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Full Length Research Paper

Biomass and lipid production of a fresh water algae *Chlorella* sp. using locally formulated media

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Abstract

***Chlorella* is a single celled photosynthetic green alga which has potential as a source of bioenergy and some value added biochemical. The organism was obtained by blooming in a 10:90 mixture of cow dung extract and pond water from fresh water pond at the African Regional Aquaculture Centre (ARAC) situated at Aluu, Rivers State, Nigeria. Blooming was enhanced by aeration using an aquarium pump and artificial illumination with fluorescent tubes (ca15µE/m²/s each).The isolate was cultured using different animal (Goat, Pig, chicken, Cow dung and Grass cutter) wastes. The growth response was monitored under different conditions (i) artificial illumination (aerated and unaerated) which were mounted in a fluorescent chamber at a height of about 30cm, (ii) natural illumination shaken intermittently, at a temperature of 28±2℃ for 21 days. The results showed that under artificial illumination (aerated) biomass as dry matter (mg/ml) and lipid content (%w/w) were respectively 3.07 and 3.80 goat wastes; 1.49 and 3.38 pig wastes, 1.69 and 6.61 cow dung waste, 1.48 and 9.72 grass cutter waste and 1.68 and 11.20 chicken wastes. Unaerated condition yielded 1.39 and 0.72 goat wastes; 1.26 and 4.18 pig wastes; 1.44 and 6.63 cow dung wastes; 1.31 and 3.40 grass cutter wastes and 1.58 and 7.17 chicken wastes in biomass as dry matter (mg/ml) and lipid content (%w/w). The natural illumination gave biomass as dry matter(mg/ml) and lipid content (%w/w) respectively as follows goat waste 1.46 and 4.85; pig wastes 1.65and 7.77 ; cow dung waste 1.97 and 10.17; grass cutter wastes 1.58 and 13.70 and chicken wastes 2.50 and 18.32. The lipid can be processed into biodiesel. There is therefore a potential of algal biotechnology in the area of renewable, alternative green energy (biofuel/bioenergy) production using inexpensive growth media formulations such as animal wastes which support the growth of *Chlorella* sp. Other value added substances such as biochemical and pharmaceuticals can also be obtained from the biomass.**

Keywords: Animal wastes, biomass, *Chlorella*, growth conditions, lipid production, renewable energy.

INTRODUCTION

Algae have received a lot of attention as new biomass source for the production of renewable energy due to their photosynthetic nature, fast growth rate, biomass and lipid production efficiency (Feng and Zhang, 2011). They can be produced through autotrophic or heterotrophic cultivation under photo heterotrophic or chemo heterotrophic conditions by using solar energy and an artificial light source. Most of their essential nutrient can be supplied by waste water and Co_2 from the atmosphere, leading to high productivity and an associated high lipid content making them a very attractive option. Algae yield per unit area does not require agricultural lands, promoting their photosynthetic nature, utilizing atmospheric Co_2, have the ability to adapt to any hostile condition and maintain productivity (Chisti, 2007; Hu *et al.*, 2008). Under favorable conditions, the growth rate is very high and they are harvested daily or every few minutes due to the fact that most of them divide every 1-2 days or once every 3-4h which serves as a basis for their potential biomass producers (Benemann and Oswald, 1996; Huntley and Redalje, 2007). The cells of most algae grow in aqueous suspension, with an efficient access to water, Co_2 and other nutrients. Constantly these algae have been used as feed for aquaculture applications because of their high content of

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nutritionally essential polyunsaturated fatty acids. This fatty acid made algae to be known as producer of a great variety of lipid hydrocarbons and other complex oils. Microalgae contain lipids and fatty acids as membrane components, storage products, metabolites and sources of energy. The lipid and fatty acid contents of microalgae vary in accordance with culture conditions. Microalgae accumulate lipids as storage material and can be stimulated under environmental stress (Feng and Zhang, 2011). Microalgae strains capable of producing high content of lipid have been screened and their lipid production metabolism has been characterized and reported (Sheehan *et al.,* 1998). Demirbas and Demirbas (2011) estimated that algae could yield oil which is 7-31 times more than that which could be obtained from a high yield terrestrial crop such as palm. Liu *et al.,* (2010) reported that different species of the green alga *Chlorella* can grow photo autotrophically, mixotrophically and heterotrophically with high biomass concentration. Based on the lipid content, *Chlorella vulgaris* and *Chlorella protothecoides* can be chosen for synthesis of biodiesel in photoautotrophic or heterotrophic culture conditions because oil from microalgae bears a low acid value. In this work, *Chlorella* was used to produce algal biomass and lipid using different animal wastes under natural and artificial illumination. The result of cultivating *Chlorella* sp. under varying growth conditions using different animal waste was investigated for algal biomass and lipid production.

