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Sustainable Upcycling of Mushroom Farm Wastewater through Cultivation of Two Water Ferns (*Azolla* spp.) in Stagnant and Flowing Tank Reactors

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Abstract: Nowadays, the increase in the wastewater generated from the mushroom cultivation sector has become a serious environmental pollution concern. Therefore, the present study aimed to assess the efficiency of two water ferns (*Azolla pinnata* and *A. filiculoides*) in phytoremediation of mushroom farm wastewater (MFW) under stagnant and flowing tank reactor systems. For this, the laboratory scale experiments were conducted using five treatments, i.e., control (absolute borewell water), S50 (15 L borewell water + 15 L MFW: stagnant mode), S100 (30 L MFW: stagnant mode), F50 (15 L borewell water + 15 L MFW: flowing mode), F100 (30 L MFW: flowing mode), separately for both *Azolla* spp. After 15 days, *A. pinnata* and *A. filiculoides* significantly ($p < 0.05$) reduced the physicochemical parameters of MFW such as pH (18.87 and 18.56%), electrical conductivity (EC: 80.28 and 78.83%), total dissolved solids (TDS: 87.12 and 86.63%), biochemical oxygen demand (BOD: 90.63 and 89.90%), chemical oxygen demand (COD: 86.14 and 85.54%), and total Kjeldahl's nitrogen (TKN: 84.22 and 82.44%), respectively, in F100 treatment. Similarly, the highest growth and biochemical parameters of *Azolla* spp. were also observed while using absolute MFW treatment in a flowing tank reactor system. Moreover, out of the two tested growth kinetic models, the logistic model showed better fitness to the experimental data and prediction of critical growth parameters compared to the modified Gompertz model. The findings of this study are novel and suggest sustainable upcycling of MFW using plant-based treatment techniques with the production of high-quality *Azolla* spp. biomass.

Keywords: *Azolla* spp.; growth kinetics; mushroom cultivation; phytoremediation; sustainable development

1. Introduction

Although mushroom production has succeeded in dealing with the tremendous agro-industrial residues disposal into the environment, it generates large volumes of wastewaters as a natural consequence of the cultivation and postharvest technologies adopted. According to "Monterey Mushrooms," on average, the production of 1 kg of white and brown mushrooms needs around 18.2 L of freshwater [1]. Thus, being a top-ranking producing country of this important farm produce, India generates an enormous quantity of wastewater from the mushroom industry. Mushroom farm wastewater (MFW) generally contains chemical fertilizers or substances containing a high load of pollutants such as total dissolved solids (TDS), biochemical oxygen demand (BOD), chemical oxygen demand (COD), nitrogen (N), phosphorus (P), etc., that may harm the environment and all life forms [2,3]. Various activities inside the mushroom cultivation farm also contribute to the release of wastewater as shown in Figure 1. Moreover, their wastewater includes concentrations of elements such as cadmium (Cd), copper (Cu), chromium (Cr), iron (Fe), manganese (Mn), lead (Pb), nickel (Ni), and zinc (Zn) [4] that pollute underground and surface waters causing human and aquatic disorders in addition to disastrous impacts on soil microflora.

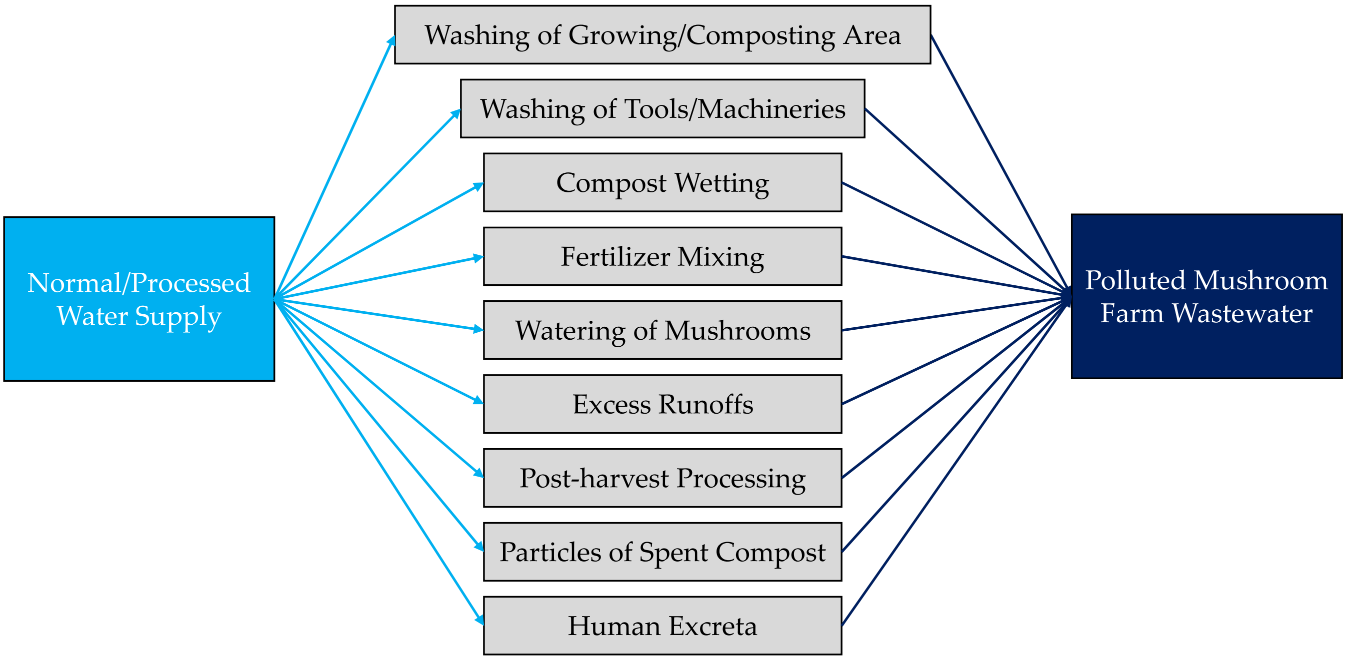

Figure 1. Various activities and sources of pollutants in wastewater released from mushroom farms.

