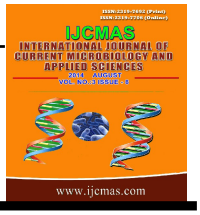
International Journal of Current Microbiology and Applied Sciences
ISSN: 2319-7706 **Volume 3 Number 8 (2014) pp. 1036-1047**
http://www.ijcmas.com



Original Research Article

Utilization of poultry waste for the cultivation of *Chlorella* sp. for biomass and lipid production

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Keywords

Algae biotechnology, biomass, *Chlorella*, lipid, poultry waste, renewable

A B S T R A C T

Chlorella sp. is a potential feedstock for a vast array of products in the food, nutraceutical, medical, pharmaceutical, agricultural and bioenergy industries and renewable material for a variety of applications in biotechnology. The investigation of using poultry waste for the cultivation of this microalga for biomass and lipid production was evaluated. About 927 ml poultry waste extract was obtained from 30g of poultry waste suspended in a liter of distilled water. Extracts from poultry waste on average contains principally PO_4^{2-} 9ppm, NO_3^{2-} 4ppm, NH_4^{+} 24ppm and SO_4^{2-} 6ppm, pH 7.0-8.5 and a conductivity of 830µOHMS/cm were used as nutrients for the cultivation of *Chlorella* sp. The isolate was cultured in 300ml of poultry waste extract, maintained under (i) sunlight (ii) aerated and (iii) unaerated conditions and finally monitored at a temperature of 28 ± 2^0C, pH 7.5 within a retention period of 21days. The best growth and highest biomass of 2.5mg/ml (dry matter) was realized after culturing in the sunlight; 1.68mg/ml in the aerated and 1.58mg/ml in the unaerated conditions. *Chlorella* sp. showed potential for lipid production in the poultry medium with about 18.32%(w/w) lipid in the wet cells in the sunlight, 11.19%(w/w) in the aerated and the unaerated condition gave 7.17%(w/w).The investigation revealed that poultry waste can be used as a suitable renewable medium for the growth of this *Chlorella* sp. There is therefore a potential of algal biotechnology in the area of renewable, alternative green energy (bioresource/bioenergy) production using inexpensive growth media formulations such as poultry wastes which support the growth of *Chlorella* sp.

Introduction

Chlorella sp. is an eukaryotic, green microalgae of the class Chlorophyceae, an aquatic relative of plants, which flourish in aerated or natural liquid cultures where the cells have sufficient access to light, carbon dioxide, and other nutrients. These microalgae primarily grown photoautotrophically; some species are able to survive heterotrophically by degrading organic substances like sugars, which are converted to biomass and subsequently lipid for biodiesel production (Rosenberg *et al*., 2008). There is currently a world-wide need to improve the way we handle wastes from its use as fertilizers in farms which causes eutrophication of water bodies affecting

drinking water quality and aquatic life; accumulation of waste in the environment threatening the health status of the populace when they inhale a lot of gases as the waste decompose because the gases contain harmful chemicals and several diseases out break which has been traced to pathogens from animal waste (Agwa, 2013). This waste must be properly managed to transform them into a multiple value added renewable energy products in a sustainable manner (Cantrell *et al.,* 2008). Taking the waste product of one process and using it as input or fuel for another process in 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 (Mckendry, 2002 a, b; Cantrell *et al*., 2007).

