

Phytoremediation of dairy wastewater using *Azolla pinnata*: Application of image processing technique for leaflet growth simulation

Madhumita Goala^a, Krishna Kumar Yadav^{b,*}, Javed Alam^c, Bashir Adelodun^{d,e}, Kyung Sook Choi^d, Marina M.S. Cabral-Pinto^f, Ali Awadh Hamid^g, Mansour Alhoshan^{c,g}, Fekri Abdurqab Ahmed Ali^g, Arun Kumar Shukla^c

^a Nehru College, Pailapool, Affiliated Assam University, Silchar, Cachar 788098, Assam, India

^b Faculty of Science and Technology, Madhyanchal Professional University, Ratibad, Bhopal 462044, M.P., India

^c King Abdullah Institute for Nanotechnology, King Saud University, P.O. Box-2455, Riyadh 11451, Saudi Arabia

^d Department of Agricultural Civil Engineering, Kyungpook National University, Daegu 41566, Republic of Korea

^e Department of Agricultural and Biosystems Engineering, University of Ilorin, PMB 1515, Ilorin 240103, Nigeria

^f Geobiotec Research Centre, Department of Geoscience, University of Aveiro, 3810-193 Aveiro, Portugal

^g Chemical Engineering Department, College of Engineering, King Saud University, P.O. Box- 2455, Riyadh 11451, Saudi Arabia

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ABSTRACT

The present study assessed the phytoremediation efficiency of water fern (*Azolla pinnata* R.Br.) in the treatment of dairy wastewater (DWW). Batch mode experimentation was done using different dilutions (0 to 100%) of DWW transplanted with five healthy leaflets of *A. pinnata*. Besides this, the *A. pinnata* growth was captured using camera vision-based image recognition and further modeled using logistic and modified Gompertz models. The findings showed that after 14 days of phytoremediation experiments, the maximum significant ($p < 0.05$) reduction efficiency of selected pollutant parameters of DWW i.e. pH (9.41%), electrical conductivity (61.42%), total dissolved solids (71.56%), total Kjeldahl's nitrogen (73.25%), and total phosphorus (65.37%) observed using 75% DWW dilution. Moreover, the periodically taken surface images were useful to recognize the position and number of leaflets which further helped to simulate *A. pinnata* growth patterns. The maximum number of leaflets ($n = 20$), fresh biomass (16.18 ± 0.42 g), dry biomass (1.47 ± 0.04 g), and chlorophyll content (3.14 ± 0.03 mg/g fwt.) was also observed using 75% DWW treatment, respectively. The logistic model was found more robust as compared to modified Gompertz to predict leaflet production (y) as revealed from model validation results i.e. coefficient of determination ($R^2 > 0.9533$) and minimum difference between experimental and model-predicted results. Thus, the combined application of phytoremediation and image processing techniques can be used to monitor and maximize plant growth performance.

1. Introduction

Environmental pollution caused by wastewater disposal has become a threatening issue, globally [1,2]. Different industrial and non-industrial sources of wastewater generation are depleting the quality of soil and water environments [3,4]. Globally, it is estimated that nearly 80% of wastewater is disposed of without appropriate treatment [5]. Moreover, developing countries are mostly affected by wastewater disposal as compared to developed countries due to a lack of proper treatment and disposal facilities [6,7]. Thus, the development of an efficient method of wastewater treatment and disposal has been the

primary necessity for both the scientific community and industrial units. Being an agricultural country, dairy is the major industrial sector of India. Currently, there are several operation dairy industries in India units producing 176.4 million-ton liters of milk [8]. A huge volume of wastewater containing a high load of nutrients is produced as a result of the production and processing of milk. Therefore, dairy industries lacking treatment facilities directly dump these wastewaters into open land or water bodies [9]. Therefore, there is an urgent need to stop open and unsafe disposal and efficiently manage wastewater pollution.

