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Algal-biomass Production from *Chlorella* sp. Using Hot and Cold Water Infusions of Poultry Droppings

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Authors' contributions

This work was carried out in collaboration among all authors. Author EE designed the study, performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Authors OKA and GOA managed and supervised the analyses of the study. Author EE managed the literature searches. All authors read and approved the final manuscript.

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ABSTRACT

Background: Microbial biomass is a valuable resource to the development of sustainable energy. However, the challenge of having an effective media for energy production have adversely affected biotechnological development. This study was aimed at comparing algal biomass produced by *Chlorella* sp. using hot and cold water extracts of poultry droppings.
Methodology: Fifteen grams (15 g) of poultry droppings was infused into 500 mL (cold and hot water) and allowed to stand for 48 h prior. Algal growth was monitored by cell dry weight and optical density readings taken at 620 nm using a spectrophotometer.
Results: Physicochemical composition of the poultry droppings for cold water infusion revealed the following: pH, 6.7; conductivity, 3404.1 µs/cm; phosphate, 25.3 ppm; nitrate,1.88 ppm; phosphate, 25.3 ppm; Mg^{2+}, 27.20 ppm; TOC, 38.03 ppm and COD, 53.8 ppm after 48 h. Whereas, the values obtained for hot extractions were: pH, 6.28; conductivity, 3.82 µs/cm; nitrate, 1.24 ppm; phosphate, 28.0 ppm; Mg^{2+}, 19.85 ppm; TOC, 64.03 ppm and COD, 553.3 ppm. The proximate composition of extract revealed crude fibre, 17.69%, ash content, 24.16%; crude fibre, 22.7%; crude protein,

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21.02%; crude fat, 3.19% and crude carbohydrate 23.02%. Microflora obtained from the biostability test revealed the presence of *Proteus* sp, *Vibrio* sp. and *Salmonella* sp. in cold extract while hot extract had *Bacillus* sp. and *Serratia* sp. The *Chlorella* sp. was identified using colonial and microscopic features. Biomass yield of 3.1 g/L and 2.8 g/l wet weight of *Chlorella* biomass was recorded for the cold and hot aqueous extracts of the poultry droppings respectively.
Conclusion: This study revealed that hot poultry droppings extract (PDE) could offer a better feedstock for biodiesel production.

Keywords: Biomass; poultry droppings; infusion; Chlorella; biostability test; sustainable energy.

1. BACKGROUND

Sustainable, cost-effective and renewable biomass-driven energy systems have dominated most scientific discuss for a while now. Greener technologies have been identified to provide a robust advancement in the attainment of energy solutions [1]. Biotechnologically driven sources of energy have been faced with challenges in carbon capture and sequestration. Microalgae are unique, multicellular and photosynthetic organisms, with rapid growth rate, even with minimal supply of nutrients [2,1]. High value products including a wide range of nutraceutical, pharmaceutical, feed supplements and energy products [3]. The minimized greenhouse and flue gas production associated with their growth and products have been identified by researchers [4, 5]. Biomass is a product of living things either macrobial or microbial; animal or plant materials, dead or living portions. Algal biomass has been bio-prospected for some novel bioactive materials that can improve the existence of man. Sadly, Zhang et al. [6] reported the under-use and untapped milleu of bio-resources trapped in several microalgae biomass. About 15% of global energy needs have been resolved by biomass sourced options [7]. There is potential for a near-future supplement or total replacement of conventional fossil fuels with algal-biofuels and one of the much-debated feedstock to achieve this feat is the green algae. According to Sarfi et al. [8] *Chlorella* can be split into two Latin words: *Chloros*-green and *ella*-small. Several species of this organism have been isolated from some extreme environments such as surfaces of natural and artificial stones where they exist as epiphytes and in water bodies where they are found as sextons and serve as food for aquatic animals. *Chlorella* has been harnessed in the production of biofuels [9], medicinal and nutraceutical [10], and fatty acids lipids [11]. Production of biomass by *Chlorella* has been reported by Agwa et al. [10] using animal droppings. The biomass productivity can be said to be a trigger-control process, this is because of nutrient limiting conditions. Higher biomass productions have been linked to the ability of the media to induce the cells to synthesize high value substances. Poultry farming is one leading aspect of the agro-industry. The droppings serve as manure for soil enrichment. It contains several organic nutrients which are used in mass cultivation of algae [12,11,5]. The aim of this research is to evaluate the rate of algal biomass production by *Chlorella sp.* using cold and hot-aqueous poultry filtrates.

