	13.5	8.5	9.7	7.4	7.3
Linolenic C_{18-3}	--	1.6	--	--	--
Behenic C_{20-0}	1.8	1.5	1.9	--	1.8
Archidonic C_{20-4}	--	1.1	--	--	--
Eicosapentaenoic C_{20-5}	--	3.1	--	--	--
Saturated fatty acids	61.8	62.3	64.7	63.9	65.4
Mono-unsaturated fatty acids	24.7	23.4	25.6	28.7	27.2
Poly-unsaturated fatty acids	13.5	14.3	9.7	7.4	7.3

*__S__ denotes Stock, **F** denotes culture illuminated with fluorescent, **R** denotes culture illuminated with red LED, **B** denotes culture illuminated with blue LED, and **W** denotes culture illuminated with white LED.

According to the results of GC-analysis summarized in table 1; both stock and culture illuminated with fluorescent have a relatively high weight percentage of poly unsaturated fatty acids (PUFA) which are 13.5 and 14.3 respectively compared to lower values resulted from white, blue and red LED systems that are 9.7, 7.4 and 7.3 respectively. Actually the limitation of unsaturated fatty acids is necessary since the oxidation stability decreases with the increase of PUFA which increases the risk of polymerization of glycerides.

The results revealed that both stock and white cool fluorescent systems produce lower saturated fatty acids percentage (61.8 and 62.3 respectively) than that produced from white, blue and red LED systems (64.7, 63.9 and 65.4 respectively). Generally saturated fatty acids and mono-unsaturated fatty acids are desired for biodiesel, since they increase the oxidation stability and cetane number.

The results of GC-analysis for examined LED systems are convergent. Therefore; they are compared according to dry weight and oil content obtained from duplicated runs occupied 15 days as shown in Table 2.

Table 2: Dry weight and oil content of three cultures

	W	B	R
Dry algal weight (g/l)	2.45	2.04	2.08
Oil content (mg/l)	166	83.2	82.7
% Oil	6.78	4.08	3.98

Table 3: protein and carbohydrate content of the white and blue LED systems

	Dry weight (g/l)	Oil content (mg/l)	Protein (mg/l)	Carbohydrates (mg/l)
W	2.45	166	368	181.3
B	2.04	83.2	306.4	161.2

It was significant that the highest biomass content and highest oil content are obtained from the system illuminated with white LED (Table 2) while the results obtained from both blue and red systems are so convergent. Carbohydrate and protein analyses are approached to enrich the added value of the selected system. Table 3 summarized the results that revealed that the maximum protein and carbohydrate amounts are obtained from the system illuminated with white LED with the specifications of light system previously detailed.

A block flow diagram of the cultivation process requirements is illustrated in Figure 3. One liter of fresh water is mixed with 2g different salts contained in BG11 nutrient in a mixer prior to cultivation to insure perfect solubility. The inoculum is fed to PBR directly to avoid wasting biomass or cell deterioration. The inlet air is 1.0 vvm to supply enough turbulence without cell corruption. Culture grows exponentially; the output is settled in a settling tank for 3-6 hr at ambient temperature. The biomass is dried to obtain 2.45 g out of 1 L culture, while the decanted media solution could be discharged into fish farms as nutrient source.

The designed system is sketched in Figure 4. The culture volume is 800 ml in a column capacity 1 L. The upper gap is designed to allow gas exchange is 20% of whole volume. Air inlet pipe is entered from the top of the column reaching the bottom, the air direction is from bottom to up this allows enough turbulence avoid sedimentation and permit easy sampling through the outlet tap. The column should be exactly parallel to white LED light source in a distance 6.3 cm to gain irradiance 81 $\mu mol.s^{-1}.m^{-2}$. The number of LED lamps is 36 lamp arranged along 70 cm ribbon length. The attached magnet moves up and down direction one time every day to avoid algal sticking on the inner wall. Samples are taken from sampling outlet to monitor the culture during a cultivation period 15 days.