Phytoremediation is a cost-effective and environmentally friendly method of wastewater treatment using hyperaccumulating plants

* Corresponding author.

E-mail addresses: envirokrishna@gmail.com (K.K. Yadav), javaalam@ksu.edu.sa (J. Alam).

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[10,11,40]. These plants have the capability to remediate various pollutants from soil and water bodies and translocate them within their roots, stem, and leaf parts [12,13]. Recently, phytoremediation technology has emerged significantly as studies on testing the efficiency of plants for pollutant removal are increasing. Based on the localization of aquatic plants, they have been categorized as free-floating, emergent, and submerged plants [14,15]. Water fern (*Azolla pinnata* R.Br.) is a free-floating macrophyte having a distinct capacity to absorb various nutrients from water bodies. Previous studies on *A. pinnata* showed its application in the biological treatment of different types of wastewater including, composite effluent [10], palm oil wastewater [16], domestic, municipal, aquaculture, and industrial [17], synthetic dyes [18], etc. Therefore, the use of the *A. pinnata* plant may give promising results in terms of removing major physicochemical and nutrient pollutants from dairy wastewater [19]. However, the growth patterns of aquatic plants depend on the availability of nutrients and also the genetic compatibility to toxicants [10,20]. It is reported that the optimum load of supplied nutrients may give better plant growth performance which further could be adjusted to optimize the wastewater treatment process. In this, the growth of a plant can be determined by various direct and indirect methods.

Recently, with the advancement of information and technology, image processing and deep learning have been evolved as efficient techniques to monitor and analyze real-time objects [21,22]. In this, an actual photographic view of the object is obtained and systems are trained to identify/count the same kind of objects. This technique has been successfully applied in various studies such as fruit sorting [23], disease identification [24], mushroom growth measurement [25,26], vegetation index measurement [27], etc. Thus, this technique combined with the phytoremediation process could possibly provide good insights into the growth measurement of plants. Besides this, kinetic models are an efficient tool for studying the growth behavior of plants and microbes [28,29]. There are several linear and non-linear growth models widely used to study these growth patterns [30,31]. Out of them, sigmoid models are “S” shaped functions which are normally followed by most plants and microbes. These models provide the best fitting result for the system where two growth phases i.e. lag and stationary are distinct. Previous studies have reported the usability of sigmoid functions in the phytoremediation process [32]. Thus, the use of such models may provide a better understanding of *A. pinnata* growth patterns under DWW treatment using the image processing technique. More specifically, phytoremediation is a well known sustainable technology that can help in restoring soil, air, and water components of our environment. However, the performance of plants used for phytoremediation purposes needs to be monitored in order to decide whether the plant is capable to grow in the given media or not. Thus, manual monitoring of plant growth is a conventional method. In this study, we introduced the image processing technique to identify the status of selected test plant species i.e. *A. pinnata*. The data obtained from camera visuals was helpful to precisely identify the number and locations of *A. pinnata* leaflets which represents a novel method of its growth monitoring. Moreover, the model analysis showed that the plant growth patterns can be critically analyzed by using the data obtained from image processing technique which could help in further maximizing both growth and pollutant removal processes. Previously, no attempt has been made to study the *A. pinnata* plant growth performance under a batch mode phytoremediation of dairy wastewater. Thus, this study adds new information which enables the integration of both phytoremediation and image recognition techniques for sustainable wastewater management.

Believe this, the present study has been designated to assess the efficiency of the *A. pinnata* plant in the treatment of different dilutions of DWW. The influence of DWW amended rates on growth and biochemical pigment of *A. pinnata* has been reported. Moreover, the growth of *A. pinnata* was monitored with help of the image processing technique and further modeled using different growth models i.e. logistic and modified Gompertz.