2. MATERIALS AND METHODS

2.1 Isolation of *Chlorella* sp.

Algal stock culture of *Chlorella* sp. was obtained from Dr. (Mrs) Agwa, O.K of the Department of Microbiology, University of Port Harcourt. The microalga was sub-cultured, spread on agar-solidified synthetic medium. Isolation medium was prepared by inoculating a 100 mL of synthetic medium by fortifying the media with 100 µg/mL chloramphenicol and 62.5 µg/mL nystatin [12]. Cultured plates were exposed to solar radiation (natural illumination) for biomass growth.

2.2 Characterisation of the *Chlorella* sp.

The samples were examined by carrying out a 10-fold dilution, placed on a grease free slide and then viewed under x40 objective lens. The isolate was characterized.

2.3 Proximate and Physicochemical Analysis of the Poultry Droppings

The dry poultry droppings were subjected to proximate analyses to ascertain the ash, carbohydrate, moisture, crude proteins, fibre, and lipid contents [13]. Ten millilitres of the poultry dropping extracts were employed for physicochemical analysis to determine the various compositions of pH, nitrate, conductivity, total organic content, phosphate, salinity, calcium

and total hardness, biochemical and chemical oxygen demand [13,14]. Final values of the compositions were mathematically calculated as stipulated by standard procedure.

2.4 Preparation of Poultry Extracts

Poultry droppings were sterilized in an autoclave at 121 psi for 15 minutes. The sterile samples were infused into a litre of hot and cold borehole water. The set-up was allowed to stand overnight for the poultry droppings and subsequently filtered to remove debris. Freshly grown microalgal stock was used to inoculate into 250 mL poultry dropping extract. The inoculated broth was exposed to natural illumination and manually aerated for 6 h.

2.5 Optimal Wavelength Selection

The poultry droppings extract and a control using (BG11 medium) were made to pass through a range of wavelengths from 325-675 nm using spectrophotometer (Spectrum Lab Germany). This study was aimed at selecting the wavelength range where treatments absorbed the least lights and deterred obstruction from the colour of the media. The optimal wavelength (one that absorbed light the least) was selected as best wavelength for the growth monitoring studies [15].

2.6 Growth Monitoring

2.6.1 Determination of cell biomass

The cell dry weight approach was used to determine the algal biomass yield. Five millilitres (5 mL) of the culture broth of the blooming set-up was transferred to a centrifuge tube and spun at 4,500×g for 15 minutes three times. The pellets were dislodged and then poured on a pre-weighed Whatman filter paper. The pellets were filtered off and the sample (filtrate) dried at 50^{o}C in a hot-air oven to constant weight before being brought to room temperature in a desiccator. The net dry cell weight was determined by measuring the arithmetic difference between the final weight of the filter paper and the initial weight [16,17].

2.6.2 Determination of cell optical density

The measure of absorbance was used to evaluate the cell density, using spectrophotometer. The optimal wavelength of 680nm was used to study the increase in absorbance of the medium, arising from a corresponding increase in *Chlorella* sp. [18].

2.6.3 Determination of cell numbers

The cell numbers from the different treatments of the poultry droppings were determined by carrying out a ten-fold dilution using 1.0 mL of the culture and 9 mL of distilled water. The diluted pure culture suspension was counted on a haemocytometer. The cell number in the culture was calculated by dividing the number of cells counted by the volume and the dilution was determined every 3-day interval. The growth rate was obtained by calculating the gradient of the growth at the exponential phase.

