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Pigments Extraction of Treated Hybrid Microalgae-Activated Sludge

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Abstract

Microalgae have the ability to grow in wastewater, remove heavy metal ions and pollutants, and can be used to produce renewable energy alternatives such as biofuels, biogas, biomethane and biohydrogen. Algae can also produce high-value non-energy pigments such as chlorophylls and carotenoids that are used in feeds, colorants, textiles, nutraceutical and pharmaceutical industries. Methanol extraction method was employed to extract the pigments from microalgal specie Chlorella vulgaris spectrophotometrically after bioremediation of synthetic tannery wastewater (STWW) in stirred-tank photobioreactors (STPBRs) operated at about 580 $\mu mol.m^{-2}.s^{-1}$ and 100 rpm for 12:12 light-dark cycles. The maximum Chl a, Chl b, total carotenoids and total pigments were determined to be 35.5091, 8.6315, 1.9521 and 41.850 $\mu g/L$, respectively and highest content increment of 66.91, 66.97, 69.11 and 69.38% in reactor A, B, C and D, respectively, was achieved during the bioremediation process.

Keywords: photobioreactors, pigments, chlorophyl, carotenoids, bioremediation

1. INTRODUCTION

Microalgae are widely employed in bioremediation processes due to their even distribution, high tolerance to environmental conditions [1] as well as their modes of nutrition and metabolic activities [2]. Algae can easily grow in the presence of moisture thereby utilizing the available energy from light to photosynthesize [3] and increase in concentration within a short period of time [4], accumulate pollutants through the cell surface [5] and absorb positively charged heavy metal ions present in the wastewater stream to their cell walls by anionic ligands [6, 7]. Several types of research reported the extraction of energy molecules from algal biomass that range from bioethanol, biodiesel, biogas, biohydrogen, methane and many other valuable energy alternatives [1, 8, 9] that make algal bioremediation more attractive. Despite these advances in the treatment prospects, the extraction of energy molecules from microalgae is associated with some setbacks including low productivities and high extraction cost that hinders the full utilization of algal renewable energy alternatives compared to conventional fossil fuels.

Several studies highlighted some ways to minimising the production cost of algal biomass extracts as [2] suggest that mass cultivation of microalgae may reduce the production cost and will also increase the quantity of valuable energy molecules extractions. [5, 10–12] indicated increased treatment efficiencies and microbial biodegradability when activated sludge was incorporated into the microalgal treatment due to symbiotic relationship that exist between algae and bacteria by production of oxygen as by-product of photosynthesis which was needed by bacterial cells for waste degradation. Also production of carbon-dioxide as a result of bacterial degradation termed as mineralization by [13], which was cheaply utilized by microalgae in photosynthesis [3]. These treatment approaches enhance the productivity and reduce the cost of stirring accounting for about 40-75% overall treatment cost [14, 15].

The potential high value non-energy molecules comprising pigments such as chlorophylls and carotenoids; lipids, proteins and many more are useful for food, nutraceutical, pharmaceuticals, personal care product industries [16, 17]. However, despite this wide application of non-energy molecules extracted from algae (e.g. chlorophylls, carotenoids, etc.), only little attention was paid to it with regards to its full extraction and utilization, which in turn may decrease the carbon footprint of microalgal cultivation system as well as reduce algal cultivation cost by increasing the quantity of extracted molecules.

Microalgae as photosynthetic organisms contain chlorophylls and carotenoids as pigments that are termed lipophilic molecules which when extracted can easily replace conventional synthetic colouring pigments. Their capability to

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replace synthetic pigments is due to the growing concerns with toxicity of synthetic pigments and the simplicity of extraction approach of these naturally occurring organic pigments as well as their wide applications [18, 19]. Recently, various methods were employed for the extraction of microalgal pigments and these include 90% Acetone [20, 21], 100% Methanol extraction method [8, 19, 22], high performance liquid chromatography [23], mechanical extraction [24] and ultrasound method [25].

The choice of extraction method depends on solvent simplicity, compatibility, cost, yield, and inertness that does not alter the microbial cell composition [24, 26] and as such, organic solvent extraction methods are considered as the best alternative [19, 21, 27]. Methanol was found to be one of the most efficient solvents to extract multiple pigments from algae due to its lysis property in destruction of cell walls that increase the extraction yield [19]. In the current study, methanol extraction process was adopted in order to quantify the amount of chlorophyll a and b, total carotenoids as well as total pigments content during the treatment of synthetic tannery wastewater (STWW) using microalgal specie Chlorella vulgaris in a hybrid microalgae-activated sludge system in semi-continuous stirred-tank photobioreactors, for a period of three weeks.