2. Materials and methods

2.1. Collection of *Azolla pinnata* and dairy wastewater

For the present study, *Azolla pinnata* biomass was collected from the Ganga canal of Dhanouri, Haridwar, India (29°56'15" N and 77°57'04" E). The juvenile leaflets of *A. pinnata* were collected in polythene bags having an aeration facility. Besides this, the treated dairy wastewater was collected from Mohan Milk Processing Plant located at Bhagwanpur, Haridwar, India (29°58'25" N and 77°46'39" E). The wastewater samples were collected in 10-liter polyvinyl chloride (PVC) cans and immediately transported to the laboratory. The samples were preserved at 4 °C until final implementation in phytoremediation experiments and laboratory analyses.

2.2. Experimental setup

The phytoremediation experiments were conducted in a 250 mL capacity circular PVC bin. Five different concentrations of DWW including, 0 (borewell water as control), 25, 50, 75, 100% (absolute) were used to grow *A. pinnata* under room condition. The average temperature, humidity, and light intensity of the experimental room were 20 °C, 55–70%, and 2000 lx, respectively. The loss of water content on bins was normalized by the addition of deionized water to become the initial volume. A LED bulb was used for continuous light exposure. The light intensity was controlled using a potentiometer and calibrated using a digital Lux meter (Mextech LX-1010B, IN). The setup of phytoremediation experiments was shown in Fig. 1. The image recognition device includes a portable Web Camera (Logitech C270, CH) connected to a Raspberry Pi 3 microprocessor. The Web Camera (WC) was vertically fitted above the circular PVC bins with help of an adjustable plastic stand. The microprocessor included a MicroSD card (16 GB; SanDisk, USA) which was used to save the captured images at a defined interval of 12 h for 14 days of phytoremediation experiments. Moreover, the image capturing system was connected to an uninterrupted power supply unit (UPS). The time-series images captured by WC and stored of MicroSD card were further processed and used for image recognition studies.

2.3. Image processing and leaflet identification/counting

For the processing of captured images, the Image Processing and Computer Vision (IPCV) package of MATLAB (2020b) software was used. MATLAB is widely used for image processing and objecting identification/classification studies. In this study, the periodically captured images showing leaflet positions were firstly subjected to the “imread/imshow” command of the IPCV package. Then, the command line was adjusted to convert the image into grayscale followed by setting a defined level of threshold to convert the non-leaflet pixels to black. Further, the complementation of the image was done using the ~ operator to eliminate the image noise and as a form of patches. Finally, the boundaries were defined using the “bwboundaries” command, and the number of viable *A. pinnata* leaflets was counted. Fig. 2 shows the flow-chart of different steps adopted for image processing and recognition.

2.4. Measurement of leaflet number, biomass, and growth parameters

As described above, the number of leaflets was counted using the image processing technique. Besides this, the fresh biomass of *A. pinnata* was measured before and after phytoremediation experiments. The fresh biomass (g) was measured with help of a digital weighing scale (Sciencetech SE-391, IN). After the termination of the experiment, the plant biomass was oven-dried and expressed as dry biomass (g). The leaflet growth of *A. pinnata* was modeled using two different sigmoid models i.e. logistic (Eq. (1)) and modified Gompertz (Eq. (2)).

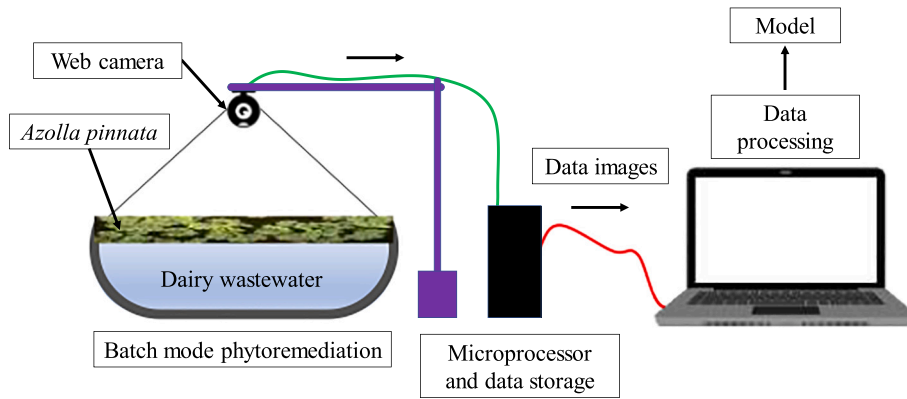


Fig. 1. Experimental setup of phytoremediation experiments equipped with image capturing accessories.

