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Cultivation of *Desmodesmus multivariabilis* for the Treatment of Cosmetic Wastewater

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Abstract: The discharge of cosmetic wastewater into the wastewater treatment systems has become an environmental concern due to high concentrations of nutrients. The current study explored the phytoremediation potential of *Desmodesmus multivariabilis*, under mixotrophic growth, to remove total organic carbon (TOC), sulfur (TS), nitrogen (TN), and phosphorus (TP) from cosmetic wastewater (CWW). The CWW was prepared using samples supplied by a local cosmetic production company (two dyes, two hair relaxers, as well as two shampoos and conditioners). The bioremediation potential of *D. multivariabilis* was tested under four different conditions: raw CWW with 0% CO_2 in the aeration stream (i.e., atmospheric air); pre-treated CWW with 0% CO_2, 2.5% CO_2, and 5% CO_2. Control experiments were run in parallel. Under mixotrophic growth, the microalga performed best at 5% CO_2 in the pre-treated CWW where TOC, TN, TP, and TS removal of >88%, >98%, >95%, and >90% were measured, respectively. The corresponding biomass (dry weight) was >203 mg/L. Relaxers promoted growth most prominently; however, it was observed that there was significant nutrient removal even in the absence of growth in all experiments. The growth followed Liebig's Law, displaying three distinct phases (CO_2 concentration, CO_2 mass transfer, and nutrient limited growth). The results demonstrated the potential for the successful bioremediation of cosmetic wastewater by *D. multivariabilis*.

Keywords: microalgal growth; cosmetic wastewater treatment; mixotrophic phyco-remediation

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1. Introduction

Cultivation of microalgae occurs in a wide range of environments due to their high tolerance to stress induced by environmental factors. Microalgae are found in saltwater, freshwater, and wastewater and can be utilized in biofuel production or wastewater treatment [1]. Microalgae are often considered to be green bio-factories that can produce bioactive compounds. Microalgae are a valuable source of nutraceuticals and renewable energy due to their high growth rate, photosynthetic ability, carbon neutral/negative properties, high content of lipids and carbohydrate, and ability to obtain nutrients from wastewater [2,3].

In recent studies, microalgal cultivation has been combined with wastewater treatments. This negates the cost of nutrients and the use of freshwater that is needed for the cultivation of biomass while remediating the wastewater [4]. Wastewater treatment plants are increasingly concerned with nutrient removal because excess nitrogen and phosphorus in the wastewater may lead to downstream eutrophication and ecosystem damage. Moreover, growing microalgae can reduce nitrogen and phosphorous in wastewater to very low levels [5]. Due to the significant involvement of carbon fixation in the algal metabolism, the process has the potential for carbon neutral or even carbon negative operation, which is an

imperative for climate change amelioration [6,7]. Significant research has been performed on the effects of greenhouse gases on climate change, and the amelioration of these effects has gained much traction [8,9].

Changes in cultivation conditions can affect microalgal growth rates, biomass growth rates, and their nutritional content, such as production of lipids and fatty acids [7,10–14]. These conditions include mode of cultivation, light/dark cycles, and nutrients such as carbon, nitrogen, phosphorus, pH, salinity, and temperature [2,4,11,15]. There are several ways to cultivate microalgae, including photoautotrophic, heterotrophic, and mixotrophic cultures [16]. Some microalgal species can grow in both photoautotrophic and heterotrophic conditions. When grown in heterotrophic conditions, green algae can use organic compounds as their source of energy in the absence of light. When growing in a mixotrophic environment, organisms may use both light and organic compounds [4,16].

Microalgae can help to fix carbon dioxide from industrial exhaust gases, as well as consume dissolved inorganic carbon from the surrounding environment for the purpose of active photosynthesis [2,17]. CO_2 can be sourced from waste gas such as biogas and flue gas. Flue gas compositions vary depending on the difference sources of fuel. Gas emitted by a plant powered by coal typically contains CO_2 in the range of 10–15% CO_2 [18,19]. In addition, CO_2 can be crucial for pH control: Each microalgal species has a specific pH range that maximizes the production of cells and lipids. This range is narrow and specific to each strain [17]. The pH of microalgal cultures rises steadily throughout the day as the microalgae take up inorganic carbon. Higher pH levels limit the availability of carbon dioxide, thus, inhibiting biomass growth. On the other hand, growing algae at a high pH can suppress unwanted biological pollutants [17].

Since most wastewater streams are low in total carbon, the supply of CO_2 (as a source of carbon) is important for photosynthesis, the fixation of carbon dioxide, and the generation of cells with desired products in microalgae. Bubbling CO_2 into algae treated wastewater was shown to increase the biomass of the microalgae and reduce nutrients and elements such as Al, As, Cd, Ni, and Zn [20].

One of the hydrodynamic variables related to the growth of microalgae and the effectiveness of the removal of CO_2 is the mass transfer of CO_2. The mass transfer of carbon dioxide from the air into the media can be a limiting factor in dense algal cultures. The rate of transfer of carbon dioxide (CO_2) from a gas to a liquid depends on many factors, such as the flow rate of the gas, the CO_2 partial pressure, the size and duration of the bubbles, and the lifetime of the gas/liquid mixture. The gas–liquid mass transfer coefficient (k_La) value (CO_2) is a general hydrodynamic parameter that is often used to evaluate the functioning of the bioreactor in the cultivation of microalgae. The high value of k_La indicates improved mass transfer of CO_2 to the microalgal culture [21]. The determination of CO_2 transfer rate is very important because it affects algal biological and physicochemical processes and the concentration of CO_2 in solution [22].

Types of wastewater that have been successfully tested for potential growth of algae include piggery wastewater, municipal wastewater, monosodium glutamate wastewater, and industrial wastewater such as pulp and paper and dairy wastewater [4,20]. An excellent example is the study on *Scenedesmus sphaerocephalus,* which was used to treat wastewater from swine farms, and it was reported that the removal of P, N, and TOC in piggery wastewater was 83%, 87%, and 12%, respectively, within 40 days [10,23].

The current state of knowledge concerning wastewater treatment from the cosmetic industry is low, as evidenced by the scarcity of publications related to this topic. The cosmetic industry generates wastewater with high levels of COD (>100,000 mg/L), BOD_5, and TOC and high concentrations of petroleum ether extracts, organic nitrogen, organic phosphorus, suspended solids, grease, oils, and detergents (>1700 mg/L) [24]. The generated wastewater contains a combination of various chemicals such as acids, alkalis, dyes, expanders, bleaching agents, and other organic and inorganic compounds. These substances can to a large extent be toxic to humans and the environment and can accumulate in many components of ecosystems [25,26].

Different types of treatment processes are used to treat cosmetic waste, mainly by coagulation/flocculation [27], ultra-filtration, advanced oxidation and catalytic wet peroxide oxidation processes [28]. Current studies on cosmetic wastewater in South Africa utilize rapid granular multimedia filtration systems. It was noted that nutrient removal was up to 98% and organics removal was up to 68% [25]. However, the widespread application of this technology has not yet been realized and the majority of cosmetic wastewater is discharged directly to the sewer and, subsequently, conventional wastewater treatment plants, where it adds to the already strained water treatment system [25]. The cheapest and most widely used cosmetic wastewater treatment method is the application of biological processes. However, the effect obtained is not always satisfactory, especially in the case of high concentrations of oils and greases, and studies have shown that surfactants, especially at higher concentrations, can significantly impair biological processes [26,29].

