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Cultivation of *Scenedesmus incrassatulus* on Poultry Waste Effluent as a Biostimulant for Growth

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Abstract: Microalgae play an effective role in the bioremediation of poultry waste. The current research is designed to study the cultivation of *Scenedesmus incrassatulus* on poultry waste effluent to improve the biochemical composition of microalgal growth. *Scenedesmus incrassatulus* was isolated, identified, and was assessed for growth performance, carbohydrate, and lipid contents on Bold's Basal medium Various concentrations of poultry waste ranging from 10 to 100 % were tested on *Scenedesmus incrassatulus* growth. The highest concentrations of poultry waste effluent prohibited the growth of microalgal culture, whereas the lowest concentration of 10 % enhanced the microalgal lipid content (26.73^{a}±.74% DW), carbohydrate content (15.75^{a}±.16 %),and induced a marginal increase in dry biomass ($.191^{a}$ ±.009 gL^{-1}).Accordingly, the practical application of microalgal growth offers prospective resource utilization.

Keywords:Poultry waste effluent, *Scenedesmus incrassatulus* , Biostimulant.

1.Introduction

The poultry industry is one of the main agricultural industries in Egypt, where investment in this industry is about 18 billion of Egyptian pounds. A significant portion of the nation's supply of animal protein (white meats and eggs) comes from this industry[1]. Poultry farms generate a significant quantity of trash at this production level. Feathers, urine, feces, and bedding materials are examples of waste items. Poultry waste is the cause of several issues. The main waste disposal methods are landfilling, incineration, and burial[2]. These methods are very dangerous for the land and water bodies. Incineration is an expensive and energy-intensive method for waste disposal and gas emissions that is again a challenge for the environment. Combustion of poultry waste during incineration produces particulate matter such as carbon dioxide (CO_2), carbon monoxide (CO), dioxins, polycyclic aromatic hydrocarbon, sulfur (SOX), and oxides of nitrogen (NOX) [3]. Poultry excreta contain nitrogenous and phosphorus compounds such as uric acid, ammonical nitrogen, carbonaceous compounds, and minerals[4, 5]. Most poultry waste is utilized as organic fertilizer, and over time, the nutrients in the soil reach natural water supplies and cause ecological problems like eutrophication. Therefore, management of poultry waste in suitable ways is needed, which must be friendly to the environment. For the healthy growth of microorganisms, wastewater that contains nutrients may be a viable substitute for expensive nutrient medium[6]. Microalgae have been proposed as possible bioremediation candidates because of their ability to survive in any season and their broad adaptability to various ecological situations[7]. Using microalgae decreases the negative effects of conventional wastewater treatments[8]. Autotrophic and heterotrophic microalgae provide the O_2 that can use to oxidize organic carbon, as well as nitrogen and phosphorus[9, 10]. However, they also supply the CO_2 that is necessary for effective microalgael cell development. The use of microalgae for

wastewater bioremediation has been studied for a variety of effluents, including agro-industrial and municipal ones[11-13]. Following pretreatment, the wastewater from poultry slaughterhouses can be utilized as a culture medium for microalgae development, which will aid in their remediation by allowing them to consume both organic and inorganic nutrients for producing their biomass. The main purpose of this research was to cultivate *Scenedesmus incrassatulus* on poultry waste effluent (PWE) to improve the biochemical constituents of microalgal cells, including lipid and carbohydrate content in an economical and environmentally sustainable manner.

2.Materials and Methods

2.1 Isolation and purification of green microalgal species

The fresh sample was obtained from a water canal near a poultry farm Daqahlia, Egypt (30º51'48.6"N31º15'21.2"E).the date when the sample collected from 10th June 2021.

The obtained sample was centrifuged at 4000 rpm for 10 min. The supernatant was decanted and the pellet was collected into a sterile test tube containing a sterilized Bold's Basal Medium (BBM)[14].The Fresh biomass was used to isolate the species following the streak plate method[15]. *S. incrassatulus* was identified under light microscope for its morphological characteristics.

