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Sustainable Use of CO_2 and Wastewater from Mushroom Farm for *Chlorella vulgaris* Cultivation: Experimental and Kinetic Studies on Algal Growth and Pollutant Removal

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Abstract: The potential use of carbon dioxide (CO_2) and wastewater released from a mushroom farm for the cultivation of *Chlorella vulgaris* microalga was investigated in this study. For this purpose, a microcontroller-based aided CO_2 capture and mixing prototype was constructed for the cultivation of *C. vulgaris* under varying concentrations of mushroom farm wastewater (0 as control, 50 and 100%). The results showed that the constructed prototype was helpful to maintain desirable CO_2 levels (6000 ppm) in the mushroom cultivation chamber with constant CO_2 supply to algal culture, i.e., 0.6% at an airflow rate of 50 mL/min. After 16 days of algal cultivation, it was observed that the maximum significant ($p < 0.05$) algal biomass production of 2.550 ± 0.073 mg/L was recorded in 50% wastewater concentration followed by 100% and control. Also, the maximum removal of selected mushroom farm wastewater pollutants, such as total dissolved solids (84.00 ± 1.37%), biochemical oxygen demand (90.17 ± 2.42%), chemical oxygen demand (91.53 ± 0.97%), total nitrogen (86.27 ± 1.60%) and total phosphorus (94.19 ± 2.33%), was achieved in 50% concentration of wastewater treatment with maximum first-order rate constant (k) values. In addition, the algal growth kinetics results showed that the logistic model fit best compared to the modified Gompertz model, based on selected validation tools, such as experimental vs. predicted values, coefficient of determination ($R^2 > 0.9938$), model efficiency (ME > 0.98) and root mean square error (RMSE < 0.03). The post-harvest characterization of algal biomass revealed that the proximate, biochemical, ultimate elements (carbon, oxygen and nitrogen) and structural properties were significantly higher in 50% treatment than those in 100% and control treatments. Therefore, the findings of this study are novel and provide significant insight into the synergistic use of CO_2 and wastewater produced by mushroom farms for algal cultivation and biological wastewater treatment.

Keywords: climate change; CO_2 capture; greenhouse; mathematical modeling; phycoremediation; zero waste mushroom farm

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1. Introduction

Carbon dioxide (CO_2) has become the most investigated greenhouse gas due to its percentage (about 76% of greenhouse gases) and association with climate change [1]. The greenhouse effect, a naturally occurring phenomenon, occurs when greenhouse gases are released into the atmosphere, trap heat and consequently raise the planet's temperature [2]. Due to several negative consequences of increasing atmospheric CO_2 levels, reducing its emissions is an essential part to mitigating the climate change impacts [3]. According to the Environmental Protection Agency (EPA, 2022), the earth has experienced a severe rise in atmospheric CO_2 in the last 150 years from five major sectors: transportation; electric power generation; industrial operations; commercial and residential; and agriculture. Agriculture-related CO_2 emissions are a serious issue that accounts for approximately 11% of the world's greenhouse gas emissions [4]. The major sources of CO_2 emissions in the agriculture sector include crop production, biomass burning, decaying, livestock raising, land-use changes, use of agrochemicals, irrigation, soil management and transport [5]. However, governments and organizations have launched several programs to solve this global issue, including supporting sustainable agriculture methods, enhancing energy efficiency and investing in renewable energy sources [6].

Mushroom farming is considered one of the important agriculture sectors contributing to CO_2 emissions. Though mushroom production helps in the recycling and management of agro-wastes for food and is regarded as the least energy- and carbon-intensive sector; still, it has a significant carbon footprint due to the release of CO_2 during fungal respiration, waste decomposition, product processing and transportation [7]. According to an estimate, 1 kg of mushroom production results in about 0.31 kg of CO_2 equivalent emissions [8]. The high CO_2 levels in the mushroom farm have several negative consequences such as poor fungal respiration, death of the mycelial network, slow fruiting, small-cap and short shelf life [9]. Besides this, wastewater released from mushroom farms contains significant amounts of organic and inorganic pollutants, including high biochemical and chemical oxygen demands (BOD and COD), total dissolved solids (TDS), carbon (C), nitrogen (N) and phosphorus (P) [10]. Mushroom farm wastewater can cause significant environmental damage due to the presence of various pollutants if disposed of inadequately [11]. Therefore, both CO_2 and wastewater released from mushroom farms need to be sufficiently managed to prevent their negative impacts.

In recent times, algal cultivation has appeared as one of the most viable options for CO_2 utilization and wastewater management [12–14]. Regulated CO_2 supply is an important factor that controls algal photosynthesis. CO_2 is vitally utilized by algal cells for the production of glucose molecules, which they use for energy, growth and reproduction [15]. CO_2 is also used by algae for cell wall development as well as for the production of proteins, lipids and other important compounds. Moreover, CO_2 can be supplied to the growth medium in several ways, including bubbling it into the water via a CO_2 injection device or adding carbonates such as sodium bicarbonate. The injection of CO_2 accelerates photosynthesis, leading to enhanced algal growth [12]. Recent studies have shown that CO_2 supply rates of 0.5–5% give optimum growth of algal species [12,15,16]. Moreover, several reports [17] have shown that algal species can be successively cultivated in various wastewaters, such as *Chlorella vulgaris* in ammonia wastewater [18], *Scenedesmus* sp. in municipal wastewater [19], *C. vulgaris* in dairy wastewater [20], *Chlamydomonas* sp. in swine wastewater [21] and *Spirulina* sp. in hospital wastewater [22].

To date, no study has explored the synergistic use of waste CO_2 and wastewater produced from mushroom production farms. Therefore, this lab-scale study aimed to assess the phycoremediation efficacy of *C. vulgaris* for the treatment of mushroom farm wastewater while using the CO_2 produced in a mushroom cultivation facility. In this study, the pollutant removal and growth of *C. vulgaris* were examined using various kinetic models, while post-harvested biomass was also characterized for proximate, biochemical, ultimate and structural properties.