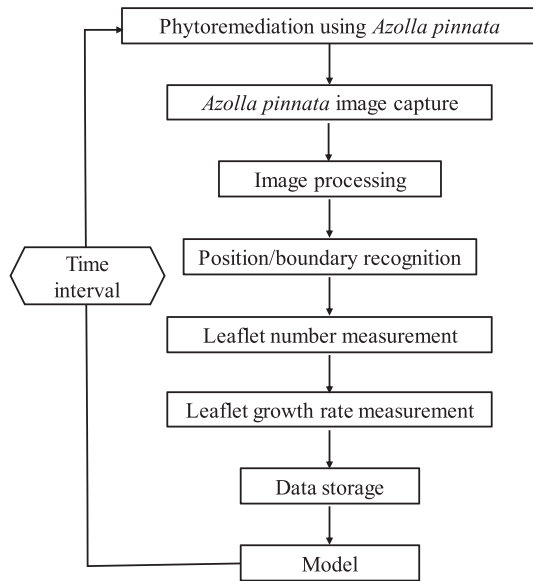


Fig. 2. Flow chart of sequential steps adopted for image capturing, processing, and model development.

$$y = \frac{y_0 - y_t}{1 + (x/x_0)^P} + y_t \quad (1)$$

$$y = P_{\exp} \left\{ -\exp \left[\frac{\mu_m}{P} (\lambda - t) + 1 \right] \right\} \quad (2)$$

where y represented the predicted number of leaflets, y and y_t were the initial and final number of leaflets, x and x_0 were the interpolated and center points of the growth phase, P was the maximum number of leaflets, μ_m was the maximum specific leaflet growth rate, λ was the lag phase while t was the time of phytoremediation experiments, respectively. For this, the “Rank Models” tool of OriginPro software was used to fit the models. The time series measured and image processed data of *A. pinnata* leaflet number was imported as X and Y columns. The fitness was decided based on the coefficient of determination (R^2), standard error, and variance criterion, and the model having minimum prediction error with acceptable R^2 values was chosen. Besides this, the total chlorophyll content (mg/g fwt.) of *A. pinnata* was determined using acetone (80%) extraction and UV-visible spectroscopic method. The absorbance was recorded at specified wavelengths i.e. 645 and 663 nm, respectively [10].

2.5. Borewell/dairy wastewater analysis and pollution load reduction efficiency

The borewell water (BWW) and dairy wastewater (DWW) used in this study were analyzed for selected physicochemical parameters including, pH, electrical conductivity (EC: dS/m), total dissolved solids (TDS: mg/L), total Kjeldahl's nitrogen (TKN: mg/L), and total phosphorus (TP: mg/L). For the water analysis, official methodologies described by the Association of Applied Chemists [41] and American Public Health Association [42] were adopted. In this regard, pH, EC, and TDS parameters were directly measured using a pre-calibrated multi-meter (ESICO 1615, IN). TKN was determined using Kjeldahl's acid-digestion and distillation method while TP was measured following the UV-vis spectroscopy method (Agilent, 60 Cary, USA). All of the laboratory grade reagents used in chemical/instrumental analyses were procured from Sigma-Aldrich, IN.

