Economically effective potential of *Chlorella* sp. for biomass and lipid production

AGWA*, O.K., IBE, S.N and ABU, G.O

Department of Microbiology, University of Port Harcourt, P.M.B.5323 Rivers State, Nigeria

ABSTRACT

Microalgae are a good source of feedstock for the production of renewable energy because of their rapid growth rate. The exploitation of the alga *Chlorella* sp. as feedstock using different animal (goat, cow, pig, grass cutter and poultry) waste as growth medium was investigated. A *Chlorella* sp. isolated from a fresh water pond was grown in the laboratory under different conditions (artificial: aerated /unaerated; natural: sunlight). The algal growth under natural illumination resulted in a higher biomass and lipid yield than that from the artificial illumination. The poultry waste under sunlit conditions gave the highest biomass yield of about 2.5g/l and 18.32% (w/w) of lipids in wet cells under mixotrophic conditions of growth. Mixotrophic conditions of growth are relatively less expensive to maintain. Biomass from the *Chlorella* sp. using inexpensive growth media formulations such as animal waste is an attractive source of valuable substrates for the neutraceutical, pharmaceutical and biochemical industries including biofuel industries.

Keywords: Animal waste, biomass, *Chlorella* sp., lipid production.

INTRODUCTION

The need for renewable energy supplies that do not cause environmental harm nor compete with food supply have heightened interest and driven economic sustainability to the development of biological sources that cannot affect food crops but can be used for energy applications (1,2). Because of their photosynthetic efficiency, faster growth rate and high biomass production compared to other crops, attention has been drawn to microalgae. Microalgae use as potential renewable energy source cannot compromise food availability, fodder and other products from plants as there is no requirement for soil fertility, arable lands and scarcity of water supplies. The use of microalgae as food source has been on for a long time, this stirred up the possibility of their use as an alternative for the production of renewable energy products. Various biomass
such as bio-wastes, food wastes, municipal wastes, agricultural and industrial as well as edible and non edible plants have been identified as alternative bio-oil sources for energy application.

Algae are large group of simple, unicellular photosynthetic organisms that have the potential to produce large amount of biomass and lipid more than terrestrial plants within a short time all the year round (3). They can be cultivated using different water bodies, non arable lands without incurring any form of land minimization or changes in environmental impact (4). The potential of growing algal biomass through autotrophic, heterotrophic or mixotrophic cultivation using organic compounds as energy and carbon sources under artificial, solar energy and carbon (iv) oxide fixing is feasible (5). Nutrients such as nitrogen and phosphorus obtained from waste water, organic effluents, and agro-based wastes can be used for microalgae cultivation and does not require pesticides or herbicide application (6). Thus microalgae cultivation has stemmed up their use for the provision of vitamins, coloring materials including carotene, chlorophyll and various pharmaceutical and nutraceutical products (7,8). Microalgal biomass can be used for the production of a variety of different energy products e.g. Liquid biofuel (9,10,11), methane (12,13), bioethanol(14,15) and biohydrogen (15,16). Biomass covers about one-tenth of the world’s primary energy demand and can be used as a replacement against the numerous challenges on earth (17). Many species of microalgae have been known to accumulate notable amounts of lipid, hydrocarbon and other complexed oils (1,18), serve as storage material, the average content varies 10-70% but under stress conditions most algal species can achieve dry matter lipid content up to 90% (19,20,21) and differ from plant oil in their high phospholipids and glycolipid concentrations. Oil productivity from microalgae depends on their rate of growth and level of biomass oil content. Any microalgae characterized by high lipid productivity are desirable for biodiesel production, and they do not compete with food in the market (22). The growth of algae serves the role of providing biomass which can lead to various energy generating processes