Poultry waste has been a traditional organic alternative source of fertilizer which is economically beneficial to use rather than chemical fertilizer, and this also reduces the environmental pollution of manure caused by the inappropriate disposal from which much needed nutrients can be retrieved and reutilized(). Poultry producers clean their poultry houses to promote the animals' health and limit the build-up of wet manure to avoid disease outbreak(). One of the possible acceptable way to utilize this farm manure is in the production of microalgae, provided the conducive conditions (light intensity, pH, and temperature) to enhance their cultivation are met. The use of poultry waste in algal cultivation maybe novel if it promotes high biomass and lipid content with efficient utilization of the renewable resource (Jacob-Lopes *et al*., 2008; Jacob-Lopes *et al*., 2009; Agwa *et al*., 2012). Algae biomass as one of the suitable feedstock for biodiesel production in recent times is not new, researches and soaring developments which are primarily due to energy crisis, climate change and environmental threats have encouraged this trend from economic assessment to engineering towards green house emission (Iyoyo *et al*., 2010). One of such category is converting waste to microalgae biomass, involving the conversion of industrial, agricultural, animal, municipal or domestic waste from a 'liability' to profit is an attractive venture. The poultry waste needs to be bioconverted in order to release the absorbable nutrients that can be fed to the algae in culture cultivation (Iyoyo *et al*., 2010). A number of researchers have demonstrated the ability of various algae to utilize animal waste as a growth medium (Wilkie and Mulbry, 2002). Several studies had revealed that poultry waste as a potential biological nutrients source and also a mineral-rich waste (Nicholson *et al.,* 1996; Amanullah, 2007). Poultry waste can be used to increase algal biomass and starve the cells by limiting nitrogen supply which would increase cellular lipid. Iyovo *et al*. (2010) have demonstrated the use of poultry waste as an important nutrient source for algal biomass enhancement when aerobically digested poultry manure is fed to the microalgae *Chlorella vulgaris* cultures. The residue rich in nutrients can be used to enhance algal cultivation, biomethane, biofertilizer, biodiesel production, and serve as feed for aquaculture.

The use of this rich waste material is a sustainable, economic utilizable opportunity, that can produce high yield of biomass, which can directly be converted into biodiesel. The investigation revealed the potential of poultry waste as a suitable bioenergy, bioresource, renewable medium for the cultivation of this microalgae *Chlorella* sp. which can subsequently be converted into various products of industrial importance.

Materials and Methods

Microorganisms and Culture Medium

The microalgae *Chlorella* sp. used in the study was obtained by blooming from water samples containing microalgae from the African Regional Aquaculture Centre (ALUU), Aluu Rivers State, Nigeria. A 10:90 mixture of cow dung extract and the fresh pond water containing *Chlorella* were subjected to aeration, using an aquarium pump. The set-ups were illuminated artificially using two white fluorescent lamps emitting ca15µEm^{-2}s^{-1}.The flasks turned leaf green after five days of incubation. A pure culture of the organism was obtained by repeated sub culturing 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 (Agwa *et al.,* 2012). The poultry wastes obtained from the livestock husbandry unit of the African Regional Aquaculture Centre (ARAC), Aluu Rivers State, Nigeria, were sundried properly, ground into fine powder using a mechanical grinder, and kept until required. Poultry waste extract was prepared by suspending 30g of poultry waste in a liter of distilled water, sterilized and filtered using whatman filter paper.

Cultivation

One milliliter of the bloomed culture was aseptically inoculated into flasks containing 300ml of poultry medium, a well defined synthetic medium consisting of 0.132 g/l Potassium nitrate, 0.066 g/l Sodium silicate, 0.066 g/l Monosodium phosphate and 0.066 g/l EDTA. The pH was adjusted to 7.5prior to autoclaving at 121^{0}C for 15mins. The Bangladesh II medium contained 2.0 g/l $NaHCO_3$, 0.05 g/l Urea, 1.0 g/l NaCl,1.50 g/l Gypsum ($CaSO_4.2H_2O$).The pH was adjusted between 7.0 – 7.5 before autoclaving at 121^{0}C for 15mins.These cultures were maintained at 28±2^{o}C in a 500ml conical flask.. The following set ups were prepared (i) Unaerated condition (ii) Aerated condition using an aquarium pump (options (i) and (ii) were illuminated artificially using two fluorescent lamps emitting ca15µEm^{-2}s^{-1} each mounted in a chamber at a height of about 30cm from the bench top) (iii) sunlight, aerated intermittently by manual shaking at 2h interval for 12h. Control flasks were set up within the different cultural conditions using poultry waste extracts. To monitor changes in algal concentration, samples were taken every 24h using aseptic techniques; samples were pooled by measuring the optical density, biomass as dry weight, cell count and lipid content from the wet algal cells

The American and public health methods (APHA, 2000) were used to determine the chemical composition of the pond water and poultry waste extracts. All experiments were by triplicates. The Statistical software of Statistical Package for Social Sciences (SPSS) was used for the statistical analysis. The Post hoc test (Scheffe and Duncan) was used to test for the significant difference at p-values < 0.05 within the groups measured at 95% confidence level.