Nowadays, phytoremediation is receiving great attention for its cost-effective, and eco-friendly technology in the remediation of pollutants found in agro-industrial wastewaters to avoid their unsafe discharge into the environment. In this context, various aquatic plants such as water ferns (*Azolla* spp.), water hyacinth (*Eichhornia crassipes*), and water lettuce (*Pistia stratiotes*), etc., have shown a high potential for remediation of a wide range of pollutants [5–7]. Previous reports pointed out that an increase in produced plant biomass was underscored proving again the success of this type of phytoremediation aiming for a safer environment [8]. Moreover, an increase in fresh biomass, chlorophyll, and relative growth rate of plants was observed when grown on these wastewaters [9,10]. Other free-floating aquatic weeds, such as *Salvinia molesta* and *Pistia stratiotes* had a considerable phytoremediation potential for domestic and industrial wastewater treatment [11,12].

Azolla spp. is considered and ranked as one of the best accumulators of pollutants, and also plays a role in the recovery of nutrients from polluted ecosystems [13]. The phytoremediation potential of several *Azolla* spp. was previously assessed within the literature. For instance, *A. filiculoides* showed high BOD, COD, and TDS removals (98.2%, 92.23%, and 90.29%, respectively), when used to treat textile (Congo red dye) wastewater [14]. Moreover, the same species showed detectable high removal efficiencies of Ni, Cd, and Pb (up to 70%) when grown in an aqueous solution [15]. Similarly, *A. caroliniana* was grown on wastewaters with Pb and Cd [16]. Although a limited negative effect of toxic elements was observed on biomass production, a high decrease in Cd percentage (to around 22%) and less reduction in Pb (to around 90%) in wastewaters were noted. *Wild A. caroliniana* was assessed for its removal potential of arsenic (As) from polluted water [17]. Authors found a high tolerance of this species for As associated with a considerable removal of this toxic element from water. Other researchers reported the growth of *A. pinnata* on integrated industrial effluent disposed of by the SIIDCUL industrial complex of Haridwar, India [5,6]. They found that these nitrogen-fixator species had a promising yield associated with considerable BOD and COD reductions, while also suitable for an optimized biogas production when whole biomass is digested.

Currently, the main focus of researchers is attributed to the treatment of agro-industrial wastes via myco- and phytoremediation. To our knowledge, no earlier interest was detectable in the treatment of MFW using *Azolla* spp. Hence, the need to find more efficient, eco-friendly, and cost-effective methods put its weight on the environmental scale. Therefore, phytoremediation of MFW using *A. pinnata* and *A. filiculoides* species could be a good trial and probable solution for its management. This study focused on MFW treatment using two *Azolla* spp. in stagnant and flowing tank reactor systems.

2. Materials and Methods

2.1. Collection of Experimental Materials

For the current investigation, the two water ferns (*A. pinnata* and *A. filiculoides*) were collected from the spring water stream at Chilla Forest Range of Rajaji National Park, Haridwar, Uttarakhand, India (29°57′54.4″ N and 78°12′01.0″ E). *Azolla* spp. were collected in transparent glass bottles (1 L) with aerated caps. *Azolla* spp. were morphologically identified using the standard keys, as described by Kumar and Nayak [18]. Then, *Azolla* spp. were individually transferred to 10 L capacity glass aquariums having 8 L of borewell water supplied with 3.10 g of nitrogen–phosphorus–potassium (NPK) fertilizer mixture and allowed for acclimatization (7 days). On the other hand, mushroom farm wastewater (MFW) was obtained from the disposal point of Kashyap Mushroom Farm located in Roorkee city, Uttarakhand, India (29°47′16.7″ N and 77°47′20.7″ E). This farm is equipped with modern technologies dedicated to round-the-year cultivation of white buttons (*Agaricus bisporus*) and milky (*Calocybe indica*) mushrooms. After moderate processing, the farm releases its wastewater into the nearby agricultural lands for crop irrigation. Purposely, the MFW was collected in 50 L capacity plastic cans and transported to a newly constructed poly-greenhouse located at Kulheri village of Saharanpur district, Uttar Pradesh, India (29°52′57.2″ N and 77°16′17.0″ E).

2.2. Experimental Design and Conditions

The phytoremediation experiments were performed from 1 to 16 March 2022. For this, transparent plastic containers of 35 L capacity were filled with 30 L working volume of MFW and used as phytoremediation reactors. The experiments were performed using a total of five working treatments (as triplicate) such as control (absolute borewell water), S50 (15 L borewell water + 15 L MFW: stagnant mode), S100 (30 L MFW: stagnant mode), F50 (15 L borewell water + 15 L MFW: flowing mode), F100 (30 L MFW: flowing mode), separately for both *Azolla* spp. (Figure 2). The flowing model reactors were equipped with a water pump (12V-7W, ARP053, Arpita Exports, Bengaluru, Karnataka, India) attached to an additional knob-based potentiometer (SEN51, Robodo Electronics, Shenzhen, China) to maintain the circular flow rate of 1.50 L/h. A total of 10 g priorly acclimatized *Azolla* spp. leaflets were added to each container and allowed to grow for 15 days under greenhouse conditions (18/6 h light/dark, 28 °C mean temperature, and 76% relative humidity).