Renewable energy products from microalgae can be more environmentally sustainable, cost effective and more profitable if combined with processes such as waste management and its utilization. Systems involving microalgal production and waste management with high level of organic compounds from waste water can be promising for the growth of microalgae (22). The traditional waste management treatment procedures have been relegated to the background because of the emerging energy opportunities that can transform livestock waste treatment from a liability to a profitable venture with multiple value-added products. The need for a consolidated waste management system that makes animal operations economically viable and environmentally benign is necessary (23,24). The use of animal manure and other organic based waste products as bioenergy feedstock for waste conversion processes have the potential to convert the treatment of livestock waste from a liability into a profitable opportunity that can generate revenue, moderate the impact of commodity prices, and diversify farm income (25). Inherent use of animal waste for microalgae cultivation would serve as a form of treatment process designed to solve odour problems, reduce volume, recover nutrients, decrease pollution potential as well as recover energy from the manure. The economics of this conversion process has made available feedstock for the production of the desired energy form to the final end – product (26,27,28). A number of researchers have demonstrated the ability of various microalgae to utilize animal waste as a growth medium (29). The reduction of nitrogen and carbon content in swine waste with an isolated alga Chlorella sp. and bacteria naturally living in liquid medium
was studied (30). Consequently (31) have demonstrated the use of poultry waste as important nutrient source for algal biomass enhancement when aerobically digested poultry manure is fed to the micro alga *Chlorella vulgaris* cultures. In this study an attempt was made to cultivate a green alga (*Chlorella* sp.) isolated from a fresh water pond using different animal waste as suitable growth medium. The aim is to optimize biomass production to a scale that would be economically cost effective for the extraction of oil for biodiesel production.

**MATERIALS AND METHODS**

**Sample Collection**
Water samples containing microalgae, poultry and pig wastes were collected into sterile clean containers from the African Regional Aquaculture Centre (ARAC), Aluu Rivers State, Nigeria; grass cutter waste was obtained from the National Biotechnology Development Agency (Centre of Excellence) Odi, Bayelsa state, cow dung wastes were collected from a cattle market and goat waste was collected from a goat pen at Alakahia village, Port Harcourt, Rivers State, Nigeria.

**Proximate Analysis**
Typical analysis of the micronutrients of the different animal wastes was done. Moisture content and crude protein determination were done (32). Crude ash, crude fibre, crude fat and total carbohydrate determination were done carried out (33).

**Preparation of animal waste extracts**
The animal wastes were sun-dried, ground into fine powder using a mechanical grinder, and kept until required. The extracts were prepared by suspending 30g of the different animal wastes in a litre of distilled water, sterilized to destroy pathogens and filtered using Whatman filter paper. The physicochemical properties of the extracts were carried out (34).

**Algal Blooming**
A 10:90 mixture of cow dung extract and the fresh pond water samples containing *Chlorella* were subjected to aeration, using an aquarium pump. The set-up was illuminated artificially using two white fluorescent lamps. The flasks turned leaf green after five days of incubation.

**Cultivation conditions and growth measurement**
One milliliter of the bloomed culture was aseptically inoculated into flasks containing *Chlorella* were maintained at 28±2°C. The following set ups were prepared (i) Unaerated condition (ii) Aerated condition using an aquarium pump supplying about 150 bubbles per minutes (35). (Options (i) and (ii) were illuminated artificially using two fluorescent lamps, emitting ca 15µEm⁻²s⁻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. Replicate samples were taken daily to monitor algal concentration by measuring the biomass as cell dry matter and lipid content from the wet algal cells.

**Cell dry matter**
Cell dry matter was determined (36). About 5ml of the growing culture was harvested by centrifugation at 3000 rpm for 10mins. The cells were washed (3x) with physiological saline dried at 50°C in a hot air oven to a constant weight.
Lipid Extraction
The wet extraction procedure was adopted (37). 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 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°C) leaving the lipid which was then reweighed using an analytical weighing balance (Setra BL-410S, USA).

RESULTS AND DISCUSSION
The proximate analysis of the different animal waste and their corresponding physicochemical properties are clearly shown in Tables 1 and 2. The highest moisture and carbohydrate content are seen in goat waste, highest lipid content is found in grass cutter waste, poultry waste exhibited the highest ash and protein content, while cow dung waste reveals the highest content of crude fibre.