Analytical Methods

The method of Anaga and Abu (1996) was adopted to perform these analyses

Algal concentration was obtained by measuring optical density (OD) at 600nm with 5ml of the growing culture using the

spectrophotometer (Spectronic 20, Genesys, Thermos, USA).

Biomass as dry matter was determined using about 5ml of the exponential growing culture, harvested by centrifugation at 3000rpm for 10mins. The cells were washed (3x) with 85% NaCl dried at 50^0C in a hot oven to a constant weight.

Cell count as cell number was determined using the Neubauer hematocytometer counting chamber with about 1ml of the culture diluted in tenfold. At least 1000 cells were counted per square and about 5 squares were counted in triplicates and the average value recorded as cells/ml.

Lipid Extraction was evaluated using the wet extraction procedure according to the protocol of Anaga (1995). Cells were harvested at the end of the retention period of 21days, by centrifuging 100ml of the culture at 3000rpm for 15mins; the supernatant was decanted into centrifuge tube leaving the wet paste at the bottom. Forty milligram of the wet cells was obtained and added 1ml of distilled water, 2.5ml methanol (Analar, England) and 1.25 ml chloroform (Analar, England), intermittently shake at room temperature for 10mins.

The mixture was centrifuged at 1000rpm for 5min, decanted into the centrifuge tube containing the initial supernatant and the pellets resuspended in 2.5ml methanol, 1.25ml chloroform, 1.0ml water, mixed, centrifuged at the same time, and the procedure repeated once. The lower chloroform phase was withdrawn into a pre-weighed 50ml Erlenmeyer flask, diluted with chloroform to 10ml and brought to dryness into a rotary evaporator (30-35^{o}C) leaving the lipid which was then reweighed using an analytical weighing balance (Setra BL-410S, USA).

Results and Discussion

The growth of *Chlorella sp*. using poultry waste extracts was investigated under three different conditions for 21days by assessing the types of micronutrients found within the waste material. Table 1 and 2 reveal the proximate and chemical composition of the poultry waste and its extracts which stimulate good blooming for the cultivation of the microalgae; however the control did not show any sign of blooming. These nutrients inherent within the waste extracts were necessary for the growth of the organism (Agwa *et al*., 2012). Most microalgae require waste water rich in minerals containing nitrogen and phosphorus as nutrients (Amanullah, 2007; Iyoyo *et al*., 2010). These findings were in agreement with the report of Grima *et al.* (1999) who showed that growth media for culturing microalgae must be inexpensive by using sea water supplemented with nitrate, phosphate fertilizers and a few other micronutrients for growing marine microalgae. Chisti (2007) reported that growth medium for algal cell cultures must constitute essential micronutrients such as nitrogen and phosphorus. Ungsethaphand *et al.* (2007) has shown that dry chicken manure can supply necessary the nutrient for the culture of *Spirulina. plantesis.*

The growth responses of cultivating *Chlorella* sp. using poultry waste, a synthetic medium and Bangladesh medium at 28 ± 2^OC for 21days are shown in the figures below. Fig 1-3 illustrates the optical density of the different conditions of growth; the poultry waste gave favorable results compared to the synthetic and Bangladesh II media. Because the poultry waste have been previously adapted to the medium there was no lag phase, but the growth response observed with the other media were very slow with a lag phase of about three days