Microalgal phyco-valorization of wastewater and waste gas is a promising technology that offers the integration of wastewater treatment, greenhouse gas generation, and the usage of fossil fuel alternatives into a single solution. Despite the immense potential that this emerging technology may hold, there has not yet been any significant commercialization [30].

The main aim of this study was to research the improvement of low-carbon cosmetic wastewater treatment with the addition of CO_2 and explore biomass growth rates and nutrient uptake due to culturing microalgae in low organic carbon cosmetic wastewater supplemented with CO_2. This study analyzed synthetic cosmetic wastewater before and after pre-treatment to identify the components of the wastewater. The concentration of carbon dioxide and the ensuing ratio of organic to inorganic carbon in the media were varied. This was achieved by diluting CO_2 with air to CO_2 concentrations at 0%, 2.5%, and 5%, respectively. The study was limited to ambient temperatures and pressures.

The research will provide a starting point for further research into treating cosmetic wastewater and waste gas streams together in an integrated process, which holds a potential economic benefit as growing algae on site may yield valuable biomass.

2. Materials and Methods

2.1. Microlagal Culture Source and Preparation

The microalgae culture was extracted from cultures isolated from the Hartbeespoort Dam reservoir, a eutrophic dam in the North West province of South Africa [31]. Algal samples used for identification were isolated using the streak plating technique and cultivated in Bold's Basal Medium with three-fold nitrogen and vitamins (3N-BBM+V) [32]. The identity of the algae was confirmed as *Desmodesmus multivariabilis* [19]. Before inoculation, algal starter cultures were cultivated to a cell density of 200–450 mg/L in 3N-BBM+V. Starter cultures were cultivated for at least three weeks before being used for inoculation.

2.2. Cosmetic Wastewater Source and Preparation

The raw wastewater contained suspended matter or particulates and possibly harmful pathogens that could interfere with the experimental results. It was, therefore, treated by filtration and sterilization (by autoclaving) before use [33]. It was filtered two to three times by using vacuum filtration. A filter flask (all glassware used, unless otherwise stated, was manufactured by Schott-Duran (Jena, Germany) and supplied by Glassworld, Johannesburg, South Africa) was connected to a vacuum pump (Sartorius (Gottingen, Germany), supplied by Labotec (Johannesburg, South Africa), and Whatman No. 1 filter paper (supplied by Glassworld, Johannesburg, South Africa) was used. The hair products were provided by the research group at L'Oréal South Africa and prepared at the Water Utilisation laboratories at the University of Pretoria, as shown in Table 1.

Table 1. Hair products supplied by L'Oréal South Africa for the preparation of synthetic cosmetic wastewater used in the study.

Routine	Products	[1]FLA Numbers
R1	Dark and Lovely Superior Moisture Plus Kit Relaxer, shampoo, and conditioner	1185566 02, 8900 SA2, 43122 SA7, 43123 SA3
R2	Wave Nouveau perm	42172 SS6, 42180 SS9, 42098 SS4
D1	Inoa black Dye	1094875, 1071397
D2	Majirel red Dye	1162585, 178914 U
SC1	Au Naturale Moisture-Replenishing Shampoo and Knot-Out Conditioner	42914 SS1, 42913 SS5
SC2	Mizani Scalp Care Shampoo and conditioner Mizani Scalp Care Conditioner	42517 SS6, 42518 SS5

[1]FLA defines Fair Labor Codes [34].

After filtration, the wastewater was characterized and then transferred to 3-L glass jars (Consol, South Africa), supplied by Plastilon (Pretoria, South Africa), which were sealed and autoclaved at 121 °C at 2 bar (using a Hirayama HV-series autoclave supplied by Labotec, Johannesburg, South Africa) for 20 min before being stored at 4 °C for long-term storage.

2.3. Carbon Dioxide Source and Preparation

Simulated flue gas (801240-IE-A–GOC MIX 1017) was obtained from African Oxygen Limited (trading as AFROX, Johannesburg, South Africa) with a certified volumetric CO_2 content of 12.35–13.65%, the balance being made up of oxygen (19–21%) and nitrogen. Analytical grade air (513207-SE-C–AIR IG ZERO), also from African Oxygen Limited, was used to dilute the flue gas to the desired concentration. Atmospheric air was used to supply two of the experiments that were not enriched with CO_2, using an off-the-shelf aquarium pump (model AP318) from Daro (Pretoria, South Africa).

2.4. Experimental Design and Planning

The lighting was supplied by a lighting rig equipped with 2 × Osram L 36W/77 Floura fluorescent bulbs per experiment, with two rigs in total per run. A light/dark cycle of 14/10 h was used to approximate optimal conditions experienced by the microalgae in their natural environment, i.e., the lighting conditions experienced by the micro-organism during the summer months. The reactors themselves were designed to minimize contact with unfiltered atmospheric air. Figure 1 and Table 2 summarize the experimental set up.

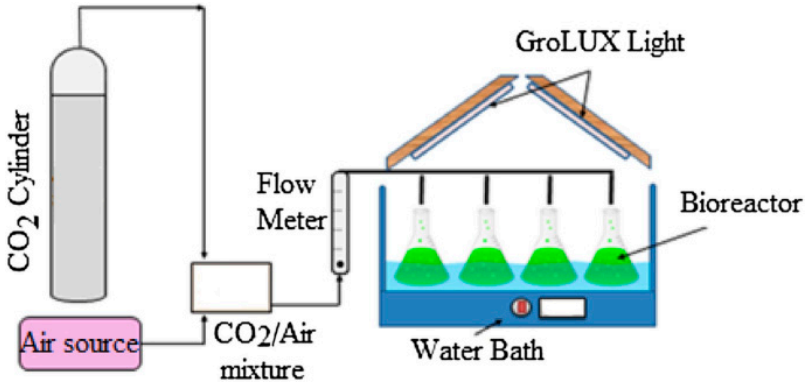

Figure 1. Experimental set up.

Table 2. Summary of the experimental set up.

Experiment	Wastewater	Aeration	Inoculation	Period	Repeats
1	Raw	0% CO_2	15 mL	14 days	3
2	Pre-treated	0% CO_2	15 mL	14 days	3
3	Pre-treated	2.5% CO_2	15 mL	5 days	3
4	Pre-treated	5% CO_2	15 mL	5 days	3
Control	Pre-treated	0% CO_2	0 mL	14 days	3

Experiments supplied with the air-CO_2 are termed "active aerated experiments", while those that only received air from the atmosphere are termed "passive aerated experiments".

2.5. Sampling and Methods of Analysis

Sampling of the reactor content was performed twice a day for the duration of the experiments, and the media was stored in 10 mL centrifuge tubes. Ultraviolet-visible spectrophotometry (WPA LightWave II, supplied by Labotech, Johannesburg, South Africa) was used to track the growth of the algae in the mixed liquor. The growth was tracked at 680, 660, and 600 nm.