3. RESULTS AND DISCUSSION

3.1 Results

The proximate composition of the poultry droppings is represented in Table 1. The ash, crude fat and protein contents (% dry weight) for the 30.0g were: 24.16±0.387, 3.19±0.352 and 21.02±0.058, respectively. Statistical analysis using one-way ANOVA ($p>0.05$). The Post Hoc studies using Tukey-HSD at 95% confidence level indicated significant difference within the tested masses of the poultry dropping for ash, moisture crude fat and protein contents.

Table 1. Proximate composition of the poultry droppings

Parameters	15.0 g
Moisture	17.69±0.256^{b}
Ash	24.16±0.387^{b}
Crude Fibre	22.7±0.17^{b}
Crude protein	21.02±0.058^{b}
Crude Fat	3.19±0.352^{a}
Carbohydrate	23.02±0.109^{b}

Values represent means ±standard error; superscripts with the same alphabet in a given row are statistically insignificant at $p < 0.05$

Tables 2 and 3 describes the physicochemical composition of the poultry droppings after hot and cold extractions, respectively. The conductivity of the hot aqueous extracts of poultry droppings decreased from 3526.67±1.156 µS/cm at 24 h to 3125±0.33 µS/cm at 48 h. Similarly, the conductivity of the cold aqueous extracts decreased from 3418.67±1.46 at 24 h to 3125±0.33 µS/cm at 72 h. These values exceeded the regulatory limits (WHO) for pond water which is < 1200. From the result, the cold

water extracts had a lower conductivity than the hot extracts. The pH increased from 6.15±0.00 to 6.74±0.075 and from 6.08±0.057 to 6.48±0.058 for cold and hot extracts, respectively. The physicochemical results for the cold extracts revealed increase in biochemical oxygen demand (BOD) from 31.12± 0.95 to 49.2±0.058 mg/kg and total organic carbon from 217.0± 4.16 to 597.67±13.29mg/kg. For hot extracts, it increased from 12.63±0.32 to 72.2±0.18 mg/kg and from 202.0±4.16 to 448.33±13.29 mg/kg in biochemical oxygen demand (BOD) and total organic carbon (TOC), respectively were observed.

The absorbance for concentration 30 g/L of the poultry droppings and the control (BG11 and Novel synthetic media) were tested for wavelength selection. The results are presented in Fig. 1. The results showed that the highest absorbance peak was obtained at wavelength of 325 nm and the lowest absorbance at 600 nm. The poultry extracts with higher colour intensity were observed to have higher absorbance than those with lower colour intensity; the control had lesser or lower absorbance. The optimum wavelength where the interference was least, at this point a wavelength of 620 nm was selected.

Figs. 3 and 5 describe the growth pattern of *Chlorella* sp. in the various concentrations of the poultry droppings extracts. All the hot aqueous extracts were characterised with exponential phases occurring from the first 72 h of the blooming duration, while the cold extracts especially the 30 g cold 24 h extract had a better bloom noticed within the first 3 days of bloom, confirmed by the characteristic greening of the media. Similarly they describe biomass production in the *Chlorella* sp. using extracts of poultry droppings. The 30 g/48 h hot extracts had

Table 2. Physicochemical composition of poultry extract for cold water infusion

Parameter	24 h Filtrate	48 h Filtrate	WHO Limit
Conductivity(µs/cm)	3418.67±1.46[a]	3404±1.15[b]	<1200
pH	6.15±0.00[a]	6.74±0.058[b]	6.5-8.5
Magnesium(mg/l)	19.85±0.29[a]	27.20±0.10[b]	0.2
Salinity	2.03±0.09[a]	2.36±0.12[b]	----
Nitrate(ppm)	1.07±0.10[a]	1.88±0.002[b]	0.2
Phosphate(ppm)	26.6±0.69[a]	25.3±0.057[b]	----
TOC(ppm)	31.12± 0.95[a]	38.03±0.13[b]	----
COD(mg/l)	451.67±0.88[a]	538±1.33[b]	----
BOD(mg/l)	217.0± 4.16[a]	396.67±0.88[b]	24
Total Hardness(ppm)	20.49±0.59[a]	18.86±0.03[b]	500
Ca-Hardness(ppm)	16.43±0.25[a]	9.91±1.71[b]	50