2. MATERIALS AND METHODS

2.1. Synthetic Tannery Wastewater (STWW)

The 50, 100 and 150mg/L concentrations of STWW was prepared by diluting the specific weight of potassium dichromate with distilled water to obtain the specified hexavalent chromium concentration as described by [6]. The entire chemicals used were of analytical grade.

2.2. Algal Cultivation

Chlorella vulgaris was isolated and cultured in 1L volumetric flask using Bold Basal's Medium (BBM) in accordance with [28], in Herbarium Laboratory, Department of Plant Biology, Bayero University, Kano, for two weeks. This culture was illuminated under 20W white fluorescent bulb in a mechanical shaker at 200rpm. During this period, the algal biomass concentration was estimated using a haemocytometer as described by [29].

2.3. Stirred-Tank Photobioreactors (STPBRs) Operation

The microalgal culture was up-scaled in three STPBRs in accordance with [3, 10]. The 21 L STPBR consisting of 16 L working volume was illuminated using red light-emitting diodes (LEDs) at an optimum irradiance of 582.7 μmol.m^{-2}.s^{-1}, 100 rpm for 12:12 light-dark cycles. Three STPBRs were inoculated with 10% activated sludge culture of the BBM-microalgal culture and labelled as B, C and D. Each of 50, 100 and 150 mg/L of STWW was introduced to B, C and D respectively; while a separate reactor A was inoculated using 50 mg/L of STWW in the dark with no enhancements of aeration and illumination. The STPBRs were wrapped with aluminium foil to concentrate light internally.

Table 1: Pigments extraction profiles for reactor A.

Test No	Chl a	Chl b	TC	TP
1	10.1584	2.2904	0.4842	12.9331
2	10.9242	2.3956	0.4399	13.7598
3	11.9633	2.2833	0.5962	14.8429
4	14.2478	2.7390	0.6939	17.6808
5	16.8851	2.2664	1.2211	20.3727
6	18.2573	4.2255	0.1531	22.6360
7	20.1488	4.1782	0.3780	24.7051
8	22.6096	3.2872	1.1295	27.0264
9	23.3341	4.3809	0.7069	28.4220
10	28.6614	6.1221	0.2762	35.0597
11	28.5356	6.5390	0.0657	35.1405
12	28.3606	6.533	0.0530	34.9474
13	28.3120	0.5358	0.0498	31.8905

2.4. Analytical Tests

Samples were collected from each reactor every 2 days and analysed for chlorophylls a and b (Chl a and b), total carotenoids (TC) and total pigment (TP) contents of the culture for the experimental duration in accordance with [8].

2.5. Pigment Extraction

A sample of 1.5 mL was collected from each STPBR and centrifuged at 17,000 xg for 5 mins, after which the supernatant was decanted. The algal pellets were then suspended in 1.5mL methanol and incubated at 45℃ for 30 mins. The methanol extracts were transferred to plastic cuvettes for spectrophotometric measurements at 470, 653 and 666 nm wavelengths and their respective absorbance values as A470, A653 and A666 were recorded using UV-Vis spectrophotometer and Chl a, Chl b, TC and TP were estimated from the following equations by [8, 30].

$$Chl\,a(\mu g/L) = 15.65A_{666} - 7.34A_{653} \quad (1)$$

$$Chl\,b(\mu g/L) = 27.05A_{653} - 11.21A_{666} \quad (2)$$

$$TC(\mu g/L) = [1000A_{470} - 2.86Chl\,a - 129.2Chl\,b]/221 \quad (3)$$

$$TP(\mu g/L) = Chl\,a + Chl\,b + TC \quad (4)$$

3. RESULTS AND DISCUSSION

Absorbance was recorded in triplicates and their mean values were used for the estimation of Chl a, Chl b, TC and TP from equations 1-4 as presented in Tables 1 – 4 for the four reactors. In reactor A, the initial Chl a concentration at the beginning of the experiment was found to be 10.1585 μg/L which increased continuously up to about first 18 days of the treatment to a maximum of 28.6614 μg/L and then declined to concentration of 28.3121 μg/L at the end of the three weeks treatment period as shown in Table 1.