A Hettich Universal 320R Centrifuge was used for biomass separation by centrifuging at 6500 RPM for 10 min. The biomass was dried using Mettler-Toledo XS205 Dual Range at 50 °C for 24 h before being weighed. This was recorded as the dry weight cell biomass. Filtration through Whatman No. 1 filter paper was performed before the samples were diluted to a ratio of 1:30 with distilled water prior to TOC analysis.

The experiments were performed until there was a noticeable decrease in growth and the cultures started exhibiting maintenance growth characteristics, i.e., metabolically active yet non-reproducing. The growth and mixed liquor were harvested after termination, and the cellular material was separated using centrifuging. The supernatant, which is cell-free, was analyzed for organic carbon, nitrogen, pH, sulfur, and phosphorus concentration.

A TOC analyzer (Shimadzu TOC-VWP, supplied by Shimadzu Corporation, Kyoto, Japan) was used for analysis of the TOC concentration of the supernatant. Total nitrogen analysis was performed using the Spectroquant 14763 and 14537 kits with the Spectroquant Nova 60 spectrophotometer (all Spectroquant kits and accessories were manufactured and supplied by Merck, Darmstadt, Germany). For chemical oxygen demand (COD) analysis, the Lovibond 2420721 kit was used with a Lovibond COD Vario photometer (Model number 209250; all Lovibond kits and accessories were supplied by Selectech, Johannesburg, South Africa). A Hanna Instruments analyzer and analysis kit (HI 93717, supplied by Hanna instruments, Woonsocket, RI, USA) was used for total phosphate analysis. Total sulphate (TS) analysis was performed using the Spectroquant 14763 and 14537 kits with the Spectroquant Nova 60 spectrophotometer (all Spectroquant kits and accessories were manufactured and supplied by Merck, Darmstadt, Germany).

The algal sample was placed in the cooled-down crucible, which was placed in the Shimadzu TOC-SSM 5000A solid TOC (total organic carbon) analyzer, which operates with analytical oxygen as an oxidizer and nitrogen as carrier gas (both gases are instrument grade, obtained from Afrox, Johannesburg, South Africa).

2.6. Definition of Data Metrics

Analysis of data was then performed, with a summary shown in Table 3:

Table 3. Definition of data metrics.

Description	Parameter	Unit	Equation/Definition	
Average biomass growth rate	$\overline{r_X}$	mg/(L.d)	$Y = \frac{M-M_i}{\Delta t}$	(1)
Specific growth rate	μ	1/d	$\mu = \frac{1}{X} \cdot \frac{dX}{dt}$	(2)
TOC removal efficiency	%TOC	%	$\%TOC = \frac{TOC-TOC_i}{TOC_i} \times 100$	(3)
COD removal efficiency	%COD	%	$\%COD = \frac{COD-COD_I}{COD_i} \times 100$	(4)
N removal efficiency	%N	%	$\%N = \frac{TN-TN_i}{TN_i} \times 100$	(5)
P removal efficiency	%P	%	$\%P = \frac{TP-TP_i}{TP_i} \times 100$	(6)
S removal efficiency	%S	%	$\%S = \frac{TS-TS_i}{TS_i} \times 100$	(7)

M, Mi (mg)—Total mass of cells produced; Initial mass of cells inoculated. TOC_i, COD_i (mg/L)—Initial concentrations of total organic carbon, chemical oxygen demand. *TOC*, *COD* (mg/L)—Final concentrations of total organic carbon, chemical oxygen demand. Δt (d)—Duration of the experiment. $\frac{dX}{dt}$ (mg/d)—Rate of growth. *TN, TP, TS* (mg/L)—Final total nitrogen, phosphate, and sulphate concentrations. TN_i, TP_i TS_i (mg/L)—Initial total nitrogen, phosphate, and sulphate concentrations.

2.7. Wastewater Characterization and Initial Growth Profiles

Growth was measured by performing both UV-Vis growth measurement and gravimetric growth measurement to have a form of inherent redundancy in the results that would ensure the robustness of the data presented [35]. To reduce the amount of scattering, which was required to build a good correlation, the results of all three wavelengths were fed into an optimization where the parameters in Equation (8) were optimized to maximize the R^2 obtained [36].

$$Optimised\ Absorptivity = 9.66OD_{680} - 1.56OD_{660} - 6.48OD_{600} \quad (8)$$

Linearized graphs of cell density vs optimized absorptivity were plotted for all six media, and results are given in Table 4. The regression of the cellular density over the absorptivity (results of the UV-Vis measurement from all the experiments were combined for every wavelength) showed a linear correlation, with the R^2 value of the fit being 0.82 and 0.97 for the relaxers, 0.96 and 0.94 for the dyes, and finally, 0.95 and 0.93 for the shampoos and conditioners.

Table 4. Graphical constants for the linearized graph of cell density (mg/L) vs optimized absorptivity in different CWW media.

Parameter	R1	R2	D1	D2	SC1	SC2
R^2	0.8179	0.9672	0.9648	0.9434	0.9503	0.9316
Slope	140.75	139.62	39.303	39.121	71.281	39.241
Intercept	4.773	5.9393	3.2167	8.7563	2.851	6.627

The wastewater characterization indicated that the dyes contained the highest initial concentration of organics in the media; Table 5 shows the results from the initial characterization.

Table 5. Initial measurements for CWW media.

	Parameter	Unit	R1	R2	D1	D2	SC1	SC2
Experiment 1	COD	mg/L	2483	4670	14,586	9968	1379	2498
	TOC	mg/L	45	47	22	71	53	50
	TN	mg/L	61	7.1	51	44	43	14
	TS	mg/L	179	248	1238	239	52	87
	TPO	mg/L	45	6	51	70.8	66	70
	pH	-	10.07	9.03	9.3	9.55	7.24	7.81
Control, Experiments 2–4	COD	mg/L	1983	4120	11,102	9662	1296	2296
	TOC	mg/L	42	33	18	67	56	46
	TN	mg/L	58	6.0	16.6	39.2	41.3	10
	TS	mg/L	124	163	1170	216	37.5	70
	TPO	mg/L	39	5.5	78.4	65.4	57	61
	pH	-	10.07	9.03	9.3	9.55	7.24	7.81

3. Results and Discussion

3.1. Growth of Profiles of Desmodesmus multivariabilis in Cosmetic Wastewater

This section of the study investigated whether *Desmodesmus multivariabilis* effectively removed the TOC, COD, and total phosphorus (TP), total sulfur (TS), and total nitrogen (TN) from cosmetic wastewater while simultaneously monitoring the resulting biomass growth. The batch reactors that were inoculated with *D. multivariabilis* showed better nutrient removal performance than the control experiment. Rapid growth was observed in experiments that were pre-treated and supplemented with CO_2.