2.2 PWE preparation

Wet poultry waste was obtained from a poultry farm. It was dried in an oven at 60 C ^{o} until solid. Then, it cooled and ground into a powder. Despite poultry waste, all feathers, sawdust, wood shavings, straw, Empty shells, infertile eggs, and deceased embryos. 10 g of poultry waste powder was weighed and dissolved in 200 mL of distilled water. Then, it was infused for 24 hrs using a magnetic stirrer. The resulting solution was filtered to get the supernatant and remove the residue. Finally, it was boiled for 1 hr, centrifugated to remove the pellets, and autoclaved at 121 C ^{o} for 20 min to be accessible for immediately use[16].

The preliminary experiment of *S. incrassatulus* cultivation on different concentrations of PWE.

The preliminary screening was performed with different concentrations of PWE to distinguish the most proper concentration for *S. incrassatulus* cultivation. In sterilized 10 mL vials, 1 mL of the microalgal pellet (10% v/v) was inoculated with various concentrations of PWE (10-100%) cultivated on BBM to attain the final volume of 5 mL.

(**Table** 1**:** :incubated for seven days at 26 ^{o} C and 16:8 h light: dark duration cycle of 50 µmol photons m^{-2} s^{-1}. At the end of the incubation period, the microalgal growth was estimated by direct cell count using a standard hemocytometer technique[17].

Table 1: Different concentrations of PWE examined for the growth of *S. incrassatulus*.

Concentration of PWE (%)	10	20	30	40	50	60	70	80	90	100	Control culture
PWE (mL)	0.5	1	1.5	2	2.5	3	3.5	4	4.5	5	-
BBM (mL)	4.5	4	3.5	3	2.5	2	1.5	1	0.5	-	5

2.3.1. Cultivation of *S. incrassatulus* on minimum concentrations of PWE

Based on the preliminary experiment, the highest concentrations (30-100%) of PWE inhibited the growth of *S. incrassatulus* culture. Accordingly, the minimum concentrations of PWE (10-25%) were tested for microalgal growth. In sterilized 2000 mL Erlenmeyer flasks, The PWE concentrations were prepared by mixing different volumes of PWE and BBM (v/v%) to attain the final volume of 1200 mL in addition to the control culture (

Table 2). Afterward, three replicates for each concentration were prepared in sterilized 500 mL Erlenmeyer flasks containing 400 mL from the previous mixture and inoculated with 40 mL of microalgal pellet. The microalgal cultures were incubated for seven days at 26 ºC and 16:8 h light: dark duration cycle of 50 µmol photons m^{-2} s^{-1} and continuous sterilized air bubbling. The microalgal growth was determined daily by measuring the optical density at 440 nm[18]. Then, the microalgal cells were harvested by centrifugation at 4000 rpm for 10 min and dried in an oven at 60° C to a constant weight and expressed as g L^{-1} [19] and therefore were utilized to assess the total lipid and carbohydrate content.

Table 2: The minimum concentrations of PWE tested for *S. incrassatulus* cultivation.

Concentration of PWE (%)	10	15	20	25	Control culture
PWE (mL)	120	180	240	300	-
BBM (mL)	1080	1020	960	900	1200

2.4. Estimation of total lipid content.

The sulfo-phospho-vanillin method was used to determine the amount of total algal lipids; therefore, the resulting color was spectrometrically measured at 530 nm[20]

2.5. Estimation of total carbohydrate content.

The total carbohydrate content of *S. incrassatulus* biomass was determined by a simple and rapid calorimetric method of phenol-sulfuric acid [21]. The developed color was measured spectrometrically at 490 nm.

2.6. Statistical analysis.

All analyses were tested and averaged in triplicate. The standard deviations (SD), and standard errors (SE) were calculated as well. The CoHort/ CoStat version 6.311 program was applied for calculating the one-way Analysis of Variance (ANOVA) followed by Least Significant Difference tests (LSD). Probabilities less than 0.05 were assumed significant (n=3).

3.Results and Discussion

3.1. Morphological identification of the isolated green microalgae

Scenedesmus incrassatulus was identified morphologically according to Bohlin [22] as shown in **Figure 1**. *S. incrassatulus* is a fresh green microalgal species that are colonial and non-motile. They are an autospore species having four multinucleated cells inside the paternal mother wall. The colony composed of (2)-4-8 fusiform, subacute cells arranged in either 1 or 2 series (alternating), median cells slightly curved, outer cells definitely curved, with the free walls strongly concave, apices of the cells with a nodular thickening, cells 5-8 µ in diameter, 17-24µ long. using florescent microscopical investigations. *S. incrassatulus* is a fresh green microalgal species that is colonial and non-motile. It is an autospore-forming species having multinucleated four cells inside the mother cell wall[23].