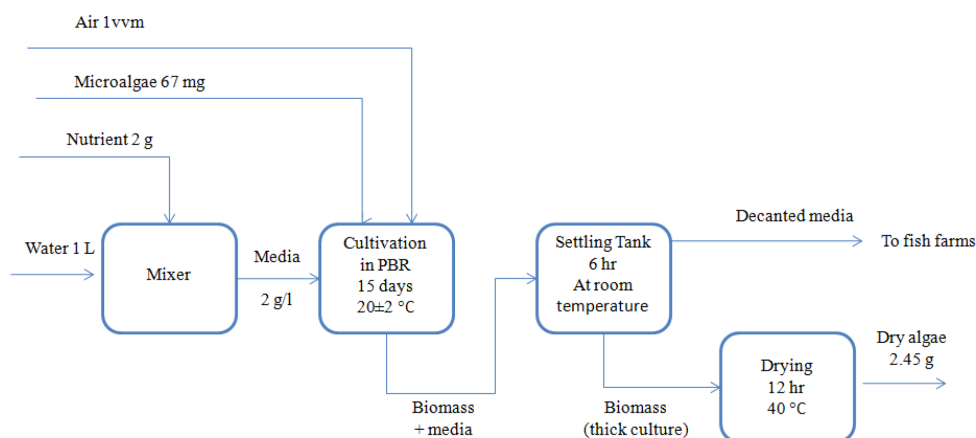

Figure 3: Block flow diagram of cultivation process

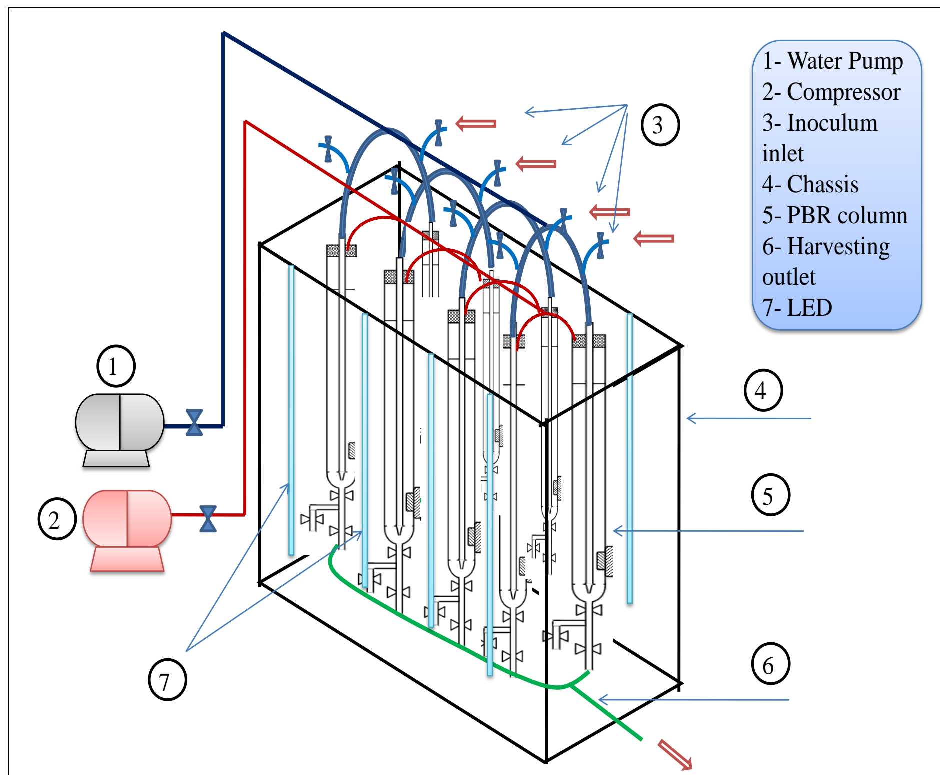

Figure 4: pilot scale Photobioreactor

Number of columns used is 8 columns arranged in one chassis. Microalgae inlet is individual for each column. The harvesting outlet pipes are designed with slope 10° towards the main outlet. This unit produces 15.68 g algae dry cells from a culture volume 6.4 L after 15 days batch cultivation.

CONCLUSIONS AND PROSPECTIVE

The main target of this work is to achieve the optimum specifications of PBR to increase the oil productivity with the most suitable oil chain structure suitable for biodiesel. Four systems with different light sources and same photon flux density were examined. The composition of fatty acid chains directly influences the biodiesel properties. The saturated fatty acids heighten the cetane number and oxidation stability. The poly unsaturated fatty acids decrease the oxidation stability. Thus, the system illuminated by white LED is the proper system, since the oil analysis exhibit few poly unsaturated fatty acid and sufficient amount of saturated fatty acid. Moreover, the highest biomass and oil content are achieved by this system. Protein and carbohydrates analysis implies the highest value among the three systems.

Finally, the optimum criteria of photobioreactor design manifested that the pilot scale can be consist of 8 bubble columns, each of volume 800 ml. For system illuminated 24 h for 15 days with white LED 0.5 W and brightness 24 lm, the LED ribbon should be fixed at a distance 6.3 cm of the column and the inner distance between lamps are 1.9 cm. The number of lamps in the ribbon is 36 lamps are arranged along 70 cm. This unit produces 31.36 g dry algae per month. This unit occupies 1m^2 land area. It can be repeated several times according to the amount of desired product.

The added value of algae is important to increase the economic income of the whole process, to overcome the cost of sterilization and cost of fresh water used. The protein could be used as animal nutrition as the toxicity analysis shows negative results of toxicity. The carbohydrates content enhances the production of bio-alcohol by fermentation or biogas via anaerobic digestion.

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