signifying the need for adaptation of these media with the microalga. There was significant difference in OD between treatments ($P<0.05$), these gave higher levels of OD as the time increases with a drop recorded after the 19th day. The biomass as dry weight monitored under artificial illumination (aerated) showed slight lag of about three days with the synthetic medium (Fig 4); while the others (artificial (unaerated) and natural illumination) cultural conditions did not have any lag phase (Figs. 5 and 6). In general, biomass values resulting from poultry waste were higher with the natural illumination (2.50mg/ml), artificial illumination (aerated (1.68mg/ml)) unaerated (1.58mg/ml). In synthetic medium artificial illumination revealed (aerated (2.22mg/ml)) unaerated (1.44mg/ml) and natural illumination (1.08mg/ml). With the Bangladesh medium artificial illumination showed (aerated (0.88mg/ml)) unaerated (0.93mg/ml) and natural illumination (0.71mg/ml). The differences in the treatments were significant ($P<0.05$). Light is a critical factor in the cultivation of microalgae, because the cells are light dependent which effectively utilizes the availability of light photon from the environment to increase its cell density (Brennan and Owende, 2009). But fluctuation in light and the concentration of the media resulted in lower population of cell (Schenk *et al*., 2008). From the result high yield were obtained from the cultivation under sunlight, because of the inherent ability of the organism to grow either photoautotrophically (Chen *et al.*, 2011), heterotrophically (Wen and Chen, 2003) and mixotrophically (Putt *et al*., 2010; Zhang *et al*., 2010). The cells have sufficient access to water, sunlight, carbon (IV) oxide, and other nutrients. Photosynthesis is the first step in the conversion of light to chemical energy and ultimately responsible for supporting all biofuel synthetic processes, converting solar energy into biomass, carbon storage products (carbohydrates and lipids) and hydrogen (Beer *et al.*, 2009). The process of producing microalgal oil consists of producing microalgal biomass that requires light, carbon dioxide, water and inorganic nutrients (nitrates, phosphates, and iron). About half of the dry weight of microalgal biomass is carbon, which is usually derived from carbon dioxide (Chisti, 2007). Using poultry waste, biomass is grown in a sustainable way, resulting in waste recovery, management, utilization and control. The combustion of biodiesel produced using this waste material has no impact on the CO_2 balance in the atmosphere, because the CO_2 emitted by the burning of biomass is offset by the CO_2 fixed by photosynthesis (Demirbas and Demirbas, 2010).

Figs.7- 9 show the lipid content obtained at the end of the growth period. The poultry extracts clearly gave the highest value of lipid 11.2%w/w with the aerated (Fig. 7); unaerated 6.17% (Fig. 8) and (18.32%) with the sunlight under natural illumination in 21 days (Fig. 9). From the result, natural illumination is the most favorable condition for the growth of *Chlorella.* In nature, microalgae accumulation of lipids increases under certain conditions, thus improving algae for industrial production.

Microalgae have long been recognized as potentially good source for biodiesel production because of their high oil content and rapid biomass production. The process of producing microalgal oil consists of producing microalgal biomass that requires light, carbon dioxide, water and inorganic nutrients (nitrates, phosphates, and iron) and this is in agreement with the results obtained from utilizing poultry waste extract under natural illumination (Chisti, 2007).

Microalgae oil is non-toxic, highly bio-degradable, utilizes wastewater for its production, can reduce carbon emission based on the cultivation conditions and feedstock, and provides a reliable and continuous supply throughout the year (Schenk *et al*., 2008).

Poultry waste is an important renewable resource for the production of biodiesel through the microalgae *Chlorella* sp. This microalga has the potential of growing in places away from the farmlands and forests, thus minimizing the damages to the ecosystem and food chain supply, can also be grown in sewage and next to power-plant smokestacks, where they can digest the pollutants and deliver the oil. With simple and inexpensive nutrient regime to culture, faster growth rate as compared to terrestrial energy crops, high biomass productivity, attractive biochemical profile and good energy content, poultry waste exhibits several important attributes for futuristic research on renewable energy.