MATERIALS AND METHODS

Algal Strain

This green alga was obtained by blooming in a 10:90 mixture of cow dung extract and pond water from fresh water ponds at the African Regional Aquaculture Centre (ARAC). Blooming was enhanced by aeration using an aquarium pump and artificial illumination with fluorescent tubes (ca.15µE/m^2/s each). A pure culture of the organism was obtained by repeated subculturing on nutrient agar using the spread method, added a mixture of chloramphenicol (62.5µg/ml) and nystatin (100µg/ml) to the culture medium to obtain a bacterial and fungal free cultures which were finally maintained on nutrient agar slopes until required. The algal strain *Chlorella* sp. was selected after preliminary screening using macroscopic and morphological characteristics (Anaga and Abu, 1996).

Culture Conditions

Different animal (grass cutter, poultry, pig, goat, cow dung) wastes were obtained from different locations within the Niger Delta Regions of Nigeria. They were processed after sun-drying using a mechanical grinder and their proximate and physico chemical properties were determined. The animal extracts were prepared by suspending 30g of the different animal wastes in a litre of distilled water, sterilized and subsequently filtered using Whatman filter paper. *Chlorella* sp. was inoculated into 500ml Erlenmeyer flasks containing 300ml of the different animal wastes extracts, incubated under artificial illumination (aerated condition using an aquarium pump) and unaerated condition) with two fluorescent light and natural illumination which were manually shaken intermittently every 2h for 12h to avoid cell sticking.

Proximate Composition

Moisture and ash were determined by the air oven method (AOAC, 1990). Crude protein was determined by the micro- Kjeldahl method (AOAC, 1990) and the conversion factor from nitrogen to protein was 6.25. Crude lipids were determined by the soxhlet extraction method of Egan *et al.* (1981). Total carbohydrate content was determined by using the Anthrone method (Osborne and Voogt, 1978). The crude fibre content was calculated by difference.

Physico-Chemical Analysis

Physico-chemical analysis was carried out using the methods recommended by AOAC, 1990. The following parameters were determined: pH, nitrate, phosphate, BOD, COD, conductivity and temperature (AOAC, 1990).

Analyses

Samples were taken daily for analyses. Optical density (OD) of the alga culture at 600nm was measured daily using a spectrophotometer (Spectronic 20, Genesys, Thermos, USA). About 5ml of the growing cultures were harvested by centrifugation at 3000rpm for 10mins, washed (3x) with physiological saline, dried at 50^{o}C in a hot oven to a constant weight to determine the dry matter (Anaga and Abu, 1996). The Neubauer cytometer counting chamber was used to determine the cell number. About 1ml of the culture was diluted in tenfold at least 5 squares were counted and the average value recorded as cells/ml. Wet extraction procedure according to Anaga (1995) was adopted for lipid extraction. Cells were harvested by centrifuging 100ml of the culture at 3000 rpm for 15mins; the supernatant was decanted into a centrifuge tube leaving the wet paste at the bottom. To about 40mg of the wet cells was added 1ml distilled water, 2.5ml methanol and 1.25ml chloroform. The mixture was mixed for 10mins, thereafter centrifuged at 1000 rpm for 5mins and the supernatant transferred into

Table 1. Proximate analysis of different animal wastes

Parameters	Goat waste	Pig waste	Cow dung waste	Grass cutter waste	Chicken waste
Moisture	28.50	7.60	6.60	11.70	8.8
Lipid	3.30	5.90	2.85	6.70	1.20
Ash	32.50	55.30	4.50	7.90	55.90
Protein	8.75	6.56	4.38	8.25	9.63
Carbohydrate	3.91	2.03	2.94	2.91	2.14
Crude Fibre	23.34	22.61	78.73	65.54	22.33