Besides this, to determine the percentage pollution load reduction efficiency of *A. pinnata* during phytoremediation of DWW, a specific removal efficiency model was used. The form of the model was given in Eq. (3):

$$\text{Reduction efficiency (\%)} = \frac{\text{Initial load} - \text{Final load}}{\text{Initial load}} \times 100 \quad (3)$$

2.6. Statistics and software

All experiments were performed in triplicate under controlled room conditions. The values were presented as mean followed by the standard deviation of triplicated analyses/experiments. The research data were analyzed using Kruskal-Wallis post-hoc, ANOVA, and non-linear regression tools. The defined level of statistical significance was prob (p) < F value of 0.05 i.e. 95% confidence interval. For this, Microsoft Office Excel (v2016, Microsoft Corp., USA), OriginPro (v2021 student learning edition, OriginLab Inc., USA), and MATLAB (v2020b, MathWorks, USA) packages were used.

3. Results and discussion

3.1. Borewell water and dairy wastewater characteristics

The BWW and DWW used in this study were analyzed for selected physicochemical and nutrient parameters as given in Table 1. It was evidenced from the results that BWW had a lesser pollution load as compared to DWW. The values of different physicochemical and nutrient parameters of DWW i.e. pH (6.53 ± 0.01), EC (6.030 ± 0.362 dS/m), TDS (1410.45 ± 34.06 mg/L), TKN (165.89 ± 9.13 mg/L), and TP (134.08 ± 8.11 mg/L) were significantly ($p < 0.05$) higher than of BWW i.e. pH (7.19 ± 0.02), EC (0.396 ± 0.010 dS/m), TDS (114.08 ± 4.08 mg/L), TKN (4.13 ± 0.25 mg/L), and TP (2.55 ± 0.05 mg/L),

Table 1Reduction efficiency (%) of physicochemical and nutrient characteristics of borewell and dairy wastewater after phytoremediation using *A. pinnata*.

Parameter	Value	Treatment					K-W p-value
		BWW	25%	50%	75%	100%	
pH	Initial	7.19 ± 0.02	7.05 ± 0.03*	6.92 ± 0.02*	6.80 ± 0.02*	6.53 ± 0.01*	<0.01
	Final	6.88 ± 0.05	6.54 ± 0.02*	6.31 ± 0.01*	6.16 ± 0.03*	6.10 ± 0.04*	<0.01
	RE (%)	4.31	7.23	8.82	9.41	6.58	-
EC (dS/m)	Initial	0.396 ± 0.010	1.380 ± 0.055*	3.240 ± 0.103*	5.010 ± 0.230*	6.030 ± 0.362*	<0.01
	Final	0.284 ± 0.024	0.883 ± 0.011*	1.533 ± 0.120*	1.933 ± 0.152*	2.683 ± 0.410*	<0.01
	RE (%)	28.28	36.01	52.69	61.42	55.51	-
TDS (mg/L)	Initial	114.08 ± 4.08	310.24 ± 10.37*	750.24 ± 22.12*	1021.13 ± 50.27*	1410.45 ± 34.06*	<0.01
	Final	65.25 ± 2.10	135.35 ± 5.03*	288.45 ± 3.98*	290.44 ± 12.50*	550.24 ± 15.86*	<0.01
	RE (%)	42.80	56.37	61.55	71.56	60.99	-
TKN (mg/L)	Initial	4.13 ± 0.25	39.24 ± 6.58*	81.13 ± 7.12*	124.24 ± 10.05*	165.89 ± 9.13*	<0.01
	Final	1.93 ± 0.76	13.24 ± 2.10*	25.24 ± 1.62*	33.24 ± 1.86*	56.24 ± 3.70*	<0.01
	RE (%)	53.27	66.26	68.89	73.25	66.10	-
TP (mg/L)	Initial	2.55 ± 0.05	28.34 ± 1.35*	64.24 ± 5.19*	90.24 ± 6.60*	134.08 ± 8.11*	<0.01
	Final	1.34 ± 0.03	12.56 ± 1.09*	24.24 ± 2.07*	31.25 ± 1.03*	57.35 ± 6.24*	<0.01
	RE (%)	47.45	55.68	62.27	65.37	57.23	-

Data presented as mean followed by the standard