Table.1 Proximate analysis of poultry waste

Parameters	Poultry waste(%)
Crude Protein	9.63
Crude Fat	1.20
Carbohydrate	2.14
Ash	55.90
Moisture content	8.80
Crude Fibre	22.33

Table.2 Chemical composition of poultry waste extract and pond water

Parameters	Poultry waste extract	Pond water
pH	7.0	6.23
NO_3^- (ppm)	4.0	8.7
NH_4 (ppm)	24.0	184.0
SO_4^{2-}(ppm)	6.0	8.0
PO_4^{3-} (ppm)	9.0	64.8
Calcium hardness (ppm)	5.4	3.45
Total hardness (ppm)	8.0	3.0
BOD (ppm)	160	2.0
COD (ppm)	1796	2.7
Conductivity (µS/cm)	830	110.0
Temperature (^oC)	29.6	30.0

Fig.1 Growth of *Chlorella* sp in broth (Aerated) Measured as Optical density.

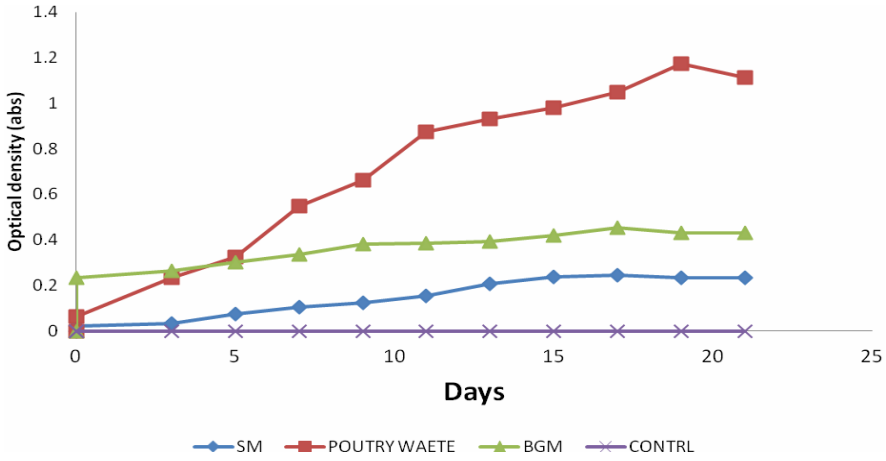


Fig.2 Growth of *Chlorella* sp in broth (unaerated) measured as Optical Density

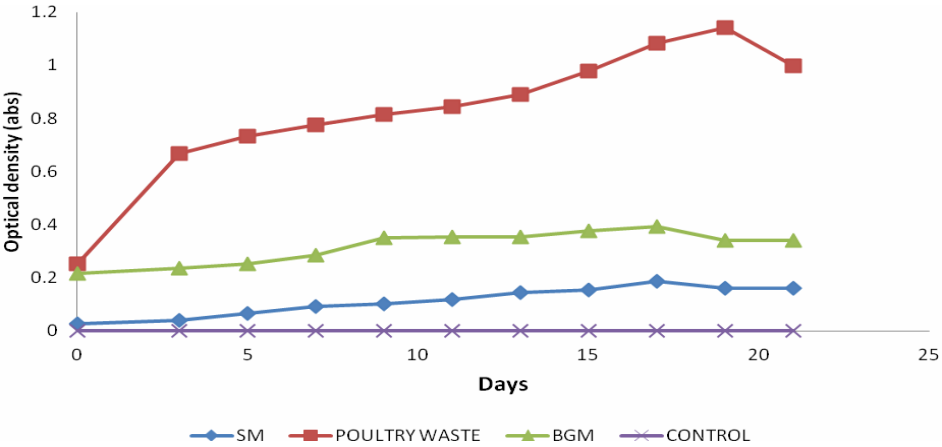


Fig.3 Growth of *Chlorella* sp in Broth (Sunlight) Measured As Optical Density.