Values represent means ±standard error; superscripts with the same alphabet in a given row are statistically insignificant at p < 0.05

Table 3. Physicochemical composition of poultry extract for hot water infusion

Parameter	24 h Filtrate	48 h Filtrate	WHO Limit
Conductivity(µs/cm)	3526±1.155[a]	3282±0.58[b]	<1200
pH	6.08±0.057[a]	6.28±0.088[b]	6.5-8.5
Magnesium(mg/l)	23.95±0.87[a]	19.85±0.15[b]	0.2
Salinity	1.74±0.38[a]	2.14±0.058[b]	----
Nitrate(ppm)	1.97±0.009[a]	1.24±0.05[b]	0.2
Phosphate(ppm)	28.24±0.69[a]	28.0±0.057[b]	----
TOC(ppm)	12.63± 0.32[a]	64.03±0.03[b]	----
COD(mg/l)	578.00±12.41[a]	553.3±1.20[b]	----
BOD(mg/l)	202.0± 4.16[a]	314.0±0.00[b]	24
Total Hardness(ppm)	12.50±0.11[a]	11.63±0.76[b]	500
Ca-Hardness(ppm)	7.5±0.10[a]	5.9±0.27[b]	50

Values represent means ±standard error; superscripts with the same alphabet in a given row are statistically insignificant at p < 0.05

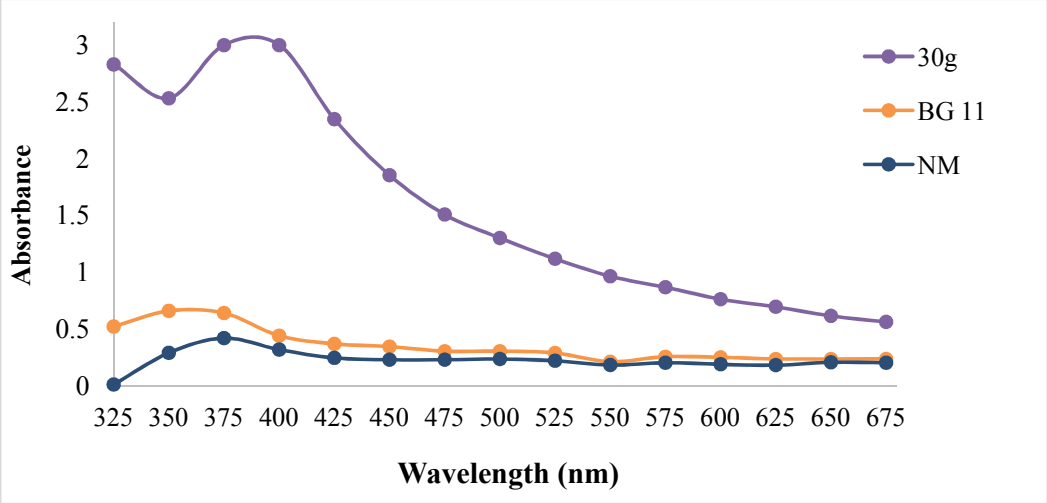

Fig. 1. Wavelength selection for the poultry extracts and control
(BG11; NM---Novel synthetic medium)