The Chl b concentration also increased from 2.2904 μg/L to a maximum of 6.5391 μg/L within the period of 20 days and then decreased to 5.5358

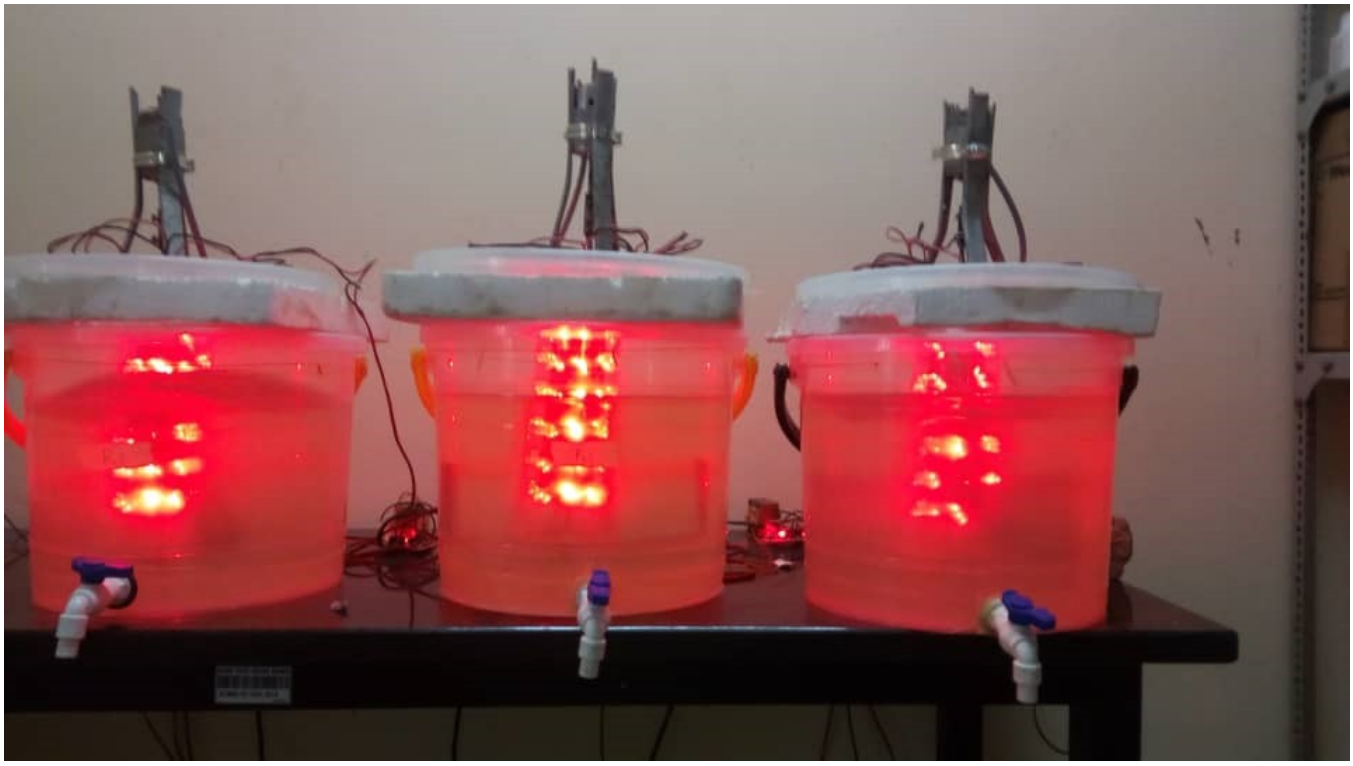

Figure 1: Stirred-Tank Photobioreactors Set-up.

Table 2: Pigments extraction profiles for reactor B.

Test No	Chl a	Chl b	TC	TP
1	10.8563	1.8558	0.7564	13.43
2	11.0347	2.3601	0.4955	13.8904
3	12.8568	2.0574	0.8344	15.7486
4	14.3221	3.2088	0.4319	17.9629
5	16.9283	3.8263	0.3403	21.0950
6	18.2573	4.2255	0.1531	22.6360
7	20.2338	4.4224	0.2930	24.9493
8	22.2256	4.2379	0.7687	27.2322
9	23.3341	4.3809	0.7069	28.4220
10	32.6555	3.7623	1.7036	38.1215
11	32.8066	3.7631	1.7284	38.2981
12	31.4983	4.6348	1.1407	37.2739
13	31.1109	3.9420	1.0542	33.9787

Table 3: Pigments extraction profiles for reactor C.