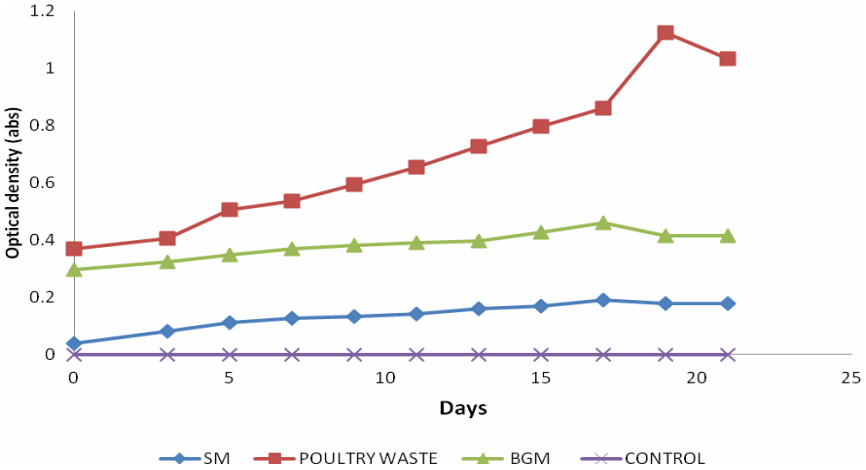


Fig.4 Growth of *Chlorella* sp in broth (Aerated) measured as dry weight

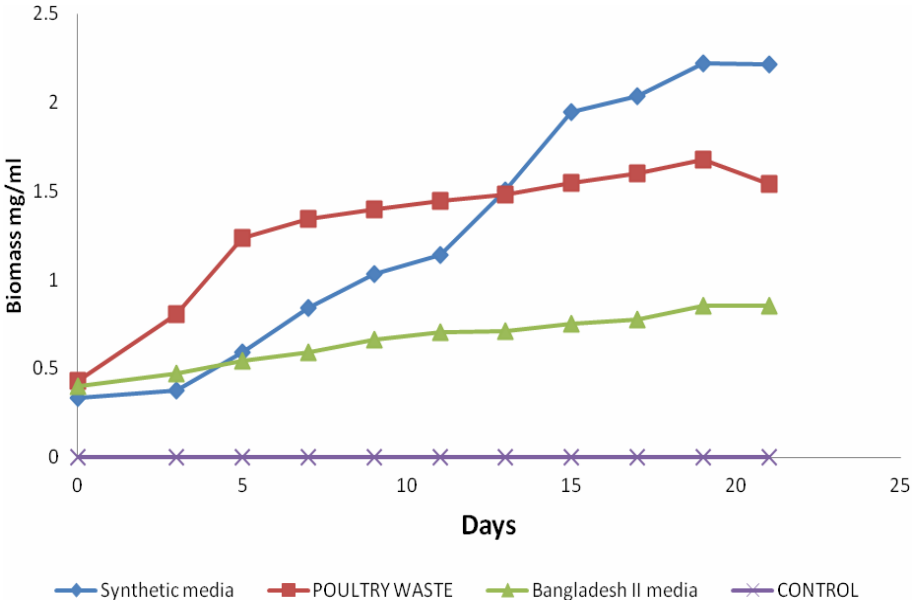


Fig.5 Growth of *Chlorella* sp in broth (Unaerated) measured as dry weight.