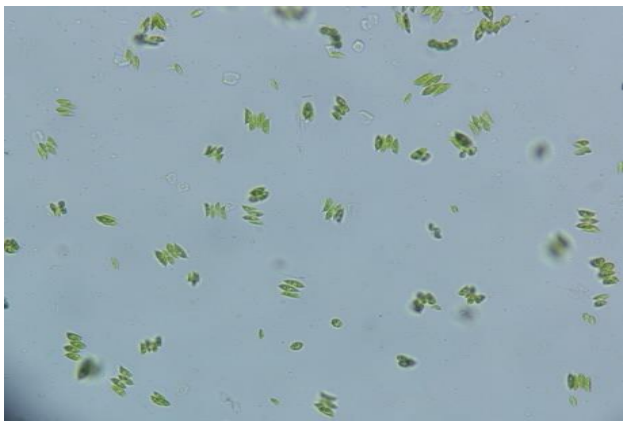

Figure 1: Morphology of *S. incrassatulus* under florescent microscope.

3.2. The growth of *S. incrassatulus* grown on different concentrations of PWE.

The microalgal cell count grown on various concentrations of PWE ranged from 10 to 100% as illustrated in **Figure 2**. The concentration of 10% PWE exhibited the highest growth of cell count (49×10^4 ^{a}±3.78 cell mL^{-1}, followed by the concentration of 20% PWE (41.66^{b}±3.41). However, the cell count of other PWE concentrations (30-100%) was significantly decreased ($p \geq 0.05$) compared to control culture ($36 \times 10^{4\ b}$ ±. 272 cell mL^{-1}) of *S. incrassatulus*. The obtained results agreed with Khiewwijit, Chainetr [24] whose results demonstrated that the microalgae scheme was effectively constructed and had a great treatment performance when cultivated on poultry waste.

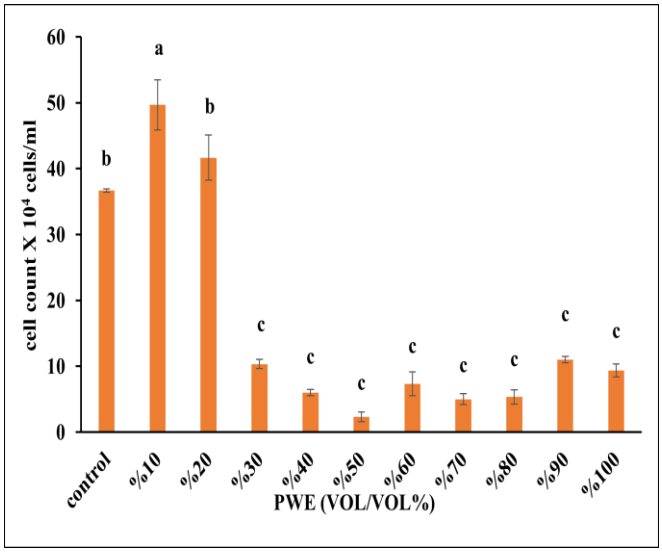

Figure 2: Cell count of *S. incrassatulus* grown on different concentrations of PWE.Different superscript letters refere to significant variation with CoHort/ CoStat version 6.311 program at $p \leq .05$, where a >b.

3.3. The growth assessments of *S. incrassatulus* grown on minimum concentrations of PWE

3.3.1. Estimation of optical density of *S. incrassatulus* cultivated on PWE.

The optical density of *S. incrassatulus* culture cultivated on PWE concentration (10-25%) was represented in **Figure3**.It was obvious that the cell optical density measurement of 10% and 15% PWE increased steadily until reaching their maximum value on 7th day (1.398^{a} ± .02 and 1.355^{a} ± .039), respectively compared to control culture (1.195^{ab} ± .136). However, the concentrations of 20% and 25% PWE achieved an ongoing increase until the third day then had a marginal decrease on the fourth day and finally increased to 1.064^{ab} ±.044 and 0.92^{b} ± .002 respectively at the end of incubation period on the seventh day.