Table 2. Physicochemical properties of animal wastes extracts

Parameters	Goat waste	Pig waste	Cow dung waste	Grass cutter waste	Chicken waste
pH	7.22	6.32	6.28	6.0	6.66
NO_3	5.06	5.06	4.6	2.61	3.83
PO_4	7.04	11.26	16.5	8.24	8.8
BOD	240	320	270	320	160
COD	844	1620	1390	1540	1796
Conductivity	272	593	370	915	827
Temperature	29.6	29.6	29.6	29.6	29.6

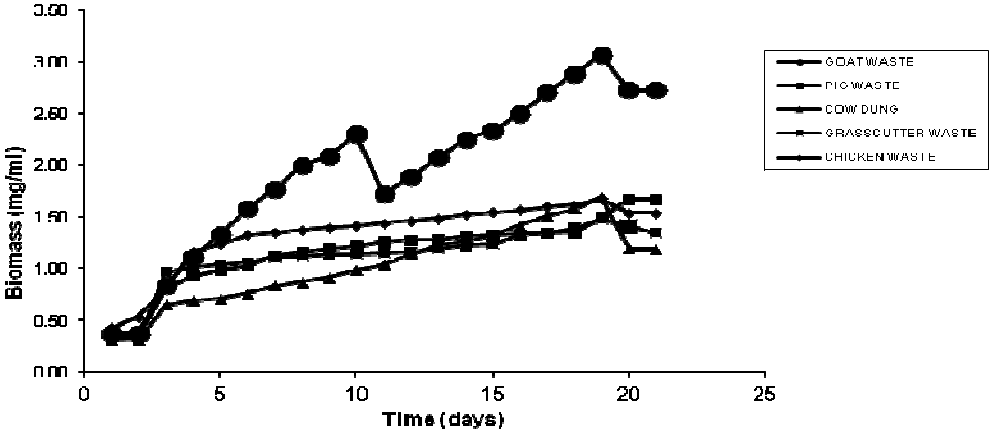

Figure 1: Growth of *Chlorella* sp. in broth media (aerated) measured as dry weight

the centrifuge tube containing the initial supernatant. To the residue at the bottom of the centrifuge tube was added another 2.5ml methanol, 1.25ml chloroform and 1.0ml water, mixed and the extraction procedure repeated. The lower chloroform phase containing the extracted lipids was transferred into a pre-weighed 50ml Erlenmeyer flask, diluted with chloroform to 10ml and brought to dryness in a rotary evaporator (30-35^{o}C) leaving the lipid which was then reweighed using an analytical weighing balance (Setra BL-410S, USA).

RESULTS

Extracts of different animal wastes were inoculated with *Chlorella* sp., monitored under different conditions for biomass and lipid production. The results of the proximate composition of the different animal wastes extracts and their subsequent physico-chemical properties are shown in table 1 and 2. This shows that the extracts contain micronutrients required for the growth of the organism. As shown in Figure 1 the different animal wastes grew up to 19 days with a 2 days lag phase except the chicken waste which did not show any lag phase but grew exponentially. Maximum growth was attained by the 19th day with a decrease by the 20th and 21st day. The growth of *Chlorell*a sp. under the unaerated condition revealed 2 days lag phase for all the animal waste extracts, and grew exponentially up to the 19th day. The chicken waste gave the highest biomass

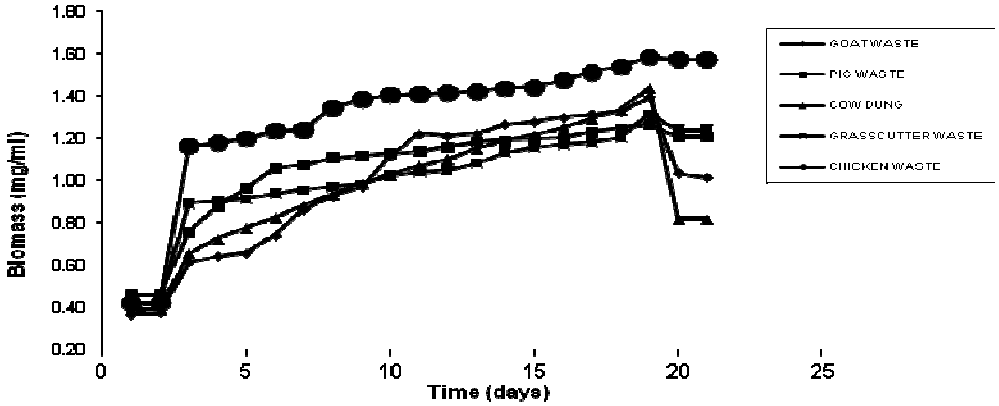

Figure 2: Growth of *Chlorella* sp. in broth media (unaerated) measured as dry weight