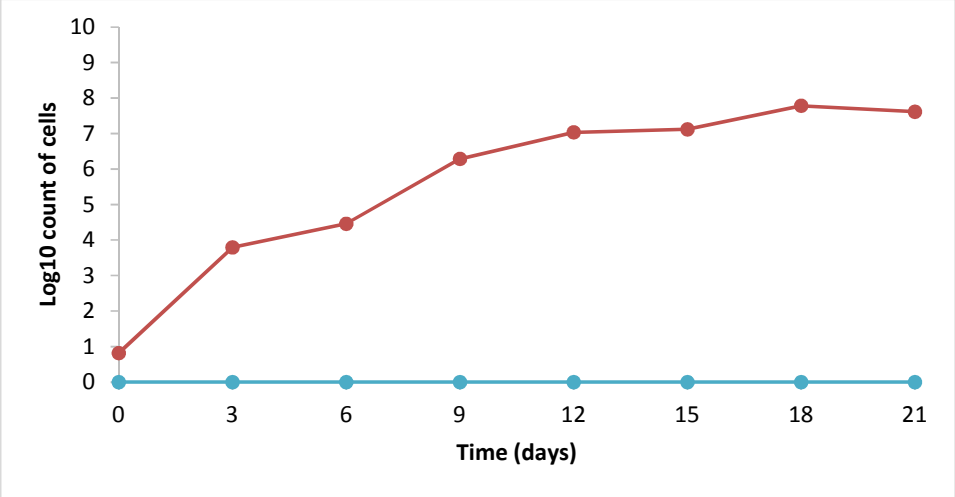

Fig. 2. Cell count of *Chlorella* sp. in 48 h cold aqueous poultry dropping extracts

a biomass production of 1.8-2.7 g/L within the first 72 h of the cultivation while the 30 g/48 h cold had a biomass concentration of about 2.7 g/L after the same period 3.2 g/L on the 9th day of the blooming. The results show a positive relationship between extraction time and infusion temperature of the poultry dropping (i.e., hot water infusion) and biomass production by the feedstock. Statistical analysis using two-way ANOVA indicates a significant biomass production using the 30 g/48 h cold and hot extracts at a 95% confidence level.

3.2 Discussion

Physicochemical composition of the 48h-extract of cold poultry droppings revealed values for COD, BOD, nitrate, phosphate as 538 mg/L, 396.67 mg/L, 1.55 ppm and 28.1 ppm, respectively. A nitrate/phosphate ratio of 1:16 compositional values of the poultry droppings agreed with Ahmad et al. (2013) who reported COD (981.6mg/L), BOD (366.67 mg/L), nitrates (18 mg/L) and phosphate (25.3 mg/L). The 48 h hot extracts had COD (881.7 mg/L), BOD (448.3 mg/L), nitrates (1.19 ppm), phosphate (28.7ppm) values, with nitrate/phosphate ratio of 1:24. This result suggests that the temperature of solvent medium was importance in achieving better extraction and agrees with Wang [19] Although hot aqueous nutrient extraction approach has been largely criticized to be both energy and time intensive, it shows high efficacy in nutrient extraction. However, the process does not

conform to the principles of ecological economics and white-energy production that more energy should not be used in production [17,20,21].

Halim et al. [22] discussed how they grew microalgae in transparent polythene bags to mimic a closed system and the set-up was manually shaken. Cost-effective materials for cultivation have been advocated for growing algae was adjudged better because of its less energy intensive [12]. In the course of this study the cells were grown in 250 mL conical flasks. The growth pattern of cold aqueous treatment were in similar pattern as the hot treatments, with resultant growth of *C. vulgaris* having a short lag phase, observed within the first 72 h of algal blooming. This gave an impression that the colour of the medium offered a resistance to absorption of light to the medium. The poultry-based media formulations had been reported to have a lower and significant growth with a longer exponential phase due to the high media adaptation by microalgae.