The existing findings agreed well with findings obtained by Tirok and Scharler [25] whose results assumed that the highest turbidity of maximum wastewater concentrations decreases the penetration power of light in the cultivation medium and therefore inhibits microalgal cell growth.

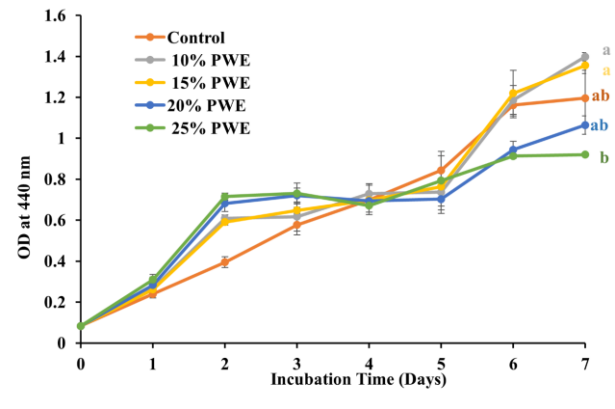

Figure3: growth curve for *S. incrassatulus* at 440nm cultivated on PWE. Different superscript letters refere to significant variation with CoHort/ CoStat version 6.311 program at p ≤ .05, where a >b.

3.3.2. Estimation of dry biomass of *S. incrassatulus* cultivated on PWE.

The results in **Figure 4**.represented the dry biomass of *S. incrassatulus* grown on minimum concentrations of PWE. The concentration of 10%, and 15% PWE maintained a significant increase in dry wt. (.191^{a}±.009 and .174^{a}±.001g L^{-1}), respectively with negligible difference with control culture (.159^{a}±.006). Conversely, the concentrations of 20% and 25% PWE exhibited a significant decrease ($p \geq 0.05$) in dry weight (.126^{b}±.0009, and .125^{b} ± .003), respectively.

The marginal increment of *S. incrassatulus* dry biomass grown on 10 % PWE was agreed with the documentation by Singh, Tyagi [6], who demonstrated that the highest dry biomass of *Chlorella pyrenoidosa* cultivated on 25 % poultry waste was (2.5 g L^{-1}) higher than the produced biomass (1.5 g L^{-1}) that cultivated on BBM as a control culture. The microalgae steadily absorbed the appropriate nutrient concentration in PWE, accumulating a large amount of biomass. However, the presence of an excess nutrient could have significantly impacted microalgae growth.

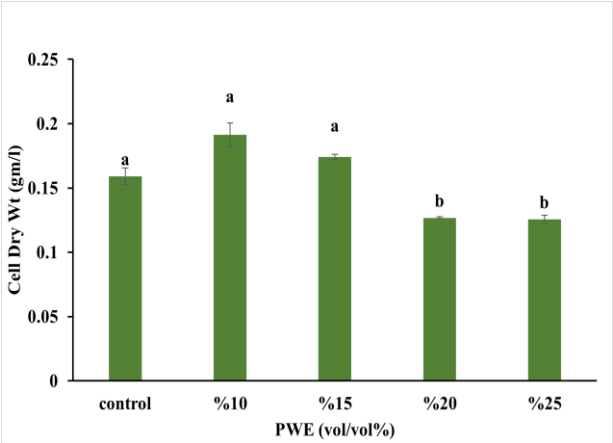

Figure 4: Estimation of dry biomass (gm/l) of *S.incrassatulus* cultivated on PWE. Different superscript letters refere to significant variation with CoHort/ CoStat version 6.311 program at p ≤ .05, where a >b.

3.4. Estimation of total lipid content of *S.incrassatulus* cultivated on PWE.

The lipid content of *S. incrassatulus* grown on minimum concentrations of PWE was displayed in **Figure 5**. The concentration of 10 % PWE maintained a significant increase (p ≤0.05) in lipid content) (26.73^{a}±.747%DW) while the concentrations of 15%,20%,25% PWE declined considerably to yield 22.25^{b}±.399 ,17.69^{b}±1.14, and 17.83^{b}±.236 respectively, compared with the control culture (21.62^{b}±.356). The obtained results agreed with the results discussed by Singh, Tyagi [6].in which the lipid content of Chlorella pyrenoidosa exhibited a high accumulation of

lipid amount (0.49 g L^{-1}) when grown on 25 % poultry waste

The stimulation of lipid content in *S. incrassatulus* may be attributed to the higher concentration of nitrogen and phosphorous in the cultural waste medium because these elements are restrictive elements of microalgal cell metabolism, which regulates the lipid accumulation in microalgae[6, 26].