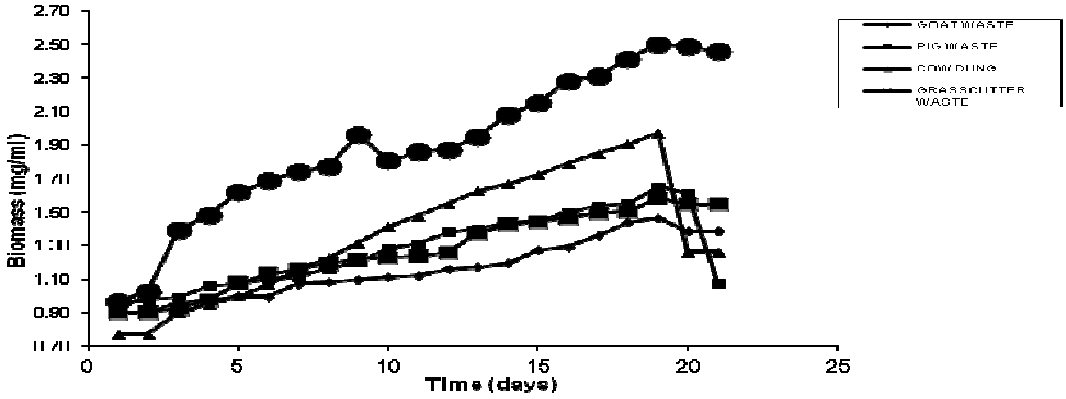

Figure 3: Growth of *Chlorella* sp. in broth media (Sunlight) measured as dry weight

dry matter of about 1.58mg/ml, while goat waste at 1.39mg/ml exhibited the least biomass dry matter. The natural illumination condition growth curve for the goat, pig and the grass cutter waste was slow with a 2 days lag phase, but the cow dung and chicken waste were typified by exponential phase, attained maximum growth by the 19th day. But it was only the cow dung waste that decreased by the 20th and 21st day (Figure 3). The population of *Chlorella* sp. in broth media were measured under the different cultural conditions are represented in Figures 4-6 which clearly shows the number of cells generated within a given period of time. The chicken waste gave the highest lipid content under the natural illumination condition (18.32%), the artificial illumination condition revealed the chicken waste with the highest lipid content of 11.20% (aerated) and 7.17% (unaerated) (Figure 7-9).

DISCUSSION

Large-scale production of biomass energy could contribute to sustainable development on environmentally, socially and economic fronts (Brennan and Owende, 2010). Biomass is one of the better sources of energy which have been focused on as an alternative energy source, since it is a renewable resource and it fixes CO_2 in the atmosphere through photosynthesis. Among biomass, algae (macro and microalgae) usually have a higher photosynthetic efficiency than other biomass (Chisti, 2007). Microalgae reproduce themselves using photosynthesis to convert sun energy into chemical energy, completing an entire growth cycle every few days (Sheehan *et al.,* 1998). Moreover they can grow almost anywhere, requiring sunlight and some simple nutrients, although the growth rates can be accelerated by the