2. Materials and Methods

2.1. Materials

For the current study, mushroom farm wastewater was collected from the disposal point of Kashyap Mushroom Farm located in Roorkee, India (29°47′16.7″ N and 77°47′20.1″ E). The wastewater was discharged from the white button (*Agaricus bisporus*) mushroom cultivation facility. The wastewater sample was carefully transported to the experimental site in 20 L polyvinylchloride plastic cans and preserved at 4 °C until final use in phycoremediation experiments. Also, *C. vulgaris* microalga was obtained from the Agro-ecology and Pollution Research Laboratory of the Department of Zoology and Environmental Science, Gurukula Kangri (Deemed to be University), Haridwar, India. *Chlorella vulgaris* was propagated in a BG-11 medium to obtain stock inoculum having a biomass density of 0.125 g/L as outlined by Kumari et al. [23].

2.2. Experimental Design and Operation

Microalgal cultivation experiments were conducted in the Agro-ecology and Pollution Research Laboratory of the Department of Zoology and Environmental Science, Gurukula Kangri (Deemed to be University), Haridwar, India. For this purpose, a previously designed chamber (1.2 × 0.6 × 0.6 m; length × width × height) was used for *A. bisporus* mushroom cultivation on a wheat straw-based substrate [24]. Breathable polypropylene bags of 10 kg capacity were used for *A. bisporus* cultivation. A total of 8 kg substrate was filled, and 3% spawn was aseptically applied in four layers. The climatic conditions of the spawn running period were adjusted as follows: temperature of 25 °C, humidity of 80% and light intensity of 700 lx. The cultivation chamber was facilitated with an air quality gas sensor module (MQ135) connected to an Arduino UNO microcontroller unit (R3, ATmega328P, Quartz Components, Jaipur, India), which was used to monitor the CO_2 levels inside the chamber. The levels of CO_2 (maximum 6000 ppm) and fresh air exchange (FAE) in the chamber were controlled via a direct current (DC) fan attached to the relay module. Also, a high-efficiency particulate air (HEPA) filter (0.003 µm; FY0194/10, Philips, Jiaxing, China) was fit inside the chamber to capture produced CO_2 and transport it to a 500 mL conical flask containing desiccant beads followed by an algal culture flask through a peristaltic pump as shown in Figure 1. The desiccant beads were situated to capture any moisture present in the air coming from the chamber. The time-course data of CO_2 production was saved on the ThingSpeak server (MathWorks Inc., Natick, MA, USA; https://thingspeak.com; accessed on 20 December 2021) by using the ESP8266 Wi-Fi module.

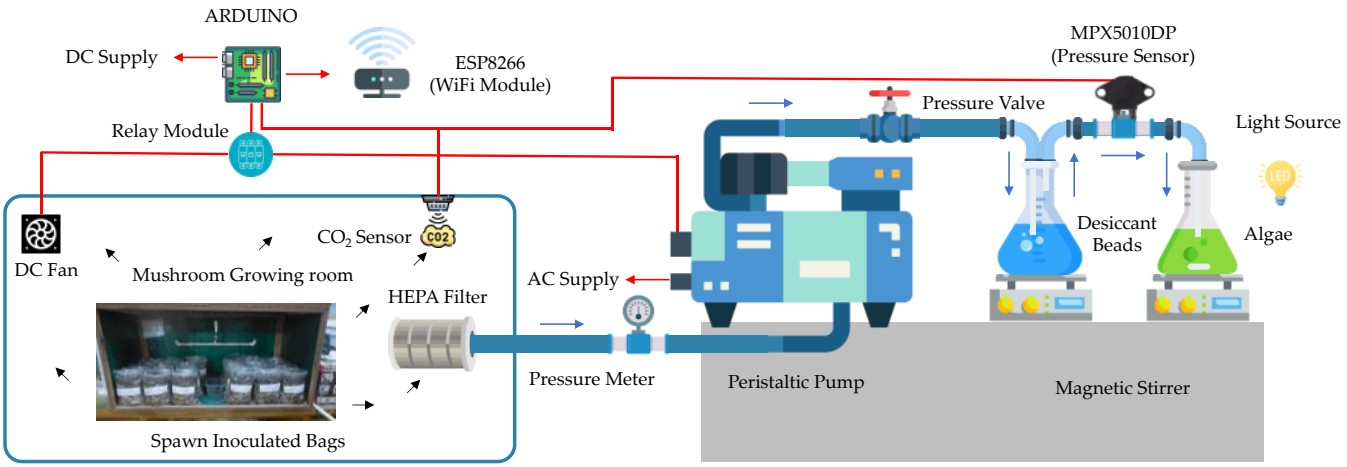

Figure 1. Experimental setup for CO_2 harvesting and sequential algal cultivation (red arrows: prototype connections; black/blue arrows: CO_2 flow directions).

For phycoremediation experiments, a total of three treatments were used to cultivate *C. vulgaris*, including control (borewell water supply), 50 and 100% mushroom farm wastewater concentrations, separately. The airflow rate in the algal photo-bioreactor (1 L culture flask) was adjusted to 50 mL/min (0.6% CO_2), and experiments lasted for 16 days. A total of 2 mL stock inoculum was added to the culture flask, and alga was propagated under artificial light (5000 lx)/dark period of 12/12 h and a temperature of 28 °C, with slow and continuous mixing using a magnetic stirrer with a hot plate (Bio Gen, New Delhi, India).

2.3. Analytical Methods

The mushroom farm wastewater used in this study was characterized (before and after phycoremediation) for the selected pollutant parameters, such as total dissolved solids (TDS), biochemical oxygen demand (BOD), chemical oxygen demand (COD), total nitrogen (TN) and total phosphorus (TP), following standard methodologies [25]. In this study, TDS was measured using a calibrated microprocessor-based digital meter (1611, ESICO, Parwanoo, India). BOD was estimated based on dissolved oxygen changes after five days as per the Walkley and Black method [26]. COD was determined by following the open-reflux digestion and spectrophotometric (650 nm) method (60 Cary, Agilent Technologies, Santa Clara, CA, USA). TN was determined using Kjeldahl's acid digestion–distillation, while TP was determined by using spectrophotometric methods, respectively [27].