Test No	Chl a	Chl b	TC	TP
1	10.8475	2.1673	0.5925	13.6074
2	11.1085	2.4597	0.5449	14.1132
3	13.2709	2.4363	0.7251	16.4324
4	15.1147	3.4474	0.3545	18.9167
5	17.9449	4.1224	0.2853	22.3527
6	18.6206	4.4665	0.1161	23.2033
7	20.8896	4.2359	0.5247	25.6503
8	22.6910	4.4275	0.7107	27.8293
9	22.9372	8.6314	1.51938	30.0493
10	35.1164	2.1739	3.1660	40.4564
11	34.4310	3.8635	1.8387	40.1332
12	34.3063	3.6259	1.9521	39.8844
13	33.1684	1.1285	1.5248	38.5745

μg/L after the three weeks. Total carotenoids being the function of chlorophylls content was initially found to be 0.4828 μg/L and then reached a maximum of 1.1298 μg/L at 14 days and subsequently reduced to 0.0498 μg/L at the end of the three weeks period. The total pigments content in A been summation of the all the other extracted pigments, it was found to increase continuously from the initial 12.9332 μg/L to the highest of 35.1405 μg/L and then decreased to 31.8905 μg/L.

In reactor B with enhancement of light and stirring, the Chl a concentration reaches highest growth of 32.8066 μg/L within the 20 days of the treatment from the initial value of 10.8564 μg/L and then decreased to 31.1109 μg/L as indicated by Table 2. The Chl b increased from the initial value of 1.8558 μg/L to a highest value of 4.6348 μg/L and then decreased to 3.9420 μg/L. The total carotenoids increased to a maximum of 1.7285 μg/L from 0.7564 µg/L and then declined to 1.0543 μg/L at the end of the treatment. The total pigment extracted from reactor B, ranges from 13.4686 μg/L to 38.2981 μg/L and then decreased to 33.9787 μg/L after the three weeks treatment period.

Chl a concentration from reactor C increased to a highest value of 35.1164 μg/L from 10.8475 μg/L during the first 16 days of the treatment as shown in Table 3, and then decreased to 33.1684 μg/L after three weeks.

In reactor D, the concentration of Chl a increased to a maximum of 35.5091 μg/L from an initial value of 10.8734 μg/L at 18 days and then

Table 4: Pigments extraction profiles for reactor D.

Test No	Chl a	Chl b	TC	TP
1	10.8734	2.2577	0.5258	13.6569
2	12.0715	1.7917	0.9727	14.8360
3	13.5980	2.4853	0.8235	16.9069
4	15.8561	3.1778	0.5523	19.5863
5	17.9449	4.1224	0.2853	22.3527
6	18.9883	4.5518	0.2832	23.8234
7	21.3791	4.4301	0.5179	26.3273
8	22.6964	5.0121	0.5091	28.2176
9	26.4497	3.2606	1.7197	31.4302
10	35.5091	2.3067	3.8389	41.6548
11	35.2874	3.4026	3.1604	41.8505
12	33.5127	4.0419	2.6739	40.2285
13	32.1789	2.7308	2.5934	37.5031

decreased to 32.1789 μg/L after three weeks of the treatment. Chl b increased to 5.0121 μg/L from 2.2577 μg/L during the first 2 weeks of the treatment and then reduced to 2.7308 μg/L at the end of the bioremediation process and the total carotenoids extracted from reactor D, ranged from the initial concentration of 0.5258 μg/L to a highest value of 3.8389 μg/L within the 18 days of the treatment period and then declined to 2.5934 μg/L at the end of the treatment.

The total pigments extracted in D ranges from a minimum concentration of 13.6569 μg/L to a maximum of 41.8505 μg/L at 18 days and then decreased to moderate value of 37.5031 μg/L after the bioremediation process as shown in Table 4

Chlorophyll concentration depends on the dominance of different algal group in the biomass population and its changes in content occurred during ontogenesis of individual cell and also the development of the whole biomass [31]. The Chl a concentration in each reactor is about 80% of the total pigment that can be observed from Table 2–4, and this confirmed the assertion that the Chl a is main photosynthetic pigment and that some algae contain only Chl a like *Nanochloropsys gaditana* [19].