<table>
<thead>
<tr>
<th>TABLE 1: Proximate analysis of different animal wastes</th>
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<tbody>
<tr>
<td>Parameters</td>
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<tr>
<td>-----------------------------------------------</td>
</tr>
<tr>
<td>Moisture</td>
</tr>
<tr>
<td>Lipid</td>
</tr>
<tr>
<td>Ash</td>
</tr>
<tr>
<td>Protein</td>
</tr>
<tr>
<td>Carbohydrate</td>
</tr>
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<td>Crude fiber</td>
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</table>

Different animal wastes inoculated with the test organism were operated for 21 days under artificial (aerated/unaerated) and natural illuminations with monitoring of algal growth as cell dry matter and lipid content. From Fig. 1, the algal growth under artificial illumination with aeration shows that there was no lag phase. The response was as follows: cow dung waste rose from 0.31mg/ml to 0.83mg/ml; grass cutter waste 0.31mg/ml to 1.11mg/ml; pig waste 0.35mg/ml to 1.12mg/ml and poultry waste 0.43mg/ml to 1.35mg/ml. The growth in the goat waste increased up to 3.07mg/ml from 0.37mg/ml in day 1 to the 19th day and decreased to 1.72mg/ml by the 11th day. The algal growth as cell dry matter under artificial illumination and unaerated increased from 0.40mg/ml to 1.00mg/ml for all the different animal wastes except...
poultry waste which grew up to 1.20mg/ml (Fig. 2). The growth of the algae in goat, pig and grasscutter wastes grew slowly under natural illumination as cell dry matter rose from 1.00mg/ml to 1.10mg/ml with a three days lag phase. But the cow dung and poultry wastes exhibited no lag phase, poultry waste cell dry matter rose from 0.97 mg/ml to 2.50mg/ml and decreased slightly to 2.46mg/ml by the 21st day.

**TABLE 2: Physicochemical properties of animal waste extracts**

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Goat Waste</th>
<th>Pig waste</th>
<th>Cow dung waste</th>
<th>Grass cutter waste</th>
<th>Poultry waste</th>
</tr>
</thead>
<tbody>
<tr>
<td>pH</td>
<td>7.22</td>
<td>6.32</td>
<td>6.28</td>
<td>6.0</td>
<td>6.66</td>
</tr>
<tr>
<td>NO₃</td>
<td>5.06</td>
<td>5.06</td>
<td>4.6</td>
<td>2.61</td>
<td>3.83</td>
</tr>
<tr>
<td>PO₄</td>
<td>7.04</td>
<td>11.26</td>
<td>16.5</td>
<td>8.24</td>
<td>8.8</td>
</tr>
<tr>
<td>BOD</td>
<td>240</td>
<td>320</td>
<td>270</td>
<td>320</td>
<td>160</td>
</tr>
<tr>
<td>COD</td>
<td>844</td>
<td>1620</td>
<td>1390</td>
<td>1540</td>
<td>1796</td>
</tr>
<tr>
<td>Conductivity</td>
<td>272</td>
<td>593</td>
<td>370</td>
<td>915</td>
<td>827</td>
</tr>
<tr>
<td>Temperature</td>
<td>29.6</td>
<td>29.6</td>
<td>29.6</td>
<td>29.6</td>
<td>29.6</td>
</tr>
</tbody>
</table>

Figure 1: Aerated growth of *Chlorella* sp. in liquid media monitored as cell dry matter under artificial illumination
The lipid content obtained at the end of an artificial illumination with aeration recorded the highest lipid content (11.19%) from poultry waste; the others were as follows: grass cutter waste 9.71%; cow dung waste 6.60%; pig waste 3.38% and goat waste 2.60% (Fig. 4). The artificial illumination of the different animal waste under un aerated condition as shown in Fig. 5 revealed low lipid content from goat waste (0.71%); grass cutter waste (3.40%); pig waste (4.18%); cow dung waste (5.63%) and poultry waste (6.17%). The lipid content under natural illumination...
resulted in increased lipid productivity from poultry waste (18.32%); grass cutter waste (13.70%); cow dung waste (10.17%); pig waste (7.77%) and goat waste (4.86%).