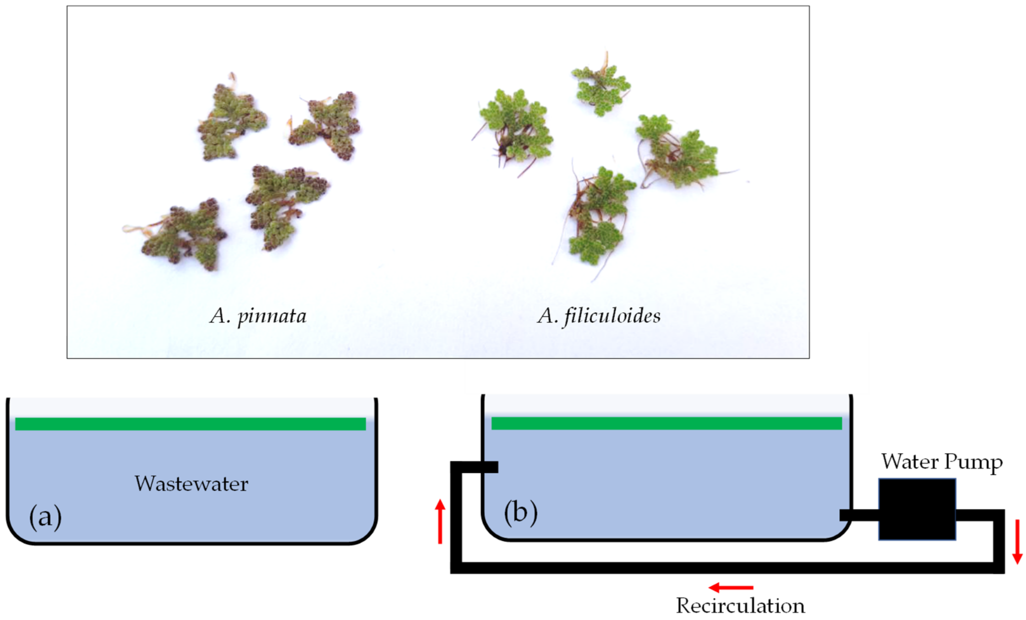

Figure 2. Experimental layout of (**a**) stagnant and (**b**) flowing tank reactors for the treatment of MFW using two *Azolla* spp. (Photographs: Pankaj Kumar and Ashish Kumar Arya).

2.3. Laboratory Analytical Methods

In this study, the borewell water and MFW were analyzed for selected quality parameters, including pH, electrical conductivity (EC), total dissolved solids (TDS), biochemical oxygen demand (BOD), chemical oxygen demand (COD), and total Kjeldahl's nitrogen (TKN) following standard analytical methodologies [19,20]. The physicochemical analysis was immediately performed after sample collection (day 0) and the termination of the phytoremediation experiment (day 15). For this, pH, EC, and TDS were measured using a microprocessor multimeter meter (1611 ESICO, India) after calibration. The net BOD_5 load was determined as a net change in the bioavailable O_2-demand through a microprocessor-based meter (1801, ESICO, Parwanoo, India). On the other hand, COD contents were determined using an open reflux digester (Scientech, Indore, India) followed by spectrophotometric measurement at 650 nm wavelength (60 Cary, Agilent Technologies, Santa Clara, CA, USA). Similarly, the TKN contents were measured by acid digestion (H_2SO_4, K2SO_4, and $HgSO_4$) followed by Nesslerization and spectrophotometric measurement at 425 nm [21]. All samples were pooled and analyzed three times. In addition to this, the harvested *Azolla* spp. were subjected to biochemical analysis for estimating the photosynthetic pigments, i.e., total chlorophyll contents and carotenoids. For this, chlorophyll contents were determined using 80% acetone as an extraction reagent followed by spectrophotometric determination at 645 and 663 nm wavelengths [5]. Similarly, acetone and petroleum ether were used for the carotenoid extraction, followed by the absorbance at 450 nm [22].

2.4. Pollutant Removal and Growth Kinetic Modeling

The net pollutant reduction by *Azolla* spp. species from MFW was calculated based on the removal efficiency index given in Equation (1) [23]:

$$\text{Removal efficiency (\%)} = [(\text{Initial load} - \text{Final load})/\text{Initial load}] \times 100 \quad (1)$$

In addition to this, the relative growth rate (RGR) of *Azolla* spp. in two different reactor systems was calculated using Equation (2) [24].

$$\text{Relative growth rate (g/g/day)} = [\text{Log(Fb)} - \text{Log(Ib)}/t_2 - t_1] \quad (2)$$

where "Fb" and "Ib" represent the final and initial fresh biomass of *Azolla* spp. at "t_2" (final) and "t_1" (initial) experimental time (days), respectively.