On the other hand, the harvested algal biomass was analyzed for selected proximate and biochemical attributes, such as moisture (%), dry weight (g), ash (%), protein (%), carbohydrate (%) and lipid contents (%), following standard methods [23]. Moreover, the dried algal biomass was further utilized for ultimate elements (C, O and N) analysis using Scanning Electron Microscope (SEM, Carl Zeiss, Oberkochen, Germany) equipped with the Energy Dispersive X-ray Spectroscopy (EDX) detector (Octane Eliter Plus, Mahwah, NJ, USA). Additionally, Fourier-Transform Infrared Spectroscopy (FTIR-8400S, Shimadzu, Columbia, MD, USA), with a spectral wavenumber range between 500 and 4000 1/cm, was used to analyze the functional groups in algal biomass.

2.4. Data Analysis

In order to understand the effectiveness of bioremediation experiments, removal efficiency is a widely used tool that depicts the pollutants eliminated from the wastewater in a stipulated period [28]. In this study, the following Equation (1) was used to calculate the pollutant removal efficiency (%) of *C. vulgaris* from mushroom farm wastewater:

$$\text{Removal Efficiency (\%)} = [(C_{t0} - C_t)/C_{t0}] \times 100 \quad (1)$$

where C_{t0} and C_t represent the initial and final concentrations (mg/L) of the pollutant. Moreover, the rate of pollutant removal from mushroom farm wastewater over time was described by a first-order kinetic model [29]. This model often assumes that the rate of removal has a rate constant that is proportional to the concentration of the pollutant present. The form of the first-order kinetic model for pollutant removal by *C. vulgaris* is given by the following Equation (2):

$$-\mathrm{d}[C]/\mathrm{d}t = k[C] \quad (2)$$

where C is the concentration of pollutants in wastewater, t is experimental time and k is the rate constant. A plot for log[C] vs. t was drawn to obtain the linear trendline ($y = ax + b$), where a refers to the rate constant (k). On the other hand, the CO_2 fixation rate of *C. vulgaris* grown in selected wastewater concentrations was calculated using the following model (Equations (3) and (4)) as previously adopted by Park et al. [30]:

$$\text{B (g/L/d)} = Y/t \quad (3)$$

$$CO_2 \text{ Fixation Rate } (g/L/d) = B \times CC \times \frac{M_{CO2}}{Mc} \quad (4)$$

where B indicates biomass productivity (g/L/d), *Y* is algae yield (g/L) and CC represents carbon content (g/g) of cultivated algal biomass, while M_{CO2} and M_C are molecular weights of CO_2 (44.01 g/mol) and C (12.01 g/mol), respectively.

In addition, the growth kinetics of *C. vulgaris* in various treatments was estimated using two models, i.e., logistic and modified Gompertz. The logistic model and the modified Gompertz model are both sigmoid function models. These models predict the algal growth rates over time and are based on the concept of nutrient limitation and the carrying capacity of the photo-bioreactor [31]. The forms of the models are given in Equations (5) and (6):

$$y = \frac{P}{1 + e^{-k\ (x-xc)}} \quad (5)$$

$$y = Pe^{-e^{(-k(x-xc))}} \quad (6)$$

where *y* is the predicted algal biomass (g), *P* is the maximum biomass production potential, *x* is the specific growth rate and *xc* is the lag phase in days. In order to study the effectiveness of logistic and modified Gompertz models, the prediction results were subjected to model validation tools including model efficiency (ME) and root mean square error (RMSE) [32], as given in Equations (7) and (8):

$$ME = 1 - \left[\frac{\sum \left(y_{predicted} - y_{experimental} \right)^2}{\sum \left(y_{predicted} - y_{mean} \right)^2} \right] \quad (7)$$

$$RMSE = \sqrt{\frac{\sum_{i=1}^{n} \left(y_{experimental} - y_{predicted} \right)^2}{n}} \quad (8)$$

All experiments were conducted in triplicate, and values were presented as mean followed by standard deviation. The data were analyzed using a one-way analysis of variance (ANOVA) test to derive significant differences in control and test treatments ($p < 0.05$). The data was analyzed and visualized in Microsoft Office 2019 (Microsoft Corp., Redmond, WA, USA) and OriginPro 2022b (Student Edition, OriginLab, Northampton, MA, USA) software packages.

3. Results and Discussion

3.1. CO_2 Generation, Fixation and Biomass Productivity of C. vulgaris

In this study, the mushroom cultivation chamber was continuously monitored for 16 days until the termination of algal cultivation experiments. It was observed from the collected data that the spawn running phase of *A. bisporus* accelerated the CO_2 levels. During the first four days, the CO_2 levels exponentially increased from 120 to 6000 ppm (Figure 2). After this period, the CO_2 production was steady and fluctuated between 4800 to 6000 ppm. Overall, the time-course trend of CO_2 production in the mushroom cultivation chamber followed a log-linear pattern [$y = 1276.7\ln(x) - 1639.9$; $R^2 = 0.7516$]. The DC fan and HEPA filter suction helped to maintain the CO_2 levels as per the prototype installed in the microcontroller unit. Being aerobic fungi, mushrooms actively utilize fresh oxygen (O_2) and emit CO_2 maximally during the spawn running phase [33]. The produced CO_2 begins to accumulate in the mushroom growing rooms and needs to be removed through fresh air exchange (FAE). A moderate CO_2 concentration (4000–6000 ppm) is preferred during the spawn run because it promotes a rapid and healthy spawn run. Chamber air with extremely high CO_2 levels may damage the mycelia by suddenly lowering the substrate pH and death of mycelia. Also, it could develop mushrooms with thick and short stipe pileus. The phycoremediation experiments were conducted for 16 days because *C. vulgaris*

gives best growth up to this period. After that, the decline phase of *C. vulgaris* starts, which makes the experiments non-feasible. Also, the spawn running of *A. bisporus* mushroom lasts up to 15–20 days, and after that, CO_2 levels need to be adjusted to not more than 1000 ppm. Thus, during the spawn running phase, CO_2 levels should be moderate, while during the fruiting stage, a decrease in CO_2 and a rise in O_2 levels are needed [9]. To replicate this experiment in an actual mushroom farm, some additional modifications in the capacity of several components and prototype design may be required.