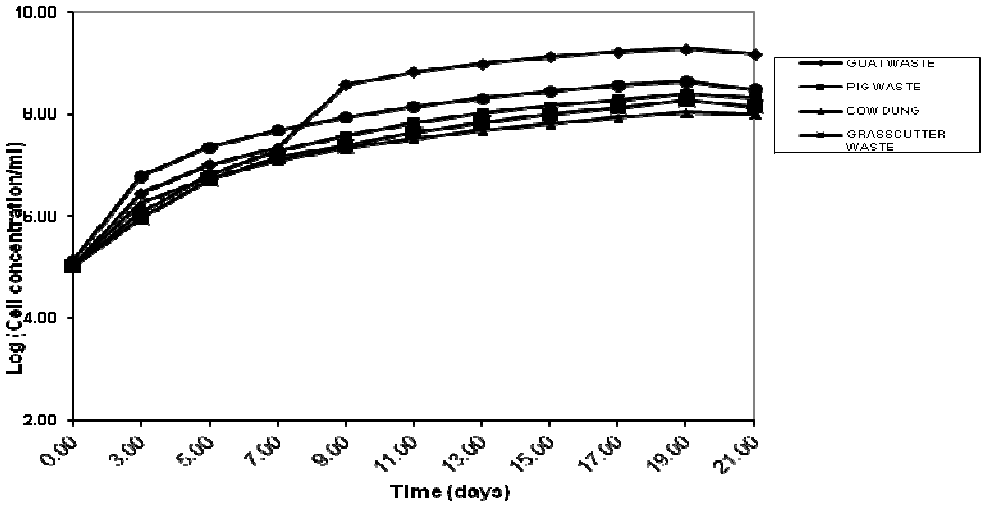

Figure 4: Population of *Chlorella* sp. in broth media (aerated)

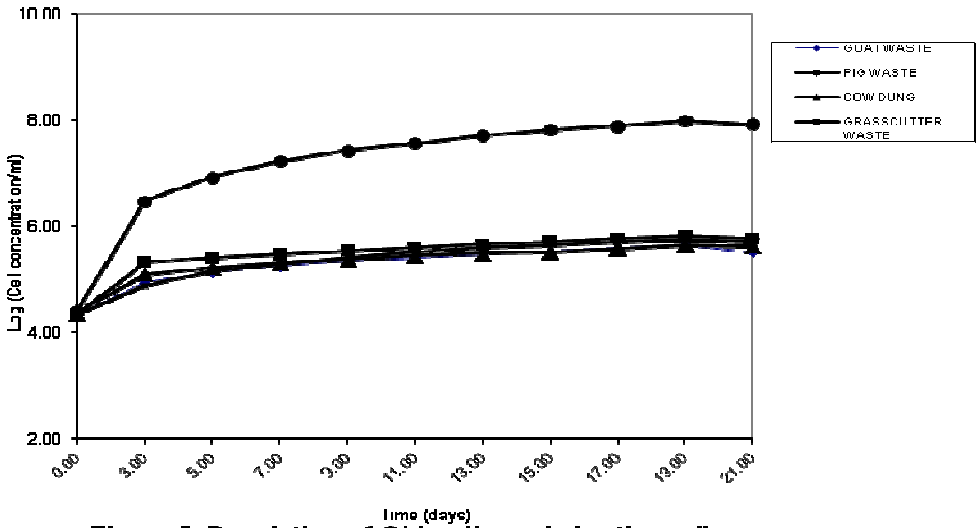

Figure 5: Population of *Chlorella* sp. in broth media (unaerated)

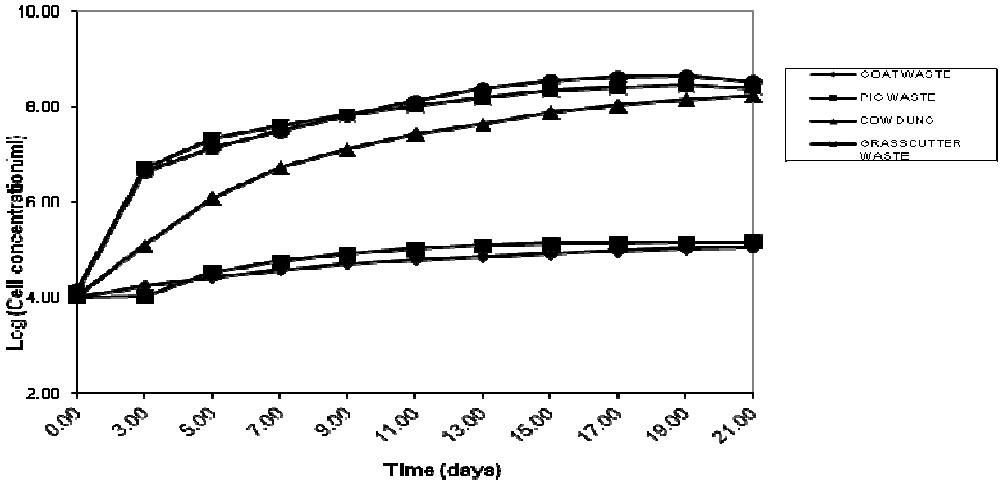

Figure 6: Population of *Chlorella* sp. in broth media (Sunlight)