The total surface coverage (%) by *Azolla* spp. was computed using MATLAB software after taking the periodical vertical surface images of the tank reactors using a web camera (w200, 720p, Hewlett-Packard, Palo Alto, CA, USA). The image was converted using the "rgb2gray" command, followed by generating a binary image of the captured objects using "imbinarize". Then, the percent surface area was calibrated and calculated by taking the sum of rotation symmetry using "Asurf" and "Asect" commands. A linear equation ($y = 0.92x + 14.64$; $R^2 = 0.98$) was drawn by taking surface coverage against the fresh biomass to interpolate *Azolla* spp. biomass at mid-experiment points (3rd, 9th, and 12th days) without disturbing the growth of *Azolla* spp. The growth performance of *Azolla* spp. was demonstrated using two sigmoidal functions viz., logistic, and modified Gompertz growth kinetic models. These models help simulate the S-shaped growth curves of microbes and plants. The non-linear curve modeling helps to determine the critical parameters that could optimize the growth performance of phytoremediation systems [25]. For this, the fresh biomass of *Azolla* spp. was used as an input parameter against sampling time (days). The forms of the models are given in Equations (3) and (4):

$$y = \frac{P}{1 + e^{-k\,(x - xc)}} \quad (3)$$

$$y = Pe^{-e^{(-k(x - xc))}} \quad (4)$$

where "y" is the predicted *Azolla* fresh biomass (g), "P" is the maximum fresh biomass production potential, "k" is the specific growth rate and "xc" is the lag phase in days.

2.5. Statistics and Software

All experiments were performed as randomized block designs of triplicated runs. The data obtained in this study were analyzed using unpaired Student's t-test and one-way analysis of variance (ANOVA) tests. For this, the computation and graphical works were performed using Microsoft Excel (Version 2019, Microsoft Corp., Redmond, DC, USA). The growth simulation and kinetic modeling were performed using OriginPro (Version 2022a, Student edition, OriginLab Corp., Northampton, MA, USA). The image processing and surface area calculations were performed using MATLAB (R2021b, MathWorks, Natick, MA, USA) software.

3. Results and Discussion

3.1. Properties of Borewell Water and MFW

The results of the physicochemical analysis of borewell water and MFW are presented in Table 1. The results indicated that MFW had significantly higher ($p < 0.05$) values of all parameters when tested using an unpaired Student's t-test. Particularly, the borewell water was characterized by a near-neutral pH value (7.13 ± 0.03) with 0.17 ± 0.01 dS/m of EC. The TDS value of borewell water was recorded as 144.82 ± 2.50 mg/L with very less values of BOD (3.18 ± 0.20 mg/L), COD (9.07 ± 0.08 mg/L), and TKN (1.55 ± 0.01 mg/L). The borewell water was free from pollutants and suitable for drinking purposes. On the other hand, MFW showed significantly higher ($p < 0.05$) pollution load in terms of alkaline pH range (8.30 ± 0.10), high value of EC (3.55 ± 0.12 dS/m), TDS (1693.40 ± 56.24 mg/L), BOD (1082.10 ± 13.85 mg/L), COD (2176.30 ± 82.66 mg/L), and TKN (255.90 ± 10.43 mg/L) pollutants. However, the pollution load of MFW exceeded the maximum safe discharge limits of the Bureau of Indian Standards (BIS) except for pH and TDS pollutants suggesting a lack of effective wastewater treatment strategies. The sources of such pollutants in MFW might be excessive use of water for washing tools, machinery, growing, composting areas, compost wetting, fertilizer mixing, irrigation of substrate, excess runoffs, post-harvest processing of mushroom and its products, human excreta, etc. (Figure 1).

Table 1. Physicochemical characteristics of borewell water and mushroom industry wastewater used in this experiment.

Properties	Borewell Water	Mushroom Farm Wastewater	Student's *t*-Test		Safe Discharge Limits ^
			t-Statistics	*p*-Value	
pH	7.13 ± 0.03	8.30 ± 0.10 *	19.57	<0.01	5.50–9.00
Electrical Conductivity (EC: dS/m)	0.17 ± 0.01	3.55 ± 0.12 *	48.61	<0.01	NA
Total Dissolved Solids (TDS: mg/L)	144.82 ± 2.50	1693.40 ± 56.24 *	47.64	<0.01	1900
Biological Oxygen Demand (BOD: mg/L)	3.18 ± 0.20	1082.10 ± 13.85 *	134.91	<0.01	100
Chemical Oxygen Demand (COD: mg/L)	9.07 ± 0.08	2176.30 ± 82.66 *	45.41	<0.01	250
Total Kjeldahl's Nitrogen (TKN: mg/L)	1.55 ± 0.01	255.90 ± 10.43 *	42.23	<0.01	100

*: Significantly different from the borewell water at $p < 0.05$; ^: surface discharge limits of Bureau of Indian Standards (BIS); NA: not available.

Remarkably, the residual compost and N-based fertilizers, (e.g., urea) are the major contributors to high BOD, COD, and TKN values of MFW. Previously, limited reports are available on physicochemical characterization of MFW. A study by Rodríguez Pérez et al. [26] characterized the MFW release from the cultivation farm of oyster (*Pleurotus* spp.) mushroom. The wastewater exhibited high loads of BOD (≈60 g/L), COD (≈30 g/L), and NH_3-N (12.50 mg/L) pollutants. This result is in agreement with the present investigation that confirms the presence of certain pollutants in MFW. Thus, the MFW collected in this study needs effective treatment through appropriate biological approaches as its BOD to COD ratio reaches a value of 0.5.