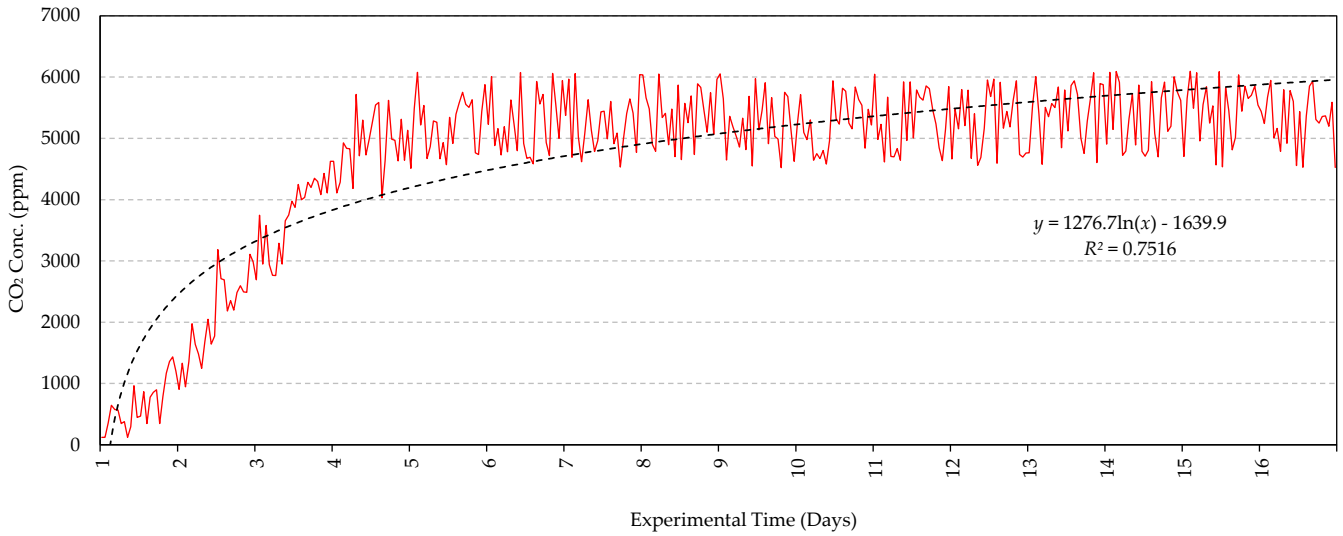

Figure 2. Time-course CO_2 levels of mushroom cultivation room as regulated by a microcontroller unit.

On the other hand, the results showed that CO_2 produced in the cultivation chamber was helpful for *C. vulgaris* cultivation. Herein, the different experimental treatments such as control, 50 and 100% showed a cumulative algal biomass production of 0.972 ± 0.025, 2.550 ± 0.073 and 2.340 ± 0.104 g/L, respectively. The biomass productivity (B) of *C. vulgaris* ranged as 0.06, 0.16 and 0.15 g/L/d for the control, 50 and 100%, while CO_2 fixation rates (CFR) were observed as 11.30, 36.03 and 29.63 g/L/d, respectively (Figure 3). This suggests that there was a significant ($p < 0.05$) increase in the B and CFR of *C. vulgaris* at 50 and 100% concentration as compared to control treatments. Algal species actively consume CO_2 for their cellular photosynthesis and other metabolic processes. In algae, CO_2 acts as a C source for the production of carbohydrates and proteins as well as helps in regulating the pH of the algal medium [30]. Therefore, the aided cultivation of algae requires a continuous supply of CO_2 in order to facilitate optimum growth. In the current study, the CO_2 captured from the cultivation chamber fulfilled the CO_2 requirements of *C. vulgaris.* Previously, limited researchers have utilized the waste CO_2 from mushroom cultivation. In a recent study by Jung and Son [34], a lab-scale experiment was conducted for synergistic utilization of CO_2 from the king oyster mushroom (*Pleurotus eryngii*) chamber for romaine lettuce (*Lactuca sativa* var. *longifolia*) cultivation. They found that both mushrooms and plants can be efficiently cultivated together while creating a gaseous equally using mathematical modeling.

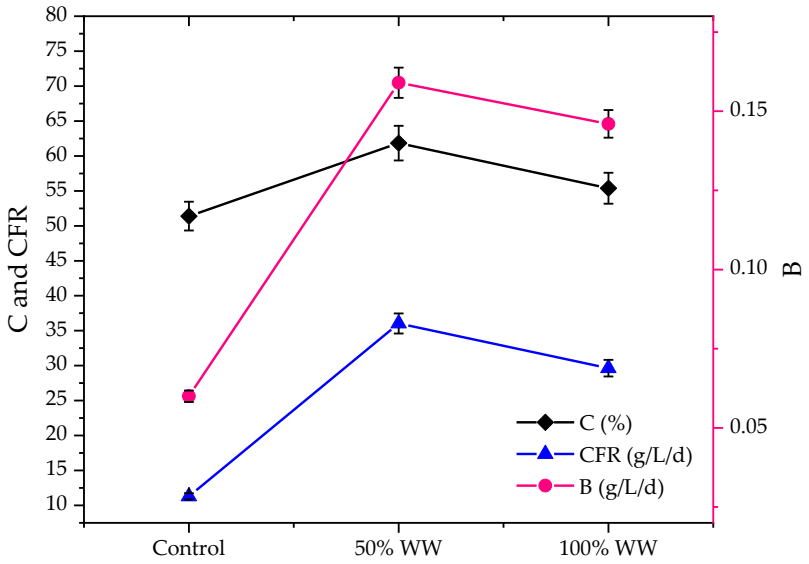

Figure 3. CO_2 fixation rate (CFR), carbon content (%) and biomass productivity (B: g/L/d) of *C. vulgaris* cultivated in different concentrations of mushroom farm wastewater (WW: wastewater).