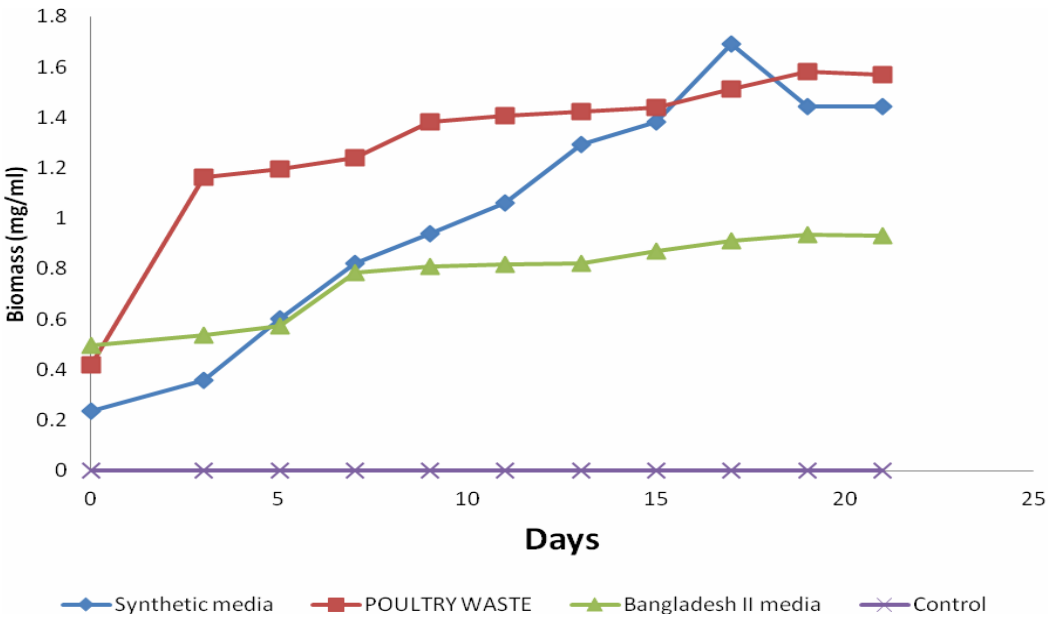
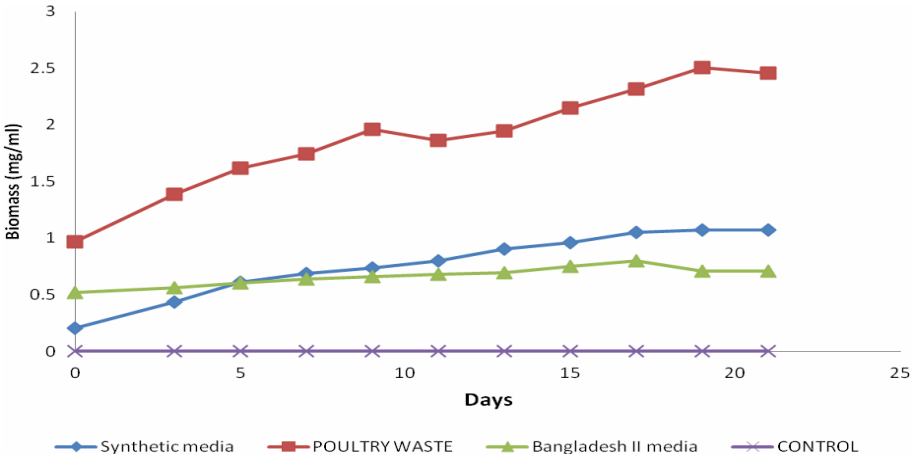


Fig.6 Growth of *Chlorella* sp in broth (sunlight) measured as dry weight



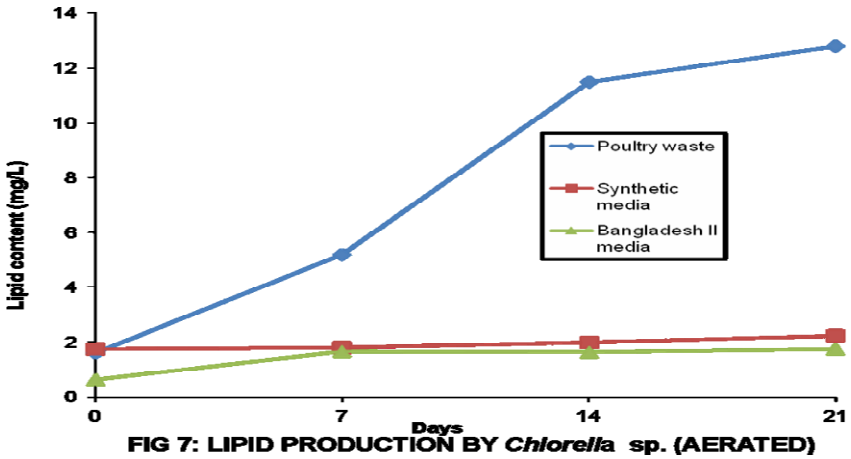


FIG 7: LIPID PRODUCTION BY *Chlorella* sp. (AERATED)