3.2. Removal of Pollutants from MFW by Azolla spp.

In the current study, the two selected *Azolla* spp. (*A. pinnata* and *A. filiculoides*) were used for the phyto-treatment of different concentrations of MFW under stagnant and flowing tank reactors. The findings showed that after a hydraulic retention time of 15 days, a substantial load of pollutants was removed by both *A. pinnata* and *A. filiculoides Azolla* spp. The initial values of physicochemical parameters were significantly ($p < 0.05$) changed after the phytoremediation experiments in all experimental treatments (Table 2). The presence of various pollutants did not only support the growth of *Azolla* spp. through biological absorption but also helped achieve their reduction from the MFW media. However, *A. pinnata* is renowned to have higher pollutant reduction efficiency compared to *A. filiculoides*. On the other hand, the flowing tank reactor systems showed a higher reduction in pollutant loads than the stagnant ones. By using a flowing tank reactor system, *A. pinnata* was capable to reduce all physicochemical parameters such as pH, EC, TDS, BOD, COD, and TKN by 18.87, 80.28, 87.12, 90.63, 84.14, and 86.38% maximally in F100 MFW treatment, respectively. Similarly, *A. filiculoides* also removed loads of pH, EC, TDS, BOD, COD, and TKN by 18.56, 86.63, 89.90, 85.54, 85.04, and 82.04% in the same treatment and reactor system, respectively (Figure 3). Overall, the increasing order of pollutant removal was identified as control < S50 < F50 < S100 < F100. Nevertheless, the removal efficiency was lesser in control and S50 treatments, which might be due to the lesser accessibility of nutrients that affected the survival capabilities of *Azolla* spp. Higher removal in the flowing tank reactor system might be due to efficient recirculation and uniform mixing of pollutants that sustain oxygen availability within the medium. On the other hand, the stagnant tank system lacked continuous recirculation, which affected the bioavailability of pollutants to the root system of *Azolla* spp.

Table 2. Changes in the physicochemical characteristics of MFW before and after cultivation of two *Azolla* spp.

Azolla spp.	Treatment		pH	EC (dS/m)	TDS (mg/L)	BOD (mg/L)	COD (mg/L)	TKN (mg/L)
A. pinnata	Control	Initial	7.13 ± 0.03	0.17 ± 0.01	144.82 ± 2.50	3.18 ± 0.20	9.07 ± 0.08	1.55 ± 0.01
		Final	6.50 ± 0.03 *	0.10 ± 0.01 *	98.35 ± 5.14 *	2.45 ± 0.50 *	3.59 ± 0.17 *	0.70 ± 0.05 *
	S50	Initial	8.13 ± 0.02	1.80 ± 0.04	845.55 ± 14.75	526.24 ± 12.38	1075.05 ± 14.20	127.19 ± 3.71
		Final	6.80 ± 0.02 *	0.76 ± 0.05 *	240.12 ± 6.07 *	110.24 ± 30.20 *	260.40 ± 8.36 *	25.30 ± 2.24 *
	S100	Initial	8.30 ± 0.03	3.59 ± 0.02	1691.10 ± 28.38	1052.47 ± 19.65	2150.10 ± 17.29	254.37 ± 3.20
		Final	7.03 ± 0.02 *	1.28 ± 0.05 *	350.33 ± 8.20 *	153.32 ± 10.80 *	388.54 ± 4.36 *	41.2 ± 4.18 *
	F50	Initial	8.12 ± 0.02	1.78 ± 0.02	846.70 ± 9.45	541.05 ± 8.77	1088.15 ± 15.65	127.95 ± 5.13
		Final	6.74 ± 0.05 *	0.42 ± 0.03 *	210.05 ± 5.60 *	92.70 ± 3.16 *	209.13 ± 7.11 *	22.44 ± 6.52 *
	F100	Initial	8.32 ± 0.01	3.55 ± 0.04	1693.40 ± 24.92	1082.10 ± 20.10	2176.30 ± 28.05	255.90 ± 7.33
		Final	6.75 ± 0.04 *	0.70 ± 0.08 *	218.09 ± 4.83 *	101.38 ± 5.04 *	301.55 ± 3.18 *	34.86 ± 5.41 *
A. filiculoides	Control	Initial	7.12 ± 0.02	0.18 ± 0.02	149.02 ± 7.10	3.15 ± 0.16	9.12 ± 0.10	1.54 ± 0.02
		Final	6.58 ± 0.02 *	0.12 ± 0.04 *	103.34 ± 4.05 *	2.54 ± 0.31 *	3.78 ± 0.20 *	0.81 ± 0.04 *
	S50	Initial	8.14 ± 0.02	1.75 ± 0.03	820.10 ± 8.34	525.45 ± 6.25	1097.63 ± 14.97	125.34 ± 3.83
		Final	6.95 ± 0.04 *	0.81 ± 0.07 *	251.40 ± 4.78 *	114.38 ± 2.61 *	265.03 ± 9.40 *	28.12 ± 2.47 *
	S100	Initial	8.32 ± 0.02	3.49 ± 0.03	1640.20 ± 10.55	1050.90 ± 12.09	2195.26 ± 26.03	250.68 ± 3.27
		Final	7.09 ± 0.02 *	1.35 ± 0.05 *	362.90 ± 6.12 *	167.04 ± 3.53 *	392.12 ± 8.16 *	48.36 ± 5.98 *
	F50	Initial	8.12 ± 0.01	1.80 ± 0.02	840.32 ± 11.58	545.53 ± 5.90	1090.35 ± 15.24	127.09 ± 5.30
		Final	6.69 ± 0.05 *	0.49 ± 0.04 *	217.50 ± 6.86 *	96.82 ± 4.10 *	210.62 ± 5.03 *	25.10 ± 6.12 *
	F100	Initial	8.35 ± 0.01	3.59 ± 0.01	1680.63 ± 15.40	1091.05 ± 20.24	2180.70 ± 27.21	254.18 ± 5.08
		Final	6.80 ± 0.03 *	0.76 ± 0.04 *	224.72 ± 8.25 *	110.18 ± 2.92 *	315.31 ± 7.72 *	38.03 ± 4.15 *

*: Significantly different from initial values at $p < 0.05$; S: stagnant; F: flowing reactor.