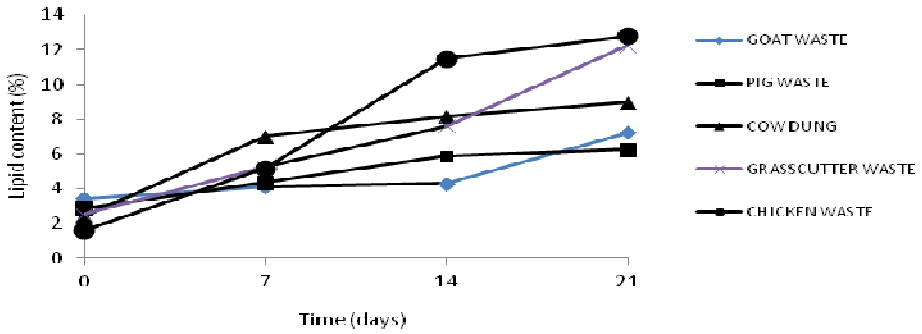

Figure 7: Lipid Production by *Chlorella* sp. in broth media (aerated)

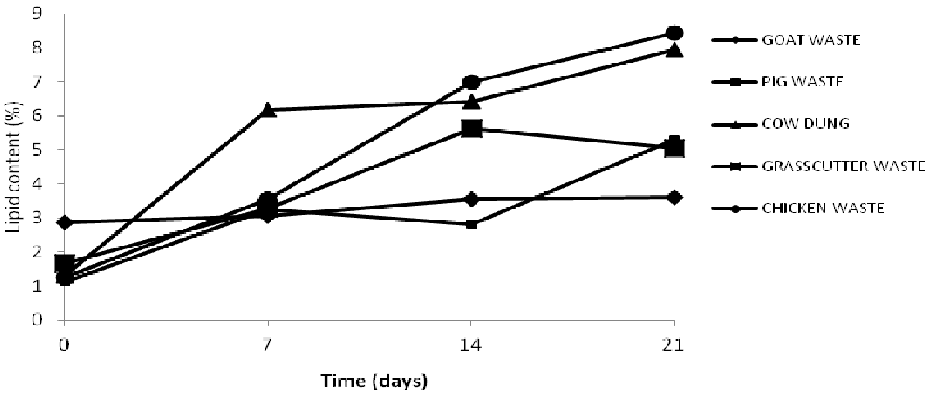

Figure 8: Lipid Production by *Chlorella* sp. in broth media (unaerated)

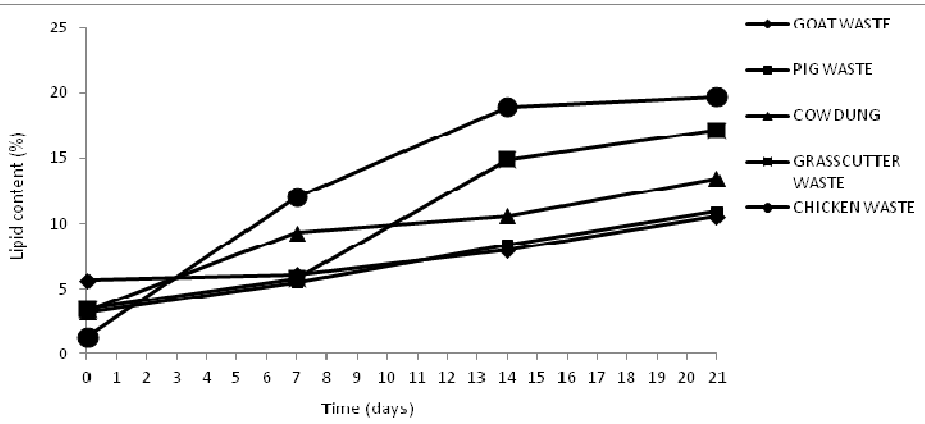

Figure 9: Lipid Production by *Chlorella* sp. in broth media (Sunlight)

addition of specific nutrients and sufficient aeration (Pratoomyot *et al*., 2005; Asian and Kaplan, 2006). The present study has demonstrated the ability of well-balanced medium to achieve high cell densities. The growth of *C. vulgaris* in different animal waste extract media were primarily followed by dry matter, counting algal cells and lipid production. To produce high quality biomass, attention must be paid to culture status and large-scale production of *Chlorella* biomass depends on many factors, the most important of which are nutrient availability, temperature and light. These factors influence the growth of *Chlorella* and the composition of the biomass produced by causing changes in metabolism (Sharma *et al*., 2011).