Cultures in Experiments 3 and 4 showcased the most rapid growth, and it was noted that the cultures reached the stationary phase in the shortest period. The cell concentrations and growth rate of cells harvested are shown in Table 6. Harvesting of the biomass was inhibited in the media with the shampoos and conditioners (SC1 and SC2) due to the presence of surfactants and the media with the dyes (D1 and D2) due to the suspended particles of the pigments. The biomass was embedded in the surfactants, and the dye pigments and flocculation of the algae was prevented. This caused the discrepancy in the values of the harvested biomass and the calculated cellular biomass. However, gravitational analysis was deemed more accurate than spectrographic analysis due to the morphology of the cosmetic wastewater.

Table 6. Biomass concentrations and average growth rates of cells harvested. The results show the average ± standard deviations of the triplicate experiments.

Parameter	Exp [1,2]	R1	R2	D1	D2	SC1	SC2
Biomass concentration (mg dry Biomass/L)	1	30 ± 0.52	11 ± 0.69	8 ± 0.70	12 ± 2.27	24 ± 1.19	12 ± 0.54
	2	79 ± 1.38	58 ± 3.55	19 ± 1.66	24 ± 4.42	55 ± 5.15	82 ± 3.61
	3	183 ± 3.17	95 ± 5.79	25 ± 2.18	37 ± 6.87	162 ± 7.92	134 ± 5.92
	4	283 ± 4.89	129 ± 7.88	42 ± 3.63	63 ± 11.64	252 ± 12.33	176 ± 7.78
$\overline{r_X}$, (mg/(L.day))	1	1.3 ± 0.02	0.5 ± 0.03	0.4 ± 0.03	0.6 ± 0.10	1.1 ± 0.05	0.5 ± 0.02
	2	3.2 ± 0.06	2.4 ± 0.14	1.0 ± 0.07	1.0 ± 0.18	4.3 ± 0.21	3.3 ± 0.15
	3	25.3 ± 0.44	13.0 ± 0.80	3.5 ± 0.30	5.2 ± 0.95	22.3 ± 1.09	18.5 ± 0.82
	4	40.7 ± 0.70	18.5 ± 1.13	6.0 ± 0.52	9.1 ± 1.67	36.3 ± 1.77	25.3 ± 1.12

[1] Experiments 1 and 2 ran for 14 days, while 3 and 4 ran for 5 days. [2] Exp 1 (0% CO_2 + RCWW), Exp 2 (0% CO_2 + CWW), Exp 3 (2.5% CO_2 + CWW), and Exp 4 (5% CO_2 + CWW).

The growth parameters i.e., total dry weight, specific biomass, and average biomass yield, are shown below in Table 6.

Figure 2 shows the general growth response of *Desmodesmus multivariabilis* at different concentrations of CO_2. These profiles indicate that the dry weight of biomass as growth

response was affected by the flow rate and concentration of CO_2 that was injected into the photobioreactor. In all cultures of Experiments 1 and 2, there was a poor growth response compared to the cultures in Experiments 3 and 4. It is remarkable to note the differences in the error bars between the different media—nearly negligible error bars were measured in R1 and R2, while significant differences in the measured biomass concentrations were observed for D1, D2, SC1, and SC2. This was likely due to the interaction between the algal biomass and the surfactants and suspended dye particles present in the media, as mentioned previously.

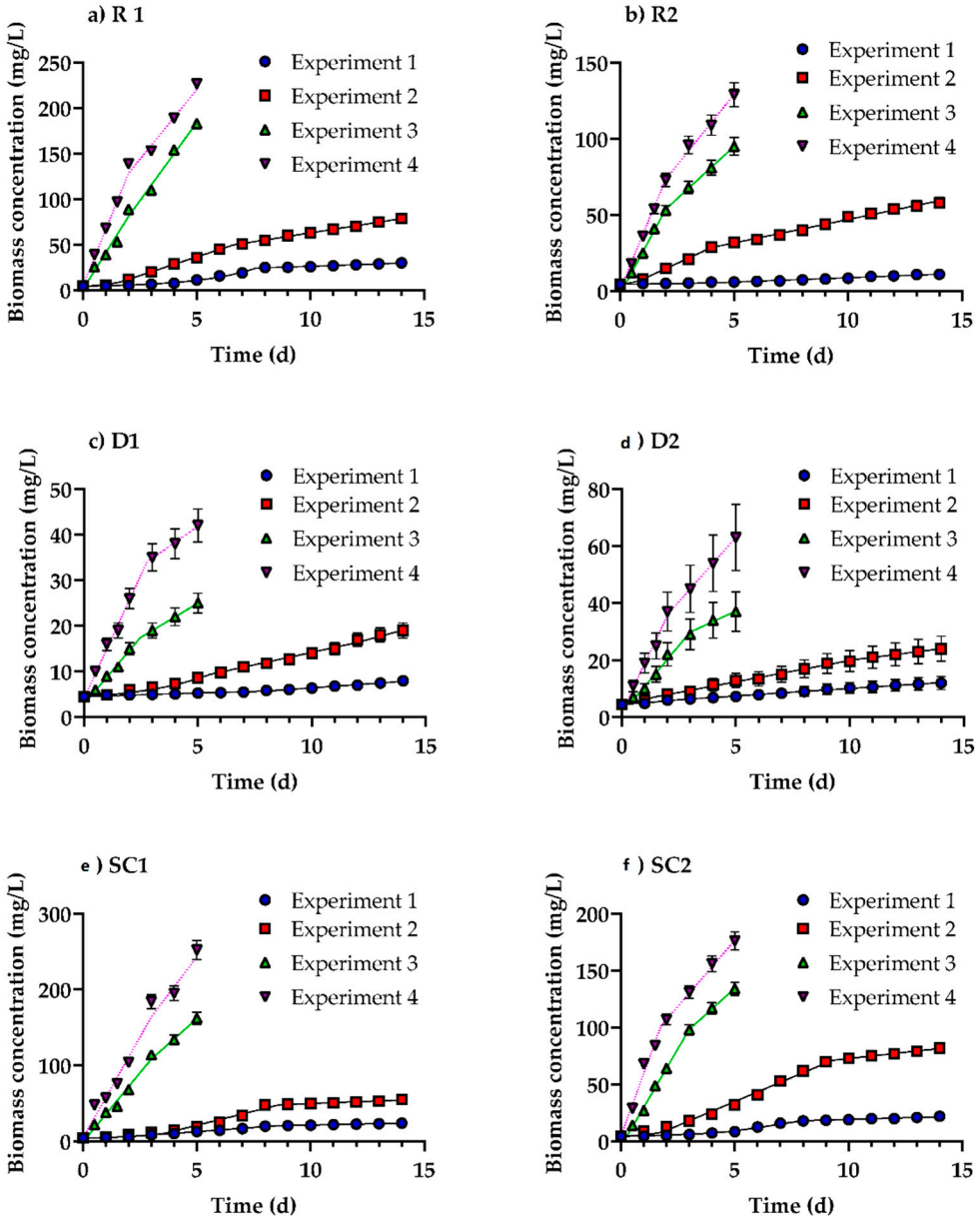

Figure 2. Growth profiles (biomass vs time) of *Desmodesmus multivariabilis* in six different media in (**a**) passive aerated experiments and (**b**) active aerated experiments. The error bars represent the standard deviations of the triplicate measurements. The shaded areas represent the 95% prediction interval (95% probability of future observations) [37]. Legend: Experiment 1 (0% CO_2 + RCWW), Experiment 2 (0% CO_2 + CWW), Experiment 3 (2.5% CO_2 + CWW), and Experiment 4 (5% CO_2 + CWW).