Recent technological knowledge has led to increased economic, alternative, renewable, sustainable, bioresource energy products. The energy is provided by the use of biological based reserves that can reduce net CO₂ emission and other pollutants causing environmental problems. An environmentally friendlier alternative energy source is the use of photosynthetic organisms to produce bioresource energy products (38). Microalgae can use solar energy for growth and have some great potential advantages (1). Microalgae can grow in water, do not compete with land – based food crops, have the benefit of reduced carbon emission with increased sufficient available feedstock, better chemical properties and are cost effective (39).

Laboratory –scale experiments were conducted to evaluate the use of different animal wastes for the growth of Chlorella sp. under different conditions of artificial (aerated/unaerated) and natural

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illumination. The waste were analyzed to make it environmentally friendly and to develop its economic use. *Chlorella* sp. was cultivated in different animal wastes at a pH of 7.5±0.2 and a temperature of 28±2°C. Artificial illumination with aeration was effected using an aquarium pump supplying about 150 bubbles per minutes (35). Various studies were conducted on *Chlorella* sp. during their growth for 21 days.

Biomass production using the different animal waste reveals the potential of the wastes as raw material resource. Artificial illumination conditions (aerated) showed that goat waste was favorable for the growth of *Chlorella* sp. at a cell dry matter of 3.07g/l, while in the unaerated condition, the poultry waste gave the highest cell dry matter of about 1.58g/l. The value obtained with the growth monitoring under natural illumination showed poultry waste at 2.50g/l cell dry matter, revealing the possibility of microalgae utilizing various animal waste as growth medium (29,31). Poultry waste as a raw material is readily available. Recently, poultry waste used in plankton production has significantly exhibited better yield against other livestock waste (40), consequently (41) strongly pointed out that waste water, municipal wastes and other related wastes are potential to promote and commercialize algae biofuels. The natural illumination would be preferable on the basis of overall cost compared with the artificial illumination which requires energy input for lighting.

The amount of lipid productivity obtained after growing *Chlorella* sp. using different animal wastes as growth medium was investigated. The natural illumination conditions revealed very high potential for the production of algal oil (20,8,42,38,3). The poultry waste had the highest productivity of 18.32%, compared with the others the responses were as follows: grass cutter 13.70%, cow dung waste 10.17%, pig and goat wastes were least with 7.77% and 4.86% respectively. The aerated condition with artificial illumination gave a similar sequence with the natural illumination; accordingly lipid production was as follows: poultry waste 11.19%, grass cutter 9.71%, cow dung waste 6.60%, pig waste 3.38% and goat waste 2.60%. The unaerated conditions resulted in an entirely different scenario as follows: poultry waste 6.17%, cow dung...
wastes 5.63%, pig wastes 4.18%, grass cutter waste 3.40% and goat waste gave 0.71%. The aeration and intermittent agitation of the liquid media used in the study enhanced the rapid multiplication of the organism and revealed the potential of lipid production of the organism. The poultry waste is clearly the best growth medium of the five media tested for the growth of *Chlorella* sp. and this may be due to the high content of nitrogen and phosphorus which enhance micro algal growth (43,44,45,31). Phosphorus and nitrogen are limiting growth nutrients for microbial growth. This can explain the use of poultry waste as an organic fertilizer and an attractive source from which nutrients can be retrieved and re-utilized.

Our preliminary results have shown the potential for microalgal biotechnology which can be harnessed and utilized for maximum productivity in the nutraceutical, pharmaceutical and energy industries. More specifically the growth process has revealed an established microbial biotechnology technique suitable for the production of biofuel (biodiesel) and a potential as an alternative, sustainable and renewable energy resource.

**CONCLUSION**

The growth of *Chlorella* sp. has been shown to present a potential opportunity factor in the form of value-added biomass providing a novel dimension to the treatment of animal waste. The result in the study showed that micro algal cultivation with different animal wastes as growth medium is a promising method for lipid production. The highest lipid content (18.32%) and 2.50mg/ml biomass productivity achieved under the natural condition, is economically attractive since the production cost can be reduced with credit to waste utilization as well as green house emission reduction and can enhance multiple waste management as waste-to-bioenergy.

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