Biomass production using the different animal wastes revealed the enormous potential of using these animal wastes as raw material (Agwa *et al*., 2012). The findings showed that the growth of *Chlorella* sp. under natural illumination gave the highest biomass of 2.50mg/ml with the chicken waste and goat waste had the lowest biomass of 1.46mg/ml. Singh (2011) reported that most of algal species are obligate phototrophs and thus require light for their growth. Different microalgal species can be adapted to a variety of environmental conditions and are best suited to it, in terms of growth characteristics with high growth rate and productivity as can be seen from the natural illumination using chicken waste (Mata *et al*., 2009). The conversion of light to chemical energy is the ultimate responsibility for driving the production of biomass for a wide variety of products (Elrad and Grossman, 2004). Light is captured by specialized light harvesting complex proteins which binds the bulk of chlorophyll in the green algae and play a role in light capture and dissipation of energy in the process of photosynthetic reactions (Horton and Ruban, 2005). But the artificial illumination (aerated) resulted in goat waste having the highest biomass of 3.07mg/ml, while the grass cutter waste had the lowest biomass of 1.48mg/ml. The unaerated artificial illumination showed the chicken waste with the highest biomass of 1.58mg/ml and pig waste with the lowest of about 1.26mg/ml. The level of aeration strongly contributes to the growth of microalgae. Aeration is necessary to prevent cells from settling, to avoid thermal stratification, to distribute nutrients and break down diffusion gradients at the cell surface, to remove photosynthetically generated oxygen and to ensure that cells experience alternating periods of light. But fluctuations in light intensity affect the specific growth rates and productivities of microalgal cultures as in the scenario of artificial illumination conditions (Richmond, 2004). The use of natural conditions for microalgae production has the advantage of using sunlight as a free natural resource. Under natural growth conditions phototrophic microalgae absorb sunlight, and assimilate carbon dioxide from the air and nutrients from the environment. Artificial illumination employs fluorescent lamps exclusively for the cultivation of phototrophic algae at pilot scale stages and allows for continuous production but at significantly higher energy input. Thus the natural illumination would be preferable on the basis of overall cost compared with the artificial illumination which requires energy input for lighting and availability of raw material (Brennan and Owende, 2010).

The work, demonstrates lipid production profile of the green microalga *Chlorella* sp. which can be significantly used for industrial production using different animal waste extracts. Similar studies have previously been published about related species, *Chlorella protothecoides* (Xu et al., 2006) and *Chlorella vulgaris* (Liang *et al*., 2009). In these studies, *C. vulgaris* showed a lower cell density (1.2 g l^{-1}) and total lipid amount (23%) compared with *Chlorella* sp. which resulted in 18.32% from chicken waste in the present study under natural illumination. In nature, microalgal accumulation of lipids increases under certain conditions, thus improving algae for industrial production and can lead to a natural accumulation of lipids in algae. While aerated gave (11.19%) and unaerated gave (6.17%) from the same waste product under artificial illumination. It can therefore be argued that *Chlorella* sp. could be more appropriate microalgae for lipid production. This is because most of microalgal organisms can use sunlight to produce lipids which can be processed into other valuable products that will provide economic benefits to different communities. Thus animal waste materials are the preferred source of nutrient for the growth of microalgae (Schnek *et al*., 2008).

CONCLUSION

Chlorella sp. in the present study was used to produce algal biomass as high as 2.50mg/ml under natural illumination and lipid content of about 18.32% using locally formulated media. Since sunlight can provide more photon density than the experimental condition. This proves that the cost effectiveness of biomass and lipid production can be achieved using this microalga (*Chlorella)* sp. without much energy input. This technology of using locally modified media involves an inexpensive resource and low cost investment in which industries could develop and the biomass could be processed into various nutraceutical and pharmaceutical products.

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