The profiles in Figure 3 were fitted using Liebig's Law [22], which proposes that the growth of an organism is limited by the rate permitted by the most limiting factor. The model can be described by Equation (9):

$$\frac{dX}{dt} = \min\left(\mu_i X,\ k_L a\left(p_{CO_2}\right)\right) \quad (9)$$

where μ_i represents the specific growth rate of the microalgae as it relates to nutrient i, $k_L a$ represents the gas-liquid mass transfer coefficient of CO_2 in the liquid, and $p_{CO}{}^2$ represents the partial pressure of CO_2 in the gas phase.

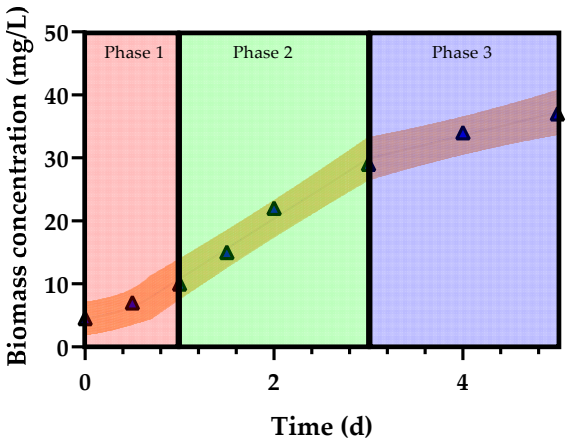

Figure 3. Separation of growth-rate-controlled and mass-transfer-controlled regions on the growth profiles. The colors represent the different growth phases—Phase 1: Red, Phase 2: Green, Phase 3: Purple.

The results demonstrated that for the conditions tested, three phases of growth could be observed. These phases are illustrated in Figure 3 below and represent a growth-rate-controlled (Phase 1), a CO_2-mass-transfer rate-controlled (Phase 2), and a final-growth-rate-controlled phase (Phase 3).

3.2. Relationship between the Observed Specific Growth Rate and CO_2 Availability

The values of the determined specific growth rates and slopes are shown in Table 7 below.

Table 7. Summary of fitted parameter values for Figure 2 using Equation (9).

Parameter	Exp [1]	R1	R2	D1	D2	SC1	SC2
μ_1 (d^{-1})	1	0.1365	0.06784	0.03742	0.1355	0.2193	0.1613
	2	0.5806	0.4655	0.1251	0.2055	0.2574	0.331
	3	1.05	1.248	0.6277	0.8407	0.8919	1.62
	4	4.168	1.765	1.081	1.195	1.105	4.168
$k_L a\left(p_{CO_2}\right)$ ($g.L^{-1}.\ d^{-1}$)	1	4.208	-	-	0.5556	2.388	3.104
	2	8.049	3.123	1.163	1.489	11.51	9.397
	3	37.52	17.25	5.056	9.747	43.08	35.17
	4	74.97	29.01	9.655	18.78	65.04	55.01
μ_3 (d^{-1})	1	0.03523	-	-	0.04561	0.03241	0.02813
	2	0.05944	0.05222	-	0.05303	0.02374	0.02915
	3	0.2298	0.1629	0.1302	0.1074	0.1796	0.1519
	4	0.2076	0.1377	0.09136	0.1825	0.2506	0.1613
R^2	1	0.9984	0.9879	0.9645	0.9971	0.9976	0.9959
	2	0.9992	0.9926	0.997	0.9966	0.9988	0.999
	3	0.9905	0.998	0.9964	0.9956	0.9941	0.9991
	4	0.9978	0.9964	0.9967	0.9964	0.9833	0.9963

[1] Exp 1 (0% CO_2 + RCWW), Exp 2 (0% CO_2 + CWW), Exp 3(2.5% CO_2 + CWW), and Exp 4 (5% CO_2 + CWW).

To determine the k_La values, the slopes of the curves in phase 2 ($k_La(p_{CO_2})$) were plotted against the partial pressure of CO_2 in the sparged gas. The fits obtained are shown in Figure 4, and the fitted k_La values are reported in Table 8; the results clearly demonstrate that mass transfer of CO_2 was the dominant rate-limiting step in phase 2.

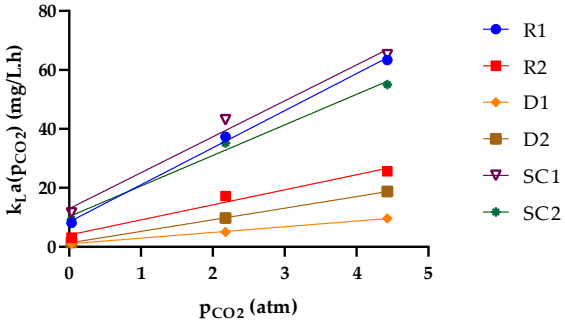

Figure 4. Determination of k_La values for the various experimental media.

Table 8. Summary of fitted parameter values from Figure 4.

Parameter	R1	R2	D1	D2	SC1	SC2
k_La ($g.L^{-1}.atm^{-1}.d^{-1}$)	12.58	5.102	1.933	3.935	12.19	10.36
R^2	0.9975	0.9739	0.9989	0.9999	0.9865	0.9920

From Table 8, it can clearly be seen that a marked difference between the k_La values was measured as it relates to the specific wastewater treated. This is interesting as it would indicate that the nature of the wastewater itself, as opposed to the reactor configuration, affects the mass transfer of CO_2 to the liquid phase. According to Kraakman et al. [38], variations in gas–liquid mass transfer were traditionally associated with changes in power applied to the system, i.e., through agitation, sparging, etc. In contrast, a more nuanced approach to the factors affecting mass transfer in biological systems is gaining traction to ensure a reduction in the power requirements of these systems. Due to the complex nature of these systems, the factors influencing mass transfer include the physico-chemical properties of the gas; the medium properties such as viscosity, salt, and organic content; the reactor characteristics (hydrodynamic behavior, surface area, etc.); and the operating conditions (e.g., gas/liquid velocity, pH, temperature) [38]. Within the system under consideration, the factors that varied between the different media runs were the wastewater properties, with significant difference measured between specifically the compositions. Barton et al. [39] found that changes of several orders of magnitude in the Henry's constant were observed due to the presence of organics in a bioreactor media.

To determine if a relationship between the observed specific growth rate and CO_2 availability existed, the values for μ_1 and μ_3 were plotted against the p_{CO_2} (Figure 5).