Chl a content were found to increase throughout the growth phase of *Chlorella vulgaris sp* in all the reactors and increases from its initial concentrations of about 10 μg/L to a maximum of 35.5091 μg/L, with percentage increment of 66.91, 66.97, 69.11 and 69.38% from reactor A, B, C and D respectively. The lowest percentage increase was observed in control reactor A with no enhancements of stirring and illumination. The increase in the Chl a content in STPBRs increases with increase in the quantity of STWW of 50, 100 and 150mg/L in reactors B, C and D respectively. This increase as a result of increasing concentration of wastewater which may be connected to the fact that the Chl content was not directly proportional to the illumination as described by [20] that the maximum Chl concentration was found in deeper hypolimnion region of the oligotrophic lake.

A reasonable increase in Chl a concentration was observed in reactor A despite the absence of light and mixing and this might be due to natural aeration that exist between microalgal cells and bacterial cells in efficient exchange of gases (carbon dioxide and oxygen). As symbiotic relationships exist between these organisms and as well as the fact that Chl concentration does not depend only on light intensities, [31] ascertained that periods with higher intensities of light were associated with averagely low concentrations of Chl content and also the Chl proliferation was also related to nutrients availability and taxonomic composition of the biomass content [20].

Relatively lower total Chl content of 75.68% of the total biovolume from green microalgae was reported by [31]. While [25] shows an increase in Chl a content of about 58.33% and [32] reported a relatively higher increase in Chl a content of about 95 and 77.77% for mesotrophic lakes and eutrophic lakes respectively and [23] reported a maximum Chl a concentration of 82.14%.

On the other hand, Chl b content in all the four reactors was found to be much lower than the Chl a, with an average of 15.99% compared to about 80% of Chl a content. Remarkable increase in Chl b content was also observed from all the reactors in the treatment, as the concentrations ranged from 64.97, 57.64, 74.96 and 54.95% for reactors A, B, C and D respectively, within 16-20 days of the treatment as also [33] reported a considerable increase in Chl a content was obtained in the 16th day of the treatment. A much less percentage increase in Chl content was observed in reactor D which may be affected by taxonomic culture conditions.

Carotenoids have been described as photosynthetic accessory pigments and play a protective role against oxygen radicals released during photosynthesis from damaging DNA by [34], a reasonable increase in total carotenoids were observed across all the reactors and ranged from 57.12, 56.24, 81.28 and 86.30% from reactor A, B, C and D respectively from 14-20 days of the treatment. The highest total carotenoid concentrations were found in reactors with higher wastewater strength of 100 and 150 mg/L. The maximum total carotenoid content of 57.12% was attained within the second week of the treatment in control reactor A.

[35] reported similar total carotenoid content increase of 64.15% which was lower than the highest total carotenoid concentrations obtained in reactor C and D, despite having higher concentration of STWW. Moreover, [36] extracted carotenoid components as β-Carotene and Lutein, with highest values for β-Carotene and Lutein to be 15.9 and 5.2 μg/kg respectively and, while a maximum Lutein increase of about 93.19%, and also 0.95 and 0.31 mg per 100 g of sample for carotene and xanthophyll was reported by [34] which were part of the total carotenoids extracted. These were specialized experiment designed for the purpose of pigment extraction whereas in this current study, the extraction of pigments was performed after STWW bioremediation process.

The quantities of pigments are extracted during the hybrid microalgae-activated sludge bioremediation of synthetic tannery wastewater cou-

pled with reasonable higher removal efficiencies of Cr(VI) ions, ammonium, nitrate and phosphates. However, these findings were limited to only co-culture of algae and bacteria to exploit their symbiotic relationship, but the capability of mono-culture of algae and bacteria to produce these pigments should be investigated and real tannery wastewater should also be employed, with the same or different treatment approach as contained in present study.

4. CONCLUSION

Following the successful bioremediation of STWW using a hybrid microalgae-activated sludge treatment system with *C. vulgaris* specie, the maximum Chl a, Chl b, total carotenoids and total pigments of 35.5091, 8.6315, 1.9521 and 41.8505 μg/L respectively were extracted and the highest pigments increment attained was 66.91, 66.97, 69.11 and 69.38% in reactors A, B, C and D after the three weeks bioremediation process. It was also observed that the highest growth rates of the biomass were observed in reactor C and D with highest concentration of STWW.

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