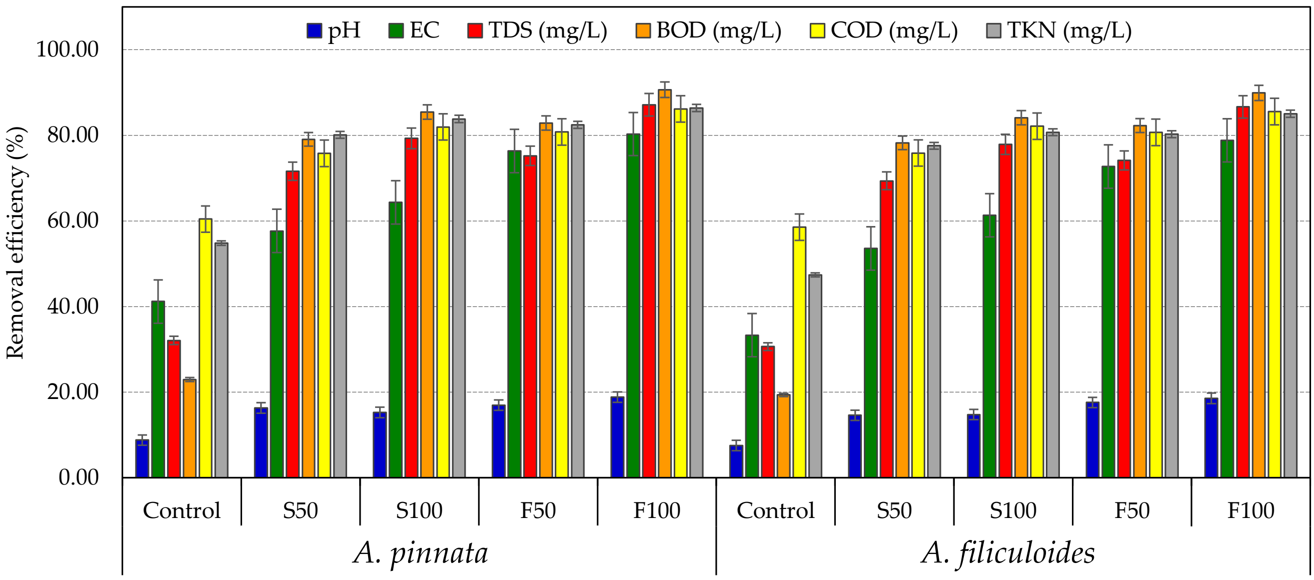

Figure 3. Pollutant removal efficiency of two *Azolla* spp. cultivated in different treatments of MFW (S: stagnant; F: flowing reactor).

Azolla spp. are ideal candidates for the phytoremediation of agro-industrial wastewaters. They act as a natural cleaner of aquatic bodies by assimilating the harmful pollutants into their vegetative parts. However, they may also act like invasive species and dominate the surface if aquatic bodies are extremely polluted, thereby affecting other residing floral and faunal communities [27]. Previous studies have reported that *Azolla* spp. can help treat various wastewaters, particularly composite industrial wastewater [5], piggery [28], etc. However, no such study is available on phytoremediation of MFW using any *Azolla* spp. Kumar et al. [5] used *A. pinnata* for the phytoremediation of composite wastewater released from an industrial complex at Haridwar, India, and reported maximum EC (>50%), TDS (>75%), BOD (>70%), COD (>72%), and TKN (>80%) reduction in 60% dilution treatment. Similarly, Lay and Iwai [28] applied *A. microphylla* for the remediation of piggery wastewater using five different concentrations. They optimized that 50:50 treatment ratio best suited for maximum pollution reduction and growth of *A. microphylla*. Thus, the present study is the first to demonstrate the sustainable management of MFW by cultivation *Azolla* spp.

3.3. Effects of MFW and Reactor Type on Growth and Biochemical Parameters of Azolla spp.

The effects of MFW and reactor type on growth and biochemical parameters of Azolla spp. were studied. The MFW used in this study was helpful for the growth of *Azolla* spp. Efficient growth of both *Azolla* spp. was observed while using absolute MFW treatment (S100 and F100); however, the flowing tank reactor depicted better growth compared to stagnant. Overall, the growth and biochemical parameters increased significantly ($p < 0.05$) with an increase in the MFW dose (Table 3). Although, the best growth performance was reported by *A. pinnata* in terms of surface coverage (84.40%), fresh biomass (110.15 g), relative growth rate (0.07 g/g/day fwt.), chlorophyll (2.40 mg/g fwt.), and carotenoids (0.34 mg/g). On the other hand, *A. filiculoides* showed moderately lesser values of surface coverage (78.82%), fresh biomass (96.10 g), relative growth rate (0.07 g/g/day fwt.), chlorophyll (2.28 mg/g fwt.), and carotenoids (0.30 mg/g). This could be linked to the strong bio-accumulative and growth capacity of *A. pinnata* compared to *A. filiculoides*. In this, the surface coverage and fresh biomass had a positive correlation with a coefficient of determination (R^2) of >0.90. All the growth and biochemical parameters were attributed to the concentration gradient of the applied MFW. Of the two reactor systems tested, the flowing (F100) was more supportive. Thus, other treatments could be considered limiting in terms of bioavailable nutrients that affect the growth of *Azolla* spp.

Table 3. Growth and biochemical changes in two *Azolla* spp. cultivated in different treatments of MFW.