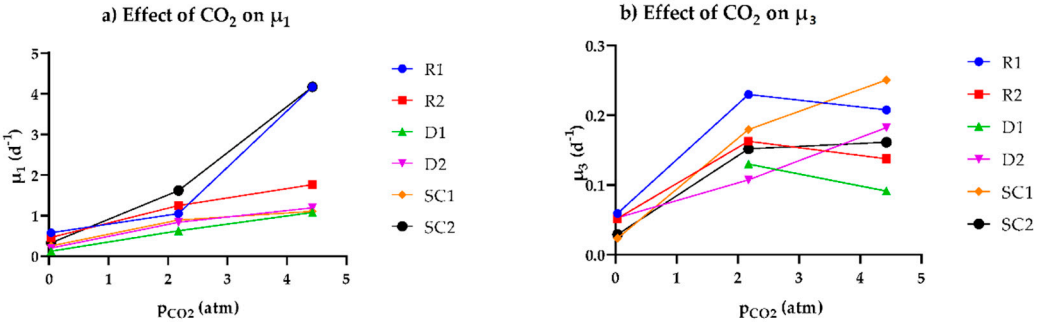

Figure 5. The comparison between the specific growth rates for (**a**) phase 1 (μ_1) and (**b**) phase 3 (μ_3) against the partial pressure of CO_2 in the gas phase.

The results suggest that a positive relationship between μ_1 and p_{CO_2} exists, while a much more tenuous relationship between μ_3 and p_{CO_2} was observed. This indicates that the rate-limiting nutrient in phase 1 is likely the CO_2, while some other nutrient (e.g., TP, TN, TOC) limited the growth in phase 3 [40].

3.3. Cell Organics Concentration Profiles in Cosmetic Wastewater

For Experiment 1 (with 0% carbon dioxide and raw wastewater), the extracellular organics depleted steadily in all six media. During the lag phase of the experiment (4 days for the dyes, 6 days for the relaxers, and 8 days for the shampoo and conditioners), the organics decreased steadily by less than an average of 10% in all the media. During the log phase (3 days for the dyes, 5 for the relaxers, and 5 days for the shampoo and conditioners), the organics depleted at a faster rate in this phase for the relaxers and the shampoos and conditioners at an average percentage of 20%. Table 9 shows the results obtained from nutrient analysis.

Table 9. Removal rates of Cell Organics in mg/(L.d).

Parameter	Experiment [1,2]	R1	R2	D1	D2	SC1	SC2
TOC	1	0.79	0.77	0.20	1.40	1.08	0.83
	2	1.67	1.30	0.38	2.26	1.65	1.85
	3	5.86	5.31	1.79	8.72	6.45	7.17
	4	7.93	5.92	1.88	9.52	7.46	7.51
	Control	0.17	0.23	0.09	0.33	0.27	0.23
COD	1	46	100	201	285	37	52
	2	97	167	379	458	56	114
	3	340	684	1777	1769	219	445
	4	460	763	1861	1913	254	465
	Control	14	35	92	69	10	17

[1] Experiments 1 and 2 ran for 14 days while control and Experiments 3 and 4 ran for 5 days. [2] Exp 1 (0% CO_2 + RCWW), Exp 2 (0% CO_2 + CWW), Exp 3 (2.5% CO_2 + CWW), and Exp 4 (5% CO_2 + CWW).

The TOC concentrations in the dyes depleted at a slower rate during the initial phase of growth at an average of 10%. Transitioning from the lag phase into the stationary phase, it was noted that the organics were consumed by 25% in R1, 23% in R2, 24% in D1, 28% in D2, 29% in SC1, and 23% in SC2. These observations were similar to those in Experiment 2 (with 0% carbon dioxide and pre-treated wastewater); the extracellular organics depleted steadily in all six media. By the end of the experiments, there was a net reduction in organics in the wastewater by 25% in R1, 23% in R2, 24% in D1, 28% in D2, 29% in SC1, and 23% in SC2.

For experiments 3 and 4 (with 2.5% and 5% CO_2 in pre-treated wastewater), the concentrations of organics initially increased in the media for R1, R2, SC1, and SC2. In experiment 3, the initial increase in the cell organics was significantly lower, being 69–78% in the relaxers and 33–47% in the shampoos and conditioners, respectively, while in Experiment 4, the organics had increased by over 193–181% in the relaxers and 196–203% in the shampoos and conditioners. Thereafter, the organics concentration gradually decreased with time, seemingly as the extracellular organics initially produced were consumed during cellular growth.

In the control experiment (with raw wastewater and no biomass activity), there was no significant change in the mass of organics in all six media. As indicated previously in Section 3.1 and adding to that observation, the harvesting of the biomass was also complicated by the presence of certain unknown organic compounds in the media and the low biomass growth rates, which inhibited the settling and flocculation of the cells. The COD removal and consumption in Experiment 4 achieved the highest removal percentage.

It was observed that the concentrations of TOC and COD decreased with time, as shown in Table 9, demonstrating the potential of *D. multivariabilis* for removal of these nutrients from the solution.

3.4. Nutrient Concentration Profiles in Cosmetic Wastewater

In the control experiment where there was no growth, nutrient removal was less than 10% in all six media, confirming these removals can be attributed to biological activity.

TN and TS consumption were observed most prominently when growth occurred. TN and TS were removed by less than 30% and 60% by the end of Experiments 1 and 2, respectively, where the growth of biomass was significantly suppressed. In Experiments 3 and 4 where significant growth was observed in the relaxers, shampoos, and conditioners, TN concentration removal was observed to be correspondingly high. It was observed that the concentrations of TN, TP, and TS decreased with time, as shown in Table 10, demonstrating the potential of *D. multivariabilis* for removal of theses nutrients from a solution.

Table 10. Removal rates of nitrogen, phosphates, and sulphates in mg/(L.d).

Parameter	Experiment [1,2]	R1	R2	D1	D2	SC1	SC2
TN	1	0.05	0.08	0.32	0.74	1.09	0.31
	2	0.06	0.21	0.41	0.98	1.38	0.59
	3	0.51	1.07	2.14	3.75	7.05	2.46
	4	0.57	1.24	3.16	4.46	8.40	2.68
	Control	0.01	0.01	0.05	0.05	0.05	0.02
TS	1	4.52	4.84	27.71	5.92	1.09	1.86
	2	5.16	8.74	34.78	7.97	2.25	3.36
	3	17.66	26.12	118.02	27.88	7.04	13.51
	4	19.57	28.93	132.88	33.62	7.94	14.21
	Control	0.47	0.84	3.57	0.76	0.18	0.27
TP	1	0.30	1.98	5.04	4.16	1.59	1.70
	2	0.28	1.69	4.64	2.65	2.56	2.58
	3	0.63	5.79	10.57	8.26	9.50	12.41
	4	0.57	6.51	10.31	9.49	11.44	13.35
	Control	0.02	0.18	0.34	0.27	0.27	0.26

[1] Experiments 1 and 2 ran for 14 days while control and Experiments 3 and 4 ran for 5 days. [2] Exp 1 (0%CO_2 + RCWW), Exp 2 (0%CO_2 + CWW), Exp 3(2.5%CO_2 + CWW), and Exp 4 (5%CO_2 + CWW).

In contrast to the TN findings, TP consumption was more pronounced in the experiments and media where growth was less significant. This could likely be explained by the precipitation of phosphates at high pH due to the elevated pH resulting from photosynthesis [41]—as observed for Exp 1 and 2 in all media (Table 10).