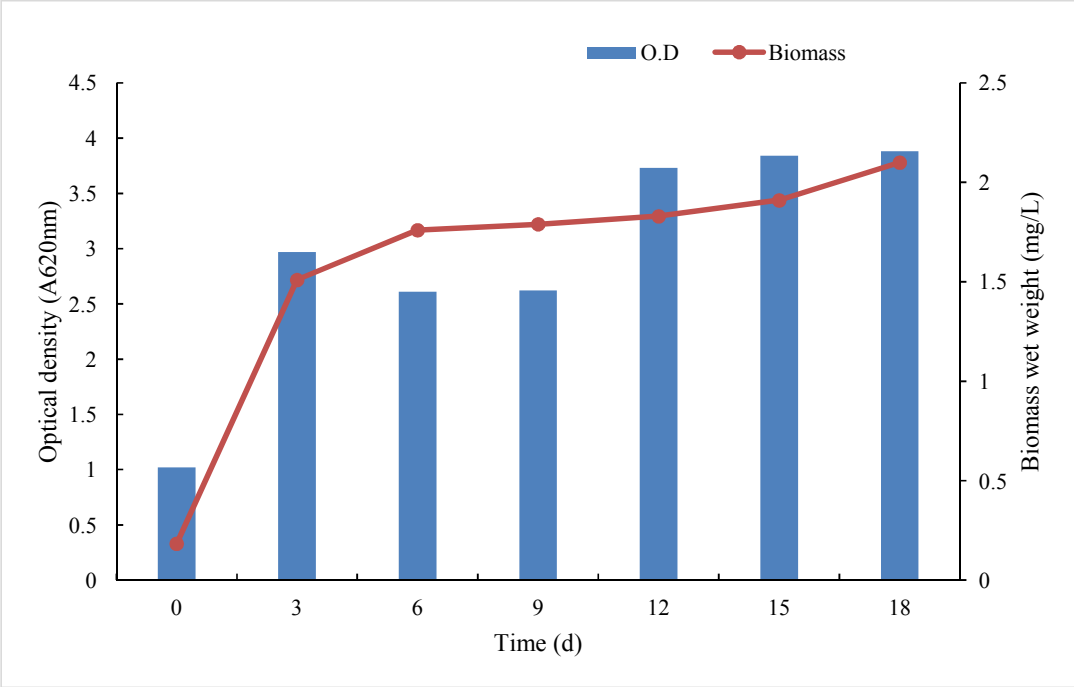

Fig. 3. Biomass and growth patterns of Chlorella sp in cold 48 hr old poultry droppings