***Azolla* spp.**	**Treatment**	**Surface Coverage (%)**	**Fresh Biomass (g)**	**Relative Growth Rate (g/g/day fwt.)**	**Chlorophyll (mg/g fwt.)**	**Carotenoids (mg/g)**
A. pinnata	Control	5.22 ± 0.10	20.33 ± 0.10	0.02	1.20 ± 0.03	0.18 ± 0.01
	S50	49.22 ± 2.06 *	59.12 ± 1.08 *	0.05	2.00 ± 0.01 *	0.21 ± 0.02 *
	S100	62.08 ± 3.50 *	80.21 ± 2.44 *	0.06	2.16 ± 0.02 *	0.28 ± 0.02 *
	F50	76.33 ± 1.72 *	63.20 ± 1.60 *	0.05	2.13 ± 0.02 *	0.25 ± 0.01 *
	F100	84.40 ± 2.03 *	110.15 ± 2.90 *	0.07	2.40 ± 0.05 *	0.34 ± 0.03 *
A. filiculoides	Control	4.50 ± 0.05	18.05 ± 0.37	0.02	1.20 ± 0.02	0.16 ± 0.01
	S50	42.60 ± 1.96 *	54.64 ± 1.10 *	0.05	1.80 ± 0.05 *	0.20 ± 0.02 *
	S100	57.10 ± 2.35 *	75.06 ± 2.02 *	0.06	2.10 ± 0.07 *	0.25 ± 0.01 *
	F50	71.09 ± 0.87 *	60.38 ± 1.45 *	0.05	2.12 ± 0.04 *	0.24 ± 0.02 *
	F100	78.82 ± 2.46 *	96.10 ± 2.14 *	0.07	2.28 ± 0.03 *	0.30 ± 0.03 *

*: Significantly different from control values at $p < 0.05$; S: stagnant; F: flowing reactor.

Aquatic plants have enormous capabilities for accumulating toxic pollutants from the aquatic bodies and spreading over them with fast multiplication. In this study, MFW implicated as nutrient media of *Azolla* spp. was helpful for their fast replication. By optimizing the nutrient proportions, higher growth is reported along with significant production of photosynthetic pigments and other phytochemical constituents. Nevertheless, no study reports the phytoremediation of MFW and its effects on the growth of *Azolla* spp. A study by Muradov et al. [29] showed that *Azolla* spp. had better phytochemical constitutes (chlorophyll $a + b$) of 6.10 µg/mL when grown in swine wastewater compared to control treatments with no wastewater addition. In addition, Mostafa et al. [12] also explored the potential of *A. pinnata* for the treatment of crude oil pollution and found that chlorophyll contents (2.78 mg/g) and carotenoid (0.17 mg/g) were improved by using a 2% treatment.

3.4. Growth Kinetic Modeling of Azolla spp. Grown in MFW

Kinetic modeling provides useful insights into understanding the critical growth patterns such as growth rate and biomass production potentials of plants growing in a phytoremediation system [30]. In the current investigation, the two tested sigmoid functions viz., logistic and modified Gompertz models showed good fitness for the time course growth patterns prediction of selected *Azolla* spp. Table 4 shows the simulated variables of logistic and modified Gompertz model for the growth of two *Azolla* spp. Results indicated that the logistic model showed better fitness to the experimental data in terms of coefficient of determination ($R^2 > 0.99$), predicted fresh biomass (y), maximum fresh biomass production potential (P: g), growth rate constant (k), and lag phase of plant's growth (xc) compared to modified Gompertz model. Comparatively, *A. pinnata* showed higher values of growth rate constant (0.40) using the logistic model compared to *A. filiculoides* (0.33) with the F100 treatment of MFW. Figure 4 depicted that both models were useful in precisely simulating the time course growth curve of *Azolla* spp. The models proved to have the ability to deal with *Azolla* spp., MFW concentration, and reactor type varying conditions. A minimum difference between the experimental and predicted data shows the high accuracy of the model that could help in real-life experiments. However, the simulated curve showed that both *Azolla* spp. displayed a lag phase, followed by a sudden increase in biomass after the 3rd day, which later become stationary after the 12th day. Herein, the first phase is termed the "establishment phase" in which the plant generally acclimatizes itself to the new MFW medium, followed by a "rapid expansion phase" in which the plant achieves its maximum growth rate, and finally an "entrenchment phase" at which plant growth becomes stationary. The product harvesting is also recommended at the entrenchment phase since after this point the system starts getting degenerating. The rapid expansion phase occurs when nutrients in the medium are abundantly available, whereas the entrenchment phase appears when resources are finite and typically utilized by the plant.

Table 4. Comparative assessment of growth kinetic models two *Azolla* spp. cultivated in different treatments of MFW.

***Azolla* spp.**	**Treatment**	**Model Variables**									
		Logistic Model					**Modified Gompertz Model**				
		R^2	y	P	k	xc	R^2	y	P	k	xc
A. pinnata	Control	0.99	20.35	22.64	0.16	1.51	0.99	20.40	24.18	0.11	1.03
	S50	0.99	60.01	65.23	0.29	6.79	0.98	60.82	77.55	0.15	5.57
	S100	0.99	82.20	88.21	0.34	7.34	0.98	83.42	104.91	0.17	6.41
	F50	0.99	64.66	74.52	0.27	8.14	0.98	65.38	99.15	0.12	7.99
	F100	0.99	112.02	116.63	0.40	7.07	0.98	113.74	129.68	0.22	5.93
A. filiculoides	Control	0.99	18.17	19.64	0.16	0.03	0.99	18.21	20.48	0.12	2.54
	S50	0.99	55.47	60.93	0.28	6.94	0.98	56.24	74.32	0.14	6.08
	S100	0.99	76.69	82.67	0.33	7.39	0.98	77.88	99.89	0.16	6.56
	F50	0.98	62.03	77.58	0.24	9.43	0.97	62.64	127.42	0.09	11.39
	F100	0.99	97.54	101.73	0.33	6.99	0.98	99.14	113.98	0.21	5.85

S: stagnant; F: flowing reactor; R^2: coefficient of determination; y: predicted fresh biomass; P: maximum fresh biomass production potential; k: specific growth rate; xc: lag phase.