In similar studies, the TN concentration was reduced by an average of 86% in the reactor grown with *C. Vulgaris.* The SO_4^{2-} and PO_4^{3-} removal efficiencies were >85% and >95%, respectively [42,43].

Figure 6 shows the summarized percentage removals of the organics and the nutrients in the cosmetic wastewater.

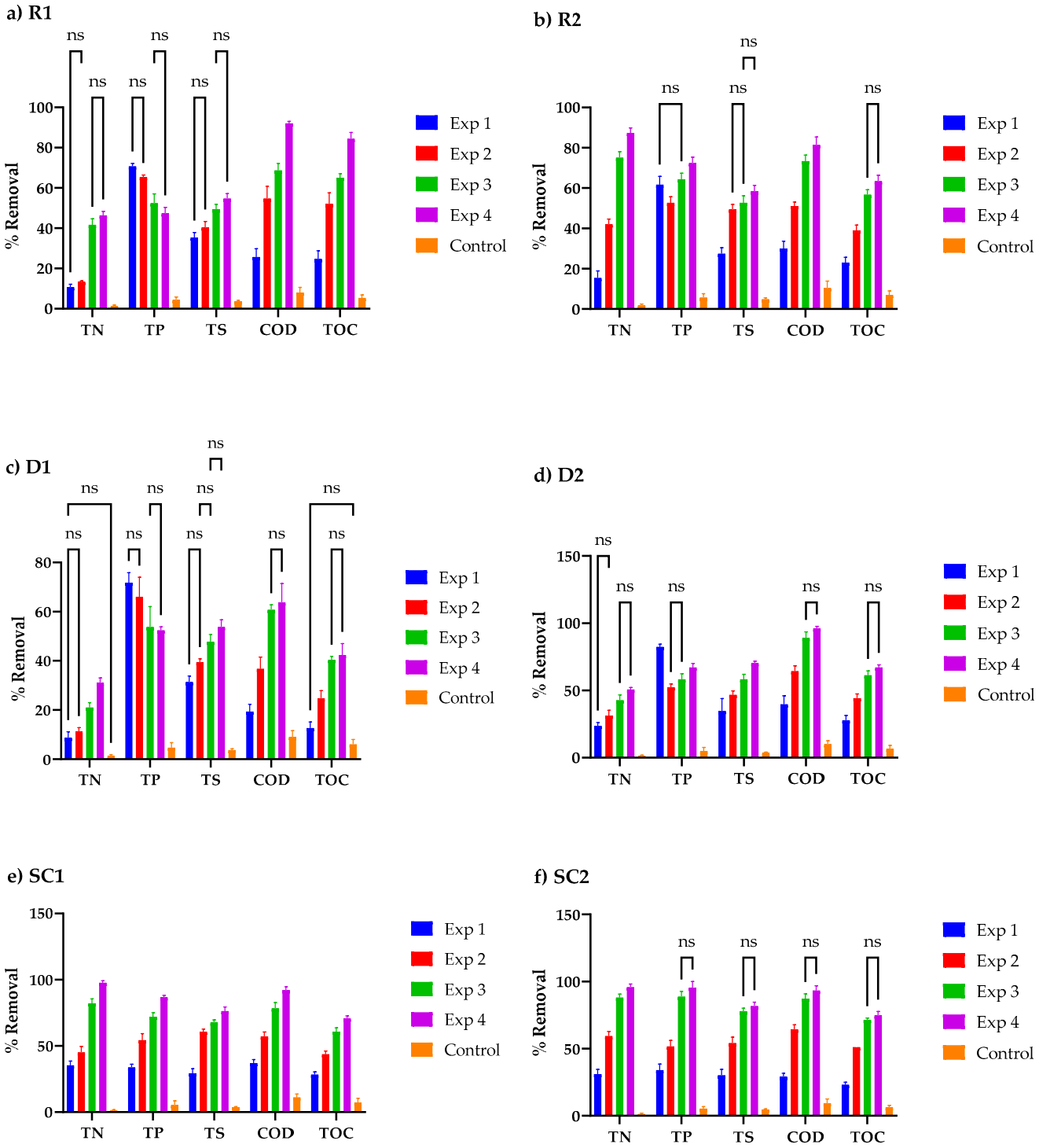

Figure 6. Percentage removal of nutrients from the media. The subplots represent: (**a**) R1, (**b**) R2, (**c**) D1, (**d**) D2, (**e**) SC1, (**f**) SC2. Error bars denote the standard deviation for the triplicate results. The brackets indicate those statistical comparisons that yielded non-significant differences at the 5% significance level.

To assess the statistical differences between the nutrient removal % for the different growth media, two-way ANOVA comparisons were performed between all experiments at a significance level of 5%. The Tukey method to correct for multiple comparisons was used [44]. The brackets in Figure 5 indicate those comparisons that were found to be not significant (all other comparisons were found to exhibit significant differences) and

demonstrate the remarkable influence that the pre-treatment method (Exp 1 vs. Exp2) and the addition of CO_2 (Exp 2 vs. Exp 3 vs. Exp 4) had on the removal of nutrients from the solution.

The most significant change in pH was noticed in Experiments 3 and 4, as seen in Table 11. The reason for the difference was that in Experiments 3 and 4 (pre-treated wastewater and carbon dioxide at 5% and 2.5%, respectively), the high rate of carbon dioxide supplementation equilibrated the media at a pH of 6.5–7.5, which is within the optimal range for effective CO_2 fixation in microalgae [14]. In Experiments 1 and 2, in the media where growth occurred, the carbon species equilibrium shifted because of the mass transfer of carbon dioxide into the cosmetic wastewater being the rate-limiting step. This led to a decrease in the dissolved inorganic carbon concentration before the equilibrium was achieved, resulting in an increase in pH.

Table 11. Final pH averages and standard deviation.

Exp [1,2]	R1	R2	D1	D2	SC1	SC2
1	12.07 ± 0.22	8.20 ± 0.05	9.60 ± 0.15	9.88 ± 0.17	10.16 ± 0.09	9.62 ± 0.09
2	10.80 ± 0.16	10.65 ± 0.03	10.74 ± 0.17	10.56 ± 0.00	11.84 ± 0.05	10.34 ± 0.10
3	6.90 ± 0.08	6.93 ± 0.02	7.10 ± 0.08	7.00 ± 0.08	6.63 ± 0.05	6.72 ± 0.08
4	6.47 ± 0.05	6.35 ± 0.09	6.83 ± 0.12	6.57 ± 0.05	6.33 ± 0.12	6.37 ± 0.10
Control	11.88 ± 0.03	8.92 ± 0.06	8.91 ± 0.04	9.44 ± 0.05	7.13 ± 0.05	7.58 ± 0.06

[1] Experiments 1 and 2 ran for 14 days while control and Experiments 3 and 4 ran for 5 days. [2] Exp 1 (0%CO_2 + RCWW), Exp 2 (0%CO_2 + CWW), Exp 3(2.5%CO_2 + CWW), and Exp 4 (5%CO_2 + CWW).