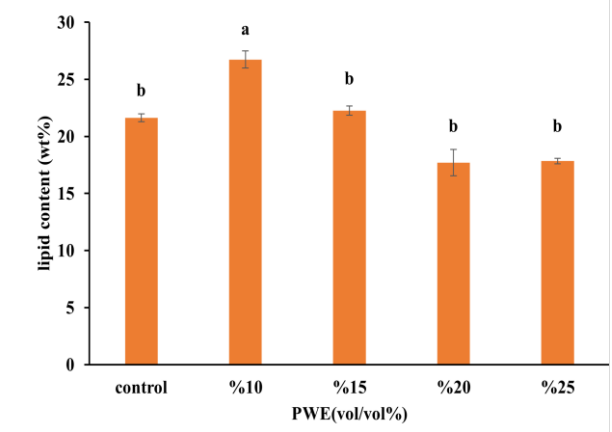

Figure 5: Estimation of lipid content (wt%) of *S. incrassatulus* cultivated on PWE. Different superscript letters refere to significant variation with CoHort/ CoStat version 6.311 program at $p \leq .05$, where a >b

3.4. Estimation of total carbohydrate content of *S. incrassatulus* cultivated on PWE.

The illustrated results in **Figure 6**, represented the carbohydrate content of *S. incrassatulus* carbohydrate content grown on minimum concentrations of PWE. The concentration of 10 % PWE displayed a significantly highest concentration, (15.76^{a} ± 0.16 %), followed by the control culture (14.1^{a}± .002 %). Although the further concentrations of PWE (15%, 20%, and 25%) demonstrated a considerable reduction in carbohydrate content to be 8.48^{b}± .95, 8.22^{b} ± .114, and 7.32^{b} ±.07, respectively. The findings are in accordance with the findings reported by Hasnain, Abideen [27] they approved that the carbohydrate content of Oedogonium sp., Cladophora sp., Ulothrix sp., and Spirogyra sp. was stimulated by growing on poultry waste exhibiting 76, 82, 80, and 89 % respectively. Carbohydrate concentration is proportional to microalgae growth, and its accumulation is determined by the availability of nutrients in the culture medium. Therefore, the growth of microalgal cell biomass increased carbohydrate content[6, 27].

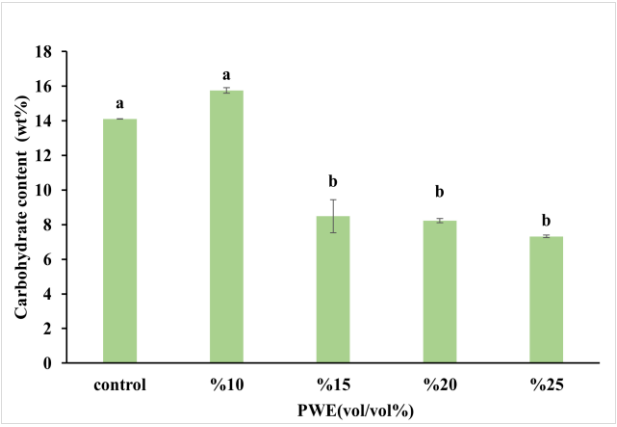

Figure 6: Estimation of carbohydrate content (wt%) of *S. incrassatulus* cultivated on PWE. Different superscript letters refere to significant variation with CoHort/ CoStat version 6.311 program at $p \leq .05$, where a >b

4.Conclusion

It was concluded that *S. incrassatulus* was recovered and cultivated on 10%PWE that was enriched with numerous micronutrients. Therefore, the constituents of micro-algal cells, specifically lipid, and carbohydrate contents were significantly stimulated,resulting in a marginal increase in dry biomass. In this context, it was cost-effective to reutilize PWE as a low-cost resource for the cultivation of microalgae.

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