3.2. Results for Growth Kinetics of C. vulgaris

Table 1 depicts the growth kinetic parameters of *C. vulgaris* cultivated in different concentrations of mushroom farm wastewater. The results showed that both sigmoidal models (logistic and modified Gompertz) were efficient in predicting the growth patterns of *C. vulgaris*. In particular, the logistic model gave better results in terms of experimental vs. predicted biomass production (y), specific biomass production (P), lag phase (xc) and specific growth rate (x) as compared to the modified Gompertz model. Figure 4 shows that the values predicted by the logistic model were quite near to the experimental values as compared to those of the modified Gompertz mode. Herein, both models depicted that *C. vulgaris* had a shorter lag phase (6.48 and 5.36 days) in absolute mushroom farm wastewater concentration (100%) as compared to other treatments, which might be due to the high availability of nutrients and their rapid uptake during the initial phase. However, both models showed that the lag phase in the control treatment was quite similar (8.96 and 8.81 days). Also, the model validation criteria, such as R^2, ME and RMSE, were optimum in the case of the logistic model (>0.9938, >0.97 and <0.03) as compared to those in modified Gompertz (>0.9920, >0.91 and <0.05). The ME value near 1 showed that models were efficient while RMSE described that model fitted in the experimental and predicted data with minimum error. The results for the growth kinetic parameters of *C. vulgaris* using logistic and modified Gompertz models can therefore be utilized to better understand the growth of this species in various mushroom farm wastewater concentrations. Overall, the logistic model is better suited to forecasting the growth of *C. vulgaris* since the best fitting results were found for the logistic model rather than the modified Gompertz model.

Recent studies showed the successful application of logistic and modified Gompertz models in the growth optimization of *C. vulgaris*. A study by Lam et al. [35] used domestic wastewater as a cultivation medium for *C. vulgaris*. They reported that both logistic and modified Gompertz models were useful for growth prediction and had $R^2 > 0.98$, RMSD < 0.02 and variance <0.01, respectively. Similarly, Ajala and Alexander [36] also investigated the growth kinetics of three algal species (*C. vulgaris*, *Scenedesmus obliquus* and *Oocystis minuta*) grown in secondarily treated-domestic effluent. They found that the modified Gompertz model gave the best-fitting results ($R^2 > 0.89$) for the prediction of the growth of all three microalgae as compared to the logistic model ($R^2 > 0.74$).

Table 1. Growth kinetic parameters of *C. vulgaris* cultivated in different concentrations of mushroom farm wastewater.

Model	Variable	Experimental Treatments		
		Control	**50%**	**100%**
Experimental	Y	0.972 ± 0.025	2.550 ± 0.073 *	2.340 ± 0.104 *
Logistic	y	1.002	2.580	2.247
	P	1.126 ± 0.045	2.646 ± 0.021	2.297 ± 0.034
	R^2	0.9938	0.9992	0.9959
	xc	8.96 ± 0.36	7.67 ± 0.06	6.48 ± 0.12
	x	0.29 ± 0.01	0.43 ± 0.01	0.48 ± 0.02
	ME	0.97	0.97	0.99
	RMSE	0.03	0.03	0.06
Modified Gompertz	y	1.026	2.645	2.349
	P	1.499 ± 0.189	2.906 ± 0.081	2.445 ± 0.051
	R^2	0.9876	0.9964	0.9920
	xc	8.81 ± 1.08	6.56 ± 0.16	5.36 ± 0.12
	x	0.13 ± 0.01	0.25 ± 0.01	0.29 ± 0.01
	ME	0.95	0.91	0.99
	RMSE	0.05	0.09	0.07

Values are mean ± SD of three replicates; *: significantly different from control treatment values at $p < 0.05$.

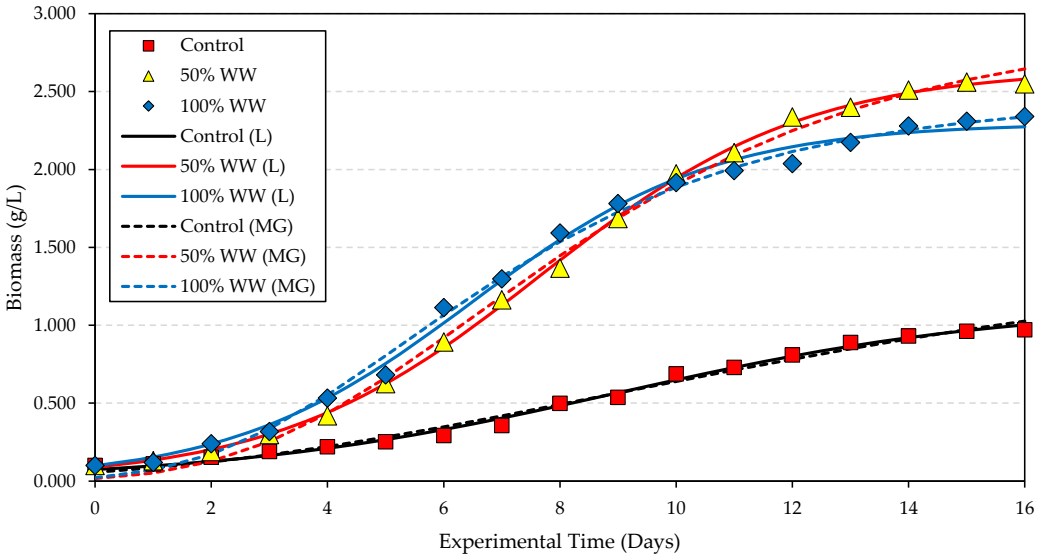

Figure 4. Experimental vs. model predicted (L: logistic; MG: modified Gompertz; WW: wastewater) growth curves of *C. vulgaris* cultivated in different concentrations of mushroom farm wastewater.