3.5. Relationship between Cell Organics, Biomass Growth, and Nutrient Uptake in Cosmetic Wastewater

In experiments 1 and 2, the obtained results show that the specific growth rate gradually increased with decreasing organics concentration. In R2, D1, and D2, the decrease in the rate of microalgal growth when there was an increase in the substrate (CO_2) concentration proved that *D. multivariabilis* can grow heterotrophically in addition to the conventional autotrophic growth. The decrease in growth performance and growth rate of algae with increasing substrate concentration indicates that *D. multivariabilis* can perform heterotrophic growth in addition to conventional autotrophic growth. The organic matters in the wastewater can act directly as essential organic nutrients or act as a secondary growth factor [45,46]. In addition, as observed in experiments 3 and 4, the results in Figure 7 initially show a pattern of increasing extracellular organics and then a steady depletion.

Growth was maximum at this condition. This may be related to the algae alternating its metabolism. When *D. multivariabilis* is under environmental stress, it releases organics in large amounts in an attempt to alter the growing conditions [47].

The other focus of this study was on inorganic carbon supplementation as a function of pH. As the microalgal growth occurred in Experiments 1 and 2, the TOC concentration decreased as the pH gradually increased. After adding the additional CO_2 to the media in Experiments 3 and 4, the pH dropped the optimum value (6.5–7.5), and the equilibrium was attained. From the outcome of this study, *D. multivariabilis* appeared to have the ability to utilize bicarbonate (HCO_3^-) as the only carbon source; however, the rate of algal biomass growth was notably lower than when carbonate (CO_3^{2-}) was present in the media [48]. Biomass production increased with decreasing pH.

Mass transfer of CO_2 from the air into the growth medium limited the growth of microalgae. Comparing the growth rates of microalgae and mass transfer demonstrated that performance was highly dependent on the microalgae metabolism, and the magnitude carried microalgae growth rate was influenced by the mass transfer of CO_2 into the cell, which would then be used for the sustainability of photosynthetic activity by microalgae.

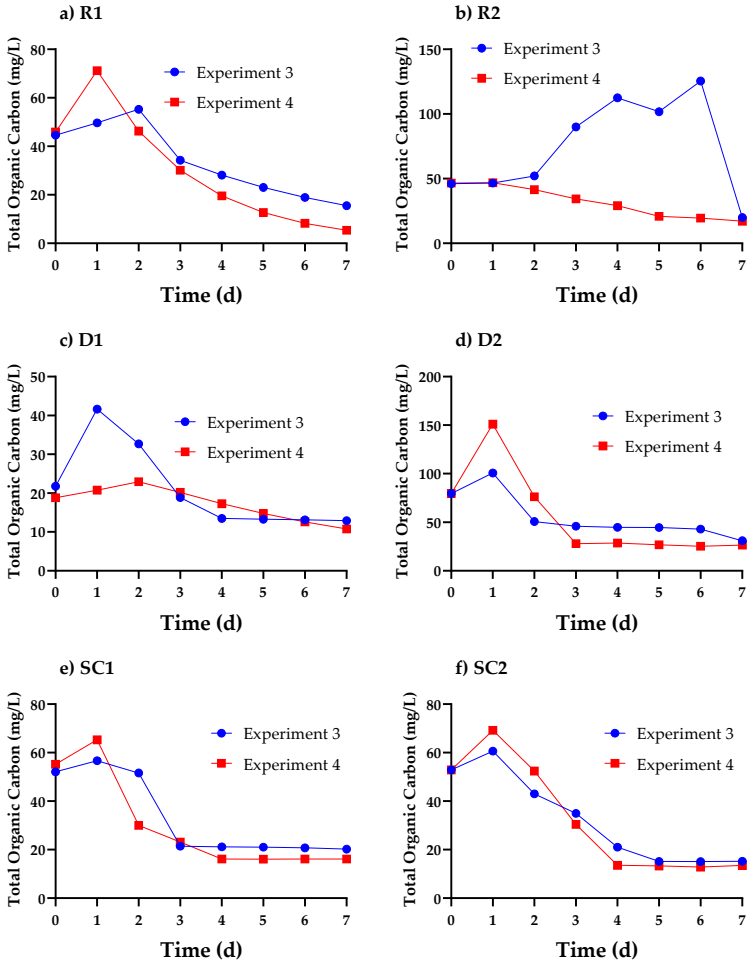

Figure 7. Extracellular organics profiles in the media for Experiments 3 and 4. The subplots represent: (**a**) R1, (**b**) R2, (**c**) D1, (**d**) D2, (**e**) SC1, (**f**) SC2.

In some cultures, the green color of the microalgae was observed, indicating the synthesis of chlorophyll. Visual monitoring was impossible in the red and black dyes. After pre-treatment, the red pigmentation in D2 was reduced, and the green algae could be seen. Studies have shown that dyes hinder growth by inhibiting photosynthesis [49], which would explain why the dyes were not a good medium for the growth of biomass. Another reason for the poor performance was the high concentration of cell organics in the initial media. Experiments with COD concentrations above 500 mg/L experienced slow growth due to COD inhibition. These high COD concentrations impacted the biomass production efficiency [41].

The growth rate in the shampoos and conditioners was slightly lower than that of the relaxers, and this can be attributed to the inhibition of cell reproduction by surfactant concentration in the media. Similar studies concluded that the toxicity of both surfactants interferes with processes involved in the cell reproduction [50]. Another significant observation was the performance of *D. multivariabilis* in Wave Nouveau perm (R2). Biomass productivity was lower than its counterparts, even though nutrient removal was maximum. This could be because of the growth inhibition caused by the limitation of the initial concentration of nitrogen and phosphate in the medium, which are crucial components of growth.

4. Conclusions

The findings in this present study indicated the potential of *D. multivariabilis* to utilize the pollutants in cosmetic wastewater as substrates. From this study, the microalgal biomass

efficiently removed TOC, TN, TS, TP, and COD from cosmetic wastewater, proving that *D. multivariabilis* can be favorably used in bioremediation.

The best medium for growth was found to be the relaxers. Surfactants in shampoo and dye components are possibly toxic to biomass, limiting the photosynthesis and erupting the cell walls of algae. The results further demonstrated that the cosmetic wastewater requires prior pre-treatment to ensure growth of microalgae, especially in the black and red-pigmented dyes. Of note is that even without significant growth, the microalgae still managed to uptake nutrients from the wastewater, therefore, treating the wastewater.

D. multivariabilis demonstrated its ability to use CO_2 as an inorganic substrate for growth and production of organics. It was shown that the growth of biomass was markedly limited by the mass transfer of carbon dioxide into the media.

In future, quantifying the extracellular polysaccharides (EPS), fatty acid methyl esters (FAME), and the intermediate samples for TN, TP, and TS would provide insight into the underlying mechanisms driving the observed algal behavior. In addition, a study exploring the use of authentic flue gas, with the same cosmetic wastewater parameters, should be performed to determine whether the toxic by-products of combustion have any significant inhibitory effect that would result in a deviation from the values obtained in this study.

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