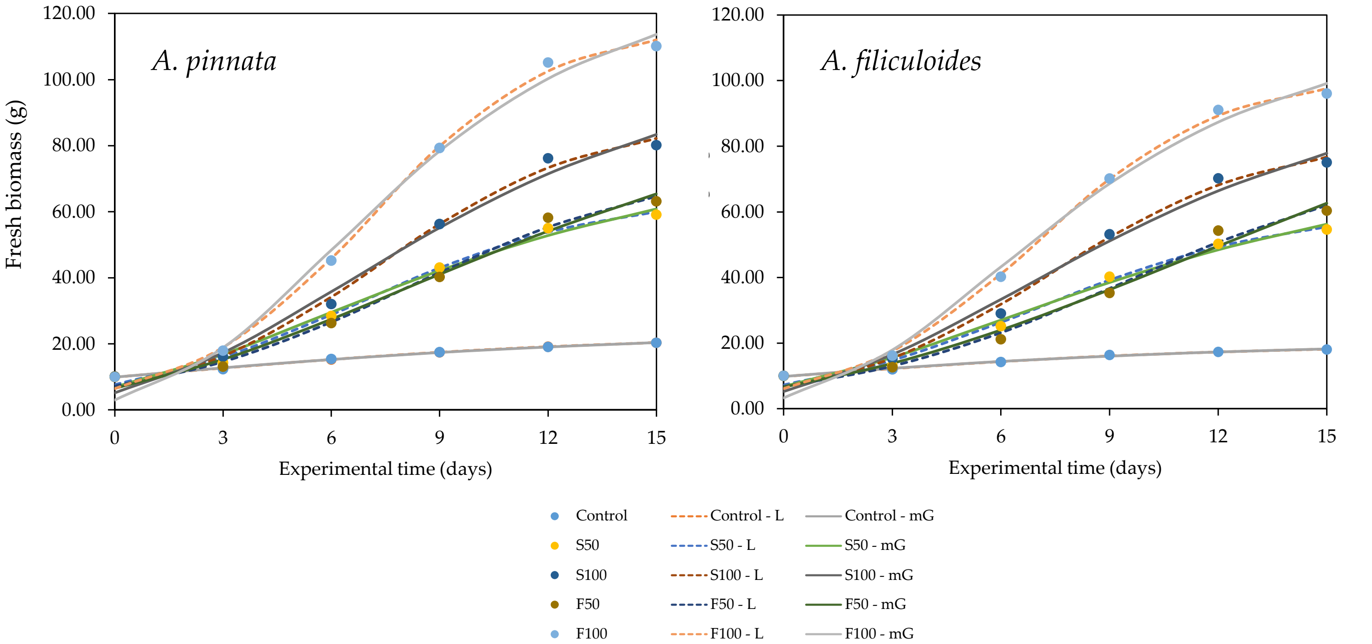

Figure 4. Comparison of experimental and predicted (S: stagnant; F: flowing reactor; L: logistic; mG: modified Gompertz) growth curves of two *Azolla* spp. cultivated in different treatments of MFW.

Previous studies have demonstrated the usefulness of sigmoidal functions in predicting the growth kinetic functions of plants growing within phytoremediation systems. A report by Goala et al. [31] cultivated *A. pinnata* in dairy wastewater for the remediation of major pollutants and studied the leaflet growth kinetics using an image recognition-based technique while implementing the logistic and modified Gompertz models for curve simulation. They reported that the logistic model showed better fitness in the experimental data with minimum error in the prediction of *A. pinnata* biomass. Another report by Yalçuk and Ugurlu [32] also studied the growth kinetics of *Typha latifolia* and *Canna indica* plants using logistic and modified Gompertz models during the treatment of landfill leachate in three types of reactors. They found that the logistic model had better fitness ($R^2 > 0.71$) compared to modified Gompertz ($R^2 < 0.14$). Therefore, the outcomes obtained from these studies are in strong agreement with the results of the present study, which indicated the application of growth kinetic models in maximizing the plant growth performance in a phytoremediation system.

4. Conclusions

The present study deals with the phytoremediation of mushroom farm wastewater (MFW) by cultivating two *Azolla* spp. in stagnant and flowing tank reactors. The findings suggested that both species (*A. pinnata* and *A. filiculoides*) significantly ($p < 0.05$) removed the pollution load of MFW after 15 days. However, the highest reduction in MFW parameters such as pH, EC, TDS, BOD, COD, and TKN was obtained using *A. pinnata* under flowing tank reactor conditions. Moreover, the maximum relative growth rate, surface coverage, fresh biomass, total chlorophyll, and carotenoids in Azolla samples were also reported in absolute MFW treatment (F100). In the two tested growth models, the logistic model showed better fitness compared to the modified Gompertz model. This is the first study that investigates the use of the green and cleaner technique for the treatment and management of MFW using *Azolla* spp. The cultivated *Azolla* spp. biomass can also be used as animal feed, resources for bioenergy production, composting, biofertilizer, etc. Further studies on the analysis and remediation of other pollutants, (e.g., pesticides, heavy metals) from MFW are highly recommended. Additionally, biochemical interactions between *Azolla* spp. and microbial communities in the MFW along with possible contamination of the harvested biomass should be investigated.

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