3.3. Proximate and Lipid Profile of Cultivated C. vulgaris

The proximate, biochemical and ultimate compositions of *C. vulgaris* grown on control and mushroom farm wastewater are reported in Table 2. However, no significant difference ($p > 0.05$) was observed between all treatments in terms of moisture and ash contents, i.e., 72.08–73.16% and 2.25–2.31%, respectively. Amin et al. [37] reported that *Chlorella* spp. contains up to 80.0% moisture content, which is relatively comparable to our findings. *Chlorella vulgaris* grown on agro-industrial by-products enclosed 8.4% ash [38], which is significantly higher than observed in the present study (2.25 ± 0.06–2.31 ± 0.05%). Jabeen et al. [39] also mentioned a 5.3% ash content in *C. vulgaris*. Moreover, the current study reported dry weight of 0.27 ± 0.03–0.68 ± 0.02 g; protein contents of 43.98 ± 0.32–48.71 ± 0.62%; carbohydrate contents of 17.10 ± 0.09–18.05 ± 0.12%; lipid contents of 6.85 ± 0.14–8.61 ± 0.28%; C contents of 51.40 ± 0.91–61.84 ± 1.40%; O contents of 21.87 ± 0.15–28.82 ± 0.70%; and N contents of 6.40 ± 0.07–7.06 ± 0.05%, which were significantly higher ($p < 0.05$) in wastewater treatments than in the control (50% > 100% > control). A dry weight content of 0.42–0.44% was

reported for *C. vulgaris* grown on hospital wastewater [22]. This value is lower than the range observed with mushroom farm wastewater in the present study (0.64 ± 0.03–0.68 ± 0.02%). The protein content of *C. sorokiniana* grown on treated wastewater reached 38.8% [40], which was lower than denoted in the present study. A comparable protein content (35.2%) to the aforementioned study was depicted by Yang et al. [41] who grew *C. vulgaris* on molasses wastewater. Whereas, significantly lower protein contents (22.2 and 22.8%) were detected in *C. vulgaris* cultivated on agro-industrial by-products and oil industry wastewater, respectively [38,42]. Another study mentioned a protein content of 27.9–35.2% in *C. vulgaris* grown on synthetic municipal secondary effluent [43]. Josephine et al. [44] reported that *C. vulgaris* encloses a carbohydrate content of 12–17%, which is comparable to the control (17.10 ± 0.09%) in the present study. However, significantly higher carbohydrate contents were observed when *C. vulgaris* was grown on membrane-treated industrial distillery and oil industry wastewaters (26.1 ± 0.6% and 40.2%, respectively) [42,45]. *Chlorella* spp. are known for their high lipid accumulation [46]. A comparable lipid content (6.56%) was observed in *C. vulgaris* grown on urban wastewater [47]. However, other studies reported significantly higher values [42,44,45]. In the present study, the increased O_2 production by *C. vulgaris* showed that this species owns the potential to act as a biological source of O_2 for aquatic life. Adamczyk et al. [48] studied the growth kinetics of *C. vulgaris* and mentioned a C content in the range of 46–51%. Such values are comparable to the control in the present study (51.40 ± 0.91%). The increased N content in *C. vulgaris* grown on mushroom farm wastewater may be due to its initial high concentrations in the latter. Such high concentrations might be the main reason behind the increased lipid content accumulation in *C. vulgaris* [49].

Table 2. Proximate, biochemical and ultimate analysis parameters of *C. vulgaris* cultivated in different concentrations of mushroom farm wastewater.

Parameters	Experimental Treatments		
	Control	50%	100%
Moisture content (%)	72.08 ± 1.85	73.16 ± 0.71 ^{ns}	72.53 ± 0.46 ^{ns}
Dry Weight (g)	0.27 ± 0.03	0.68 ± 0.02 *	0.64 ± 0.03 *
Ash (%)	2.25 ± 0.06	2.31 ± 0.05 ^{ns}	2.28 ± 0.04 ^{ns}
Protein (%)	43.98 ± 0.32	48.71 ± 0.62 *	46.09 ± 0.75 *
Carbohydrate (%)	17.10 ± 0.09	18.05 ± 0.12 *	17.68 ± 0.24 *
Lipid (%)	6.85 ± 0.14	8.61 ± 0.28 *	7.34 ± 0.19 *
Carbon (%)	51.40 ± 0.91	61.84 ± 1.40 *	55.39 ± 0.82 *
Oxygen (%)	21.87 ± 0.15	28.82 ± 0.70 *	25.87 ± 0.51 *
Nitrogen (%)	6.40 ± 0.07	7.06 ± 0.05 *	6.93 ± 0.09 *

Values are mean ± SD of three replicates; * and ns: significantly and non-significantly different from control treatment values at $p < 0.05$.

Moreover, the ionization (intensity; abscissa) and counts (energy; ordinate) EDX spectra of control, 50 and 100% concentration treatments are given in Figure 5. It was reported that a higher elemental presence could be easily detected by higher counts [50]. In the present study, the highest counts for C, followed by O and N. Such observation can be confirmed by the results of Table 2, which makes EDX an accurate and fast tool for the compositional evaluation of *C. vulgaris.* The EDX spectra observation showed that C, O and N were more concentrated in wastewater treatments than in the control (50% > 100% > control). Moreover, the EDX spectra of all treatments showed very low counts at high ionization values, which can be linked to minor impurities, mostly metal elements. In addition, the observation of microscopic images denoted a reduction of pore sizes in *C. vulgaris* grown in wastewater compared to control ones. Thus, further investigation should be performed to detect the possible effect of other elements found in wastewater on the BET surface area of *C. vulgaris* BET surface area may affect the phycoremediation potential of *C. vulgaris* by reducing the nutrient uptake rate from wastewater [51].