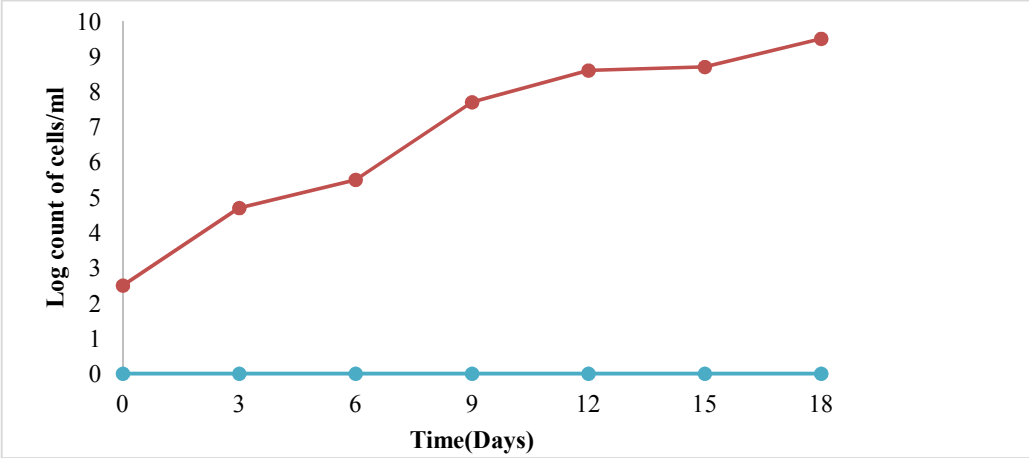

Fig. 4. Cell count of *Chlorella* sp. in 48 h hot poultry dropping extracts

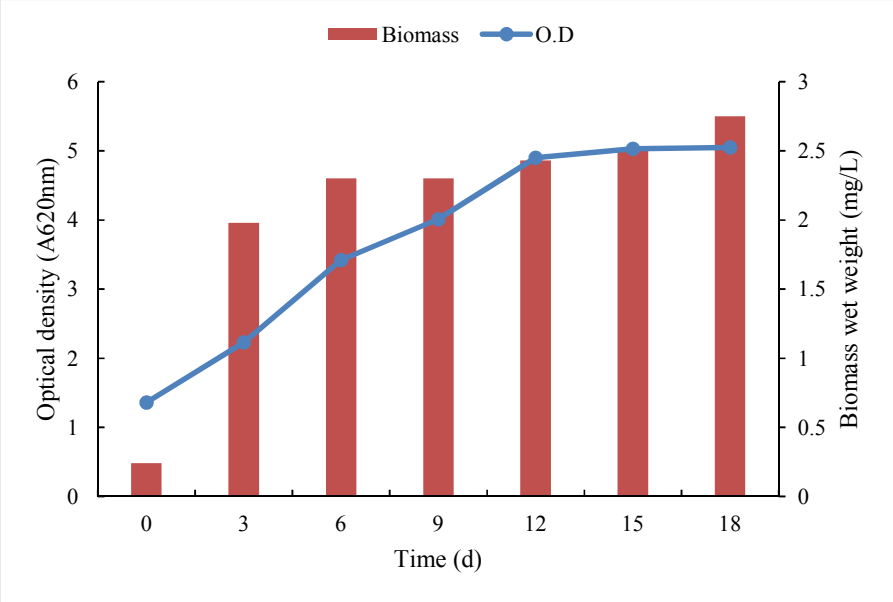

Fig. 5. Biomass and growth patterns of *Chlorella* sp. under 48 h hot poultry dropping extract

The biomass yield (wet weight) was best at 3.8g/L was observed for 48h cold water infusion followed by the 2.1g/l for 48h hot water infusion, 1.5g/L was recorded for the synthetic medium, the other treatments had close biomass yield to the best at 3.6g/L, which further explains that hot water extraction could be an economic approach [16]. The reports of Cheung et al. [23] and Wang et al. [24] revealed a lower algal yield of 1.0g/L *Chlorella* from medium developed from poultry droppings. Although, the hot extracts provided a good media formulation with the absence of a distinctive lag phase and an exponential phase, which lasted within 72 h of the cultivation, the use of hot aqueous extracts may not be ecologically acceptable for a large-scale cultivation and production of microalgae. Statistical analysis using the Post Hoc with Tukey HSD at $p< 0.05$ showed that the 48 h cold water extract had a better media adaptation and blooming.

The 30 g-48 h cold extracts had exponential phase on the 3rd day with a growth rate of 1.78 mg/l of *Chlorella* biomass. Conversely, the 48 h cold extracts had a very early exponential phase within the first 48 h of blooming, becoming visible on the first 72 h of blooming with its characteristic green colouration. Its growth rate was higher at 2.62 mg/l per day, doubling that of the other treatments. This indicates that the density of the media played a role in the blooming as calculated using Fig. 3. The results suggest that the heat of treatments has an effect on the physicochemical quality of the extracts. The findings in this study agree with the reports of Wang et al. [24] *Chlorella* sp. in four waste-waters showed an exponential growth phase on the 72 h of cultivation. Supports that, 7-day log phase by marine green algae; this was similar with the results of most of hot water extracts of higher concentrations having growth rates lower than 0.024 mg/l per day.

4. CONCLUSION

Poultry droppings extract can be harnessed in the large-scale cultivation of microalgae due to the presence of nutrients. The efficacy of the sterilization asserts the possible biostability, which could impair accuracy of results. Temperature of extraction as observed in this study, could play a vital role in the mass cultivation of algae using agro-based waste although this may does not support the principle of ecological economics where it is not recommended that energy input should be minimized in the production of energy. This study revealed poultry-based media could result in high biomass yield compared to the synthetic medium.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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