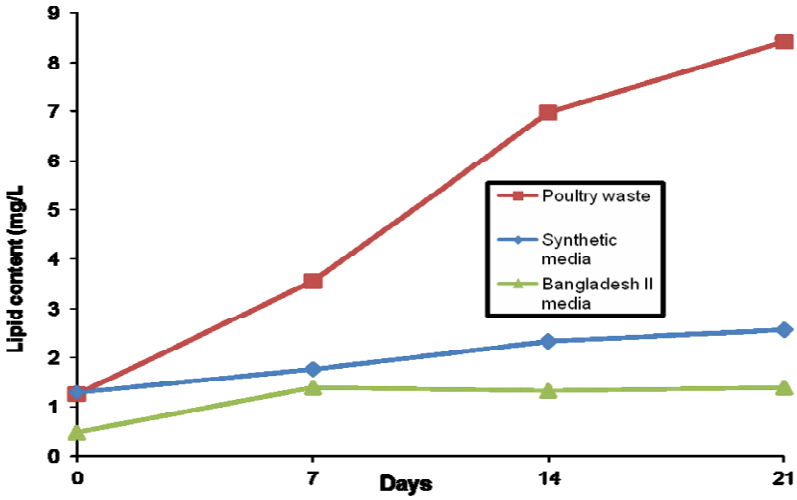


FIG 8: LIPID PRODUCTION BY *Chlorella* sp. (UNAERATED)

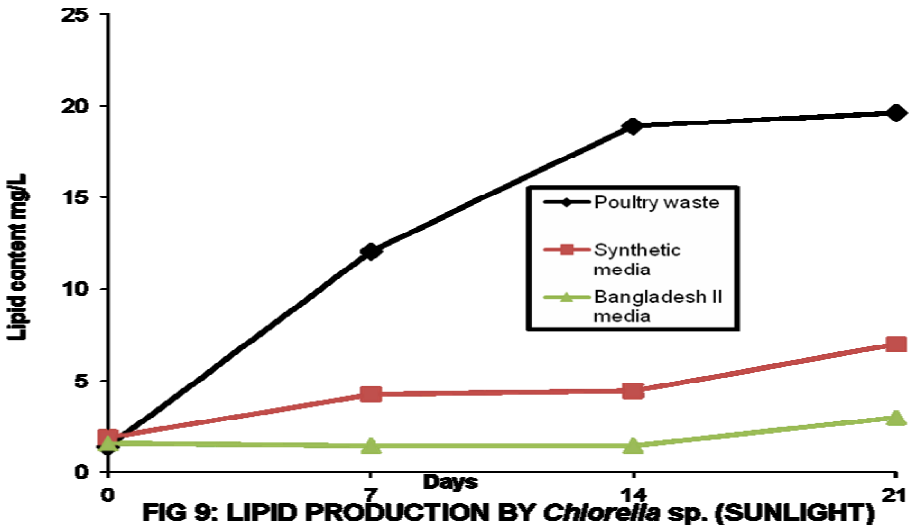


FIG 9: LIPID PRODUCTION BY *Chlorella* sp. (SUNLIGHT)

Microalgae have long been recognized as potentially good source for biodiesel production because of their high oil content and rapid biomass production. The process of producing microalgal oil consists of producing microalgal biomass that requires light, carbon dioxide, water and inorganic nutrients (nitrates, phosphates, and iron) and this is in agreement with the results obtained from utilizing poultry waste extract under natural illumination (Chisti, 2007). Microalgae oil is non-toxic, highly bio-degradable, utilizes wastewater for its production, can reduce carbon emission based on the cultivation conditions and feedstock, and provides a reliable and continuous supply throughout the year (Schenk *et al*., 2008).

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