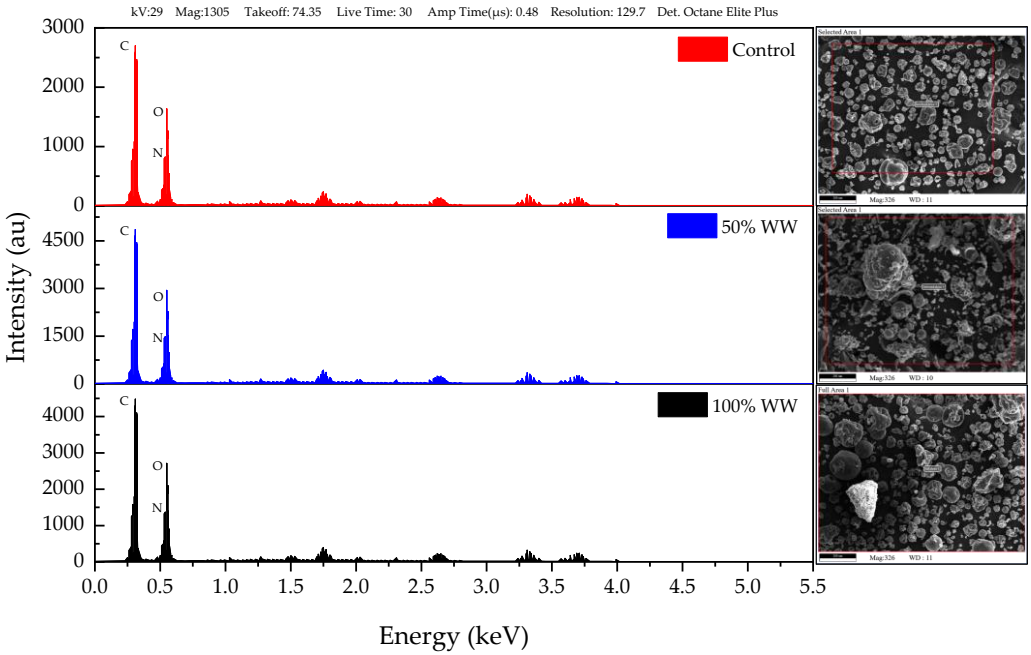

Figure 5. EDX graphs for ultimate element analysis (C, O and N) of *C. vulgaris* biomass cultivated in different concentrations of mushroom farm wastewater (WW: wastewater).

Figure 6 shows three FTIR spectra that were averaged for the comparison between control, 50 and 100% concentration treatments. All FTIR spectra were typically similar in terms of the trend with a higher transmittance percentage in control compared to 50% and 100% (an average of 4–7%) (control > 100% > 50%). Positive IR transmittance peaks refer to positive wavelength peaks of absorption and likewise negative ones. A general decreasing pattern for all spectra was detectable at 1950–2950 IR transmittance regions, which suggests asymmetric stretching vibration of CH_3 and CH_2 of acyl chains [52], outlining the lipidic profile of *C. vulgaris*. Also, an O–H bending might have occurred, which refers to the high-water content (moisture content). The positive increasing pattern observed at 3500–3750 IR transmittance regions suggests O–H stretching. The positive peaks of the three spectra at 970 IR transmittance region refer to the symmetric stretching of di-anionic acid phosphate monoester in phosphorylated proteins. Whereas, the positive peaks at 1300 IR transmittance region suggest the formation of amide III components of proteins [52]. The highest positive peaks observed at 1850–1900 IR transmittance may refer to C=C bending between lipids or fatty acids, or a second-order bending. The negative peaks among the three spectra at the 3350 IR transmittance region refer to O–H, N–H and C–H bending. Whereas, the positive spectra peaks at 3600–3700 IR transmittance regions refer to O–H and N–H stretching vibrations.

3.4. Removal of Pollutants from Mushroom Farm Wastewater

The kinetics study of pollutant removal by *C. vulgaris* was conducted using a first-order rate equation (Table 3). Moreover, a comparison between control and wastewater treatments detected the possible significant difference in terms of initial and final pollutants concentrations and removal efficiency. The highest pollutants concentrations were initially detected in 100%, followed by 50% and control. A significant ($p < 0.05$) difference between treatments in terms of pollutant concentrations was detected after *C. vulgaris* cultivation for 16 days. More precisely, wastewater treatments showed significantly higher ($p < 0.05$) TDS, BOD, COD, TN and TP concentrations at the end of the cultivation cycle. The results also showed exceptional pollutants removal, i.e., 76.95–84.00%, 78.89–90.17%, 86.39–91.53%, 80.58–86.27% and 91.21–94.14% for TDS, BOD, COD, TN and TP, respectively. The following decreasing order of pollutants removal efficiency was denoted: 50% > 100% > control. Also, the pollutants were removed in the following decreasing order of treatments:

TP > COD > BOD > TN > TDS. Moreover, the high R^2 values ($R^2 > 0.80$) depicted a good fit of the developed kinetic model, being a suitable tool for pollutant removal from mushroom farm wastewater using *C. vulgaris.* Such a kinetic model also showed promising robustness on crops grown on industrial wastewater [53].

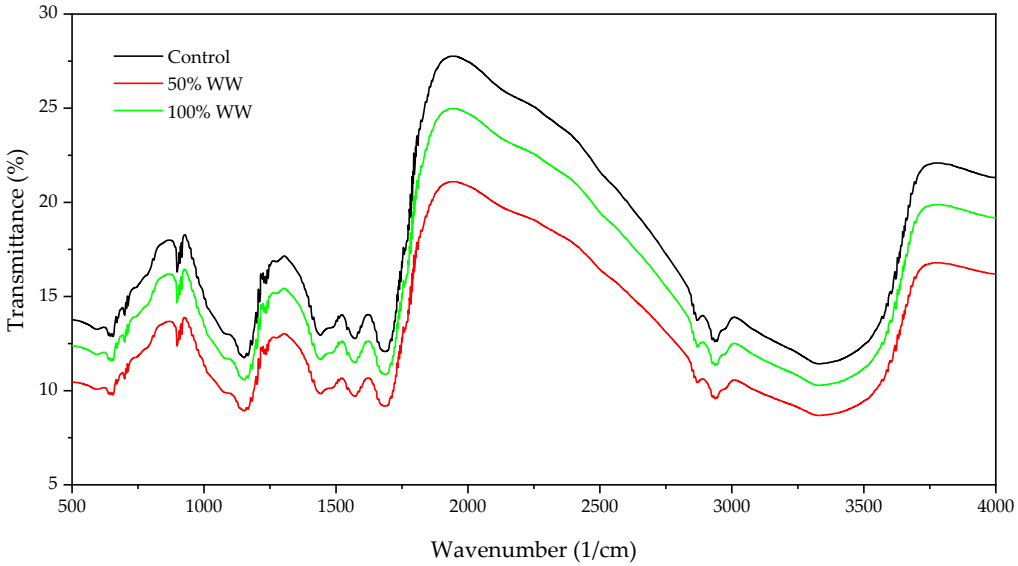

Figure 6. FTIR spectra of *C. vulgaris* biomass cultivated in different concentrations of mushroom farm wastewater (WW: wastewater).

Table 3. Kinetic parameters of *C. vulgaris* in different wastewater concentrations.

Parameters	Variable	Experimental Treatments		
		Control	**50%**	**100%**
Total Dissolved Solids (TDS: mg/L)	Initial	98.28 ± 1.63	982.02 ± 8.09	2108.72 ± 24.05
	Final	23.45 ± 0.70 *	157.08 ± 12.42 *	486.08 ± 19.62 *
	Removal Efficiency	76.14 ± 0.13	84.00 ± 1.37	76.95 ± 2.03
	Equation	$y = -0.0437x + 2.013$	$y = -0.0559x + 3.0717$	$y = -0.0464x + 3.3745$
	R^2	0.9439	0.9288	0.9263
Biochemical Oxygen Demand (BOD: mg/L)	Initial	3.10 ± 0.09	623.08 ± 20.40	1138.05 ± 14.50
	Final	1.45 ± 0.13 *	61.24 ± 7.28 *	240.25 ± 11.36 *
	Removal Efficiency	53.23 ± 1.67	90.17 ± 2.42	78.89 ± 0.95
	Equation	$y = -0.0240x + 0.534$	$y = -0.0694x + 2.9222$	$y = -0.0461x + 3.09$
	R^2	0.8869	0.9426	0.9731
Chemical Oxygen Demand (mg/L)	Initial	12.27 ± 0.10	1270.25 ± 45.09	2608.56 ± 81.45
	Final	4.90 ± 0.23 *	107.57 ± 8.12 *	355.00 ± 19.04 *
	Removal Efficiency	60.07 ± 1.08	91.53 ± 0.97	86.39 ± 1.30
	Equation	$y = -0.0273x + 1.0929$	$y = -0.0777x + 3.2178$	$y = -0.0606x + 3.5061$
	R^2	0.9647	0.9247	0.9482
Total Nitrogen (TN: mg/L)	Initial	1.09 ± 0.02	168.80 ± 5.14	310.42 ± 9.11
	Final	0.25 ± 0.04 *	23.18 ± 3.67 *	60.28 ± 6.84 *
	Removal Efficiency	77.06 ± 0.05	86.27 ± 1.60	80.58 ± 2.09
	Equation	$y = -0.0457x + 0.0929$	$y = -0.0612x + 2.297$	$y = -0.0512x + 2.5844$
	R^2	0.9391	0.9409	0.9137
Total Phosphorus (TP: mg/L)	Initial	2.80 ± 0.08	67.24 ± 2.58	141.56 ± 5.64
	Final	0.82 ± 0.10 *	3.91 ± 1.02 *	12.44 ± 2.31 *
	Removal Efficiency	70.71 ± 1.27	94.19 ± 2.33	91.21 ± 0.97
	Equation	$y = -0.0383x + 0.491$	$y = -0.0815x + 1.9904$	$y = -0.0731x + 2.2742$
	R^2	0.9367	0.9358	0.9440

Values are mean ± SD of three replicates; *: significantly different from initial values of respective treatment values at $p < 0.05$.

Several studies have reported the phycoremediation properties of *Chlorella* spp. in pollutant removal from different types of wastewaters. For instance, Alalawy et al. [54] found that *C. vulgaris* removed 93.0–94.0% COD from hospital wastewater. Whereas, *C. vulgaris* removed 59.0% COD and 93.0% TN from swine wastewater [55]. Lee et al. [56] reported the removal of 92.0% COD and 100.0% TP from undiluted piggery wastewater using a mixture of *C. sorokiniana*, *Coelastrella* sp. and *Acutodesmus nygaardii*. Moreover, Lim et al. [57] investigated the phycoremediation potential of *C. vulgaris* in textile wastewater. They found 38.3–62.3%, 44.4–45.1% and 33.1–33.3% for COD, TN and TP removal efficiencies, respectively. Furthermore, the kinetic studies of textile wastewater using *C. pyrenoidosa* were investigated [29]. The findings of that research revealed 63.0% BOD removal efficiency. Malla et al. [58] reported 90.0–98.0%, 60.0%, 75.0%, 70.0–80.0% and 60.0–70.0% of TDS, BOD, COD, TN and TP removal efficiencies, respectively, from primary and tertiary treated wastewater after 12 days of phycoremediation using *C. minutissima*. Li et al. [45] mentioned high removal efficiencies of 72.2, 80.0 and 94.0% for COD, TN and TP, respectively, from membrane-treated industrial distillery wastewater, remediated with *C. vulgaris*. However, the present study is the first to investigate its potential in the phycoremediation of mushroom farm wastewater. In this regard, Kumar et al. [10] have previously grown two *Azolla* spp. aiming phytoremediation of such wastewater. They reported high removal efficiency of pollutants. Particularly, *A. pinnata* and *A. filiculoides* succeeded in the removal of 65.0–85.0%, 78.0–90.0%, 75.0–85.0% and 78.0–85.0% for TDS, BOD, COD and TN, respectively. Such removal efficiencies were lower or comparable to those observed with *C. vulgaris*, suggesting the latter is a better candidate for pollutant removal from mushroom farm wastewater.

4. Conclusions

In conclusion, the current study has shown that wastewater and CO_2 from mushroom farms can be sustainably used for *C. vulgaris* cultivation. According to the current results, the level of CO_2 production reached >6000 ppm in the mushroom cultivation chamber, which was used to fulfill the respiration requirement of *C. vulgaris*. However, the 50% concentration of mushroom farm wastewater gave the highest growth, proximate, biochemical, ultimate and structural parameters of *C. vulgaris* with maximum removal of pollutants as compared to those in 100% and control treatments. The first-order reaction-based kinetic model was helpful to simulate the rate constant for pollutant removal. Also, the logistic model yielded best-fitting results for the growth optimization of *C. vulgaris* as compared to the modified Gompertz model. Overall, this study suggests that the proposed approach can be used to advance environmentally friendly algal cultivation with efficient management of waste CO_2 and wastewater released from mushroom farms. Future studies on the utilization of produced algal biomass for secondary purposes, such as biofuel and biochar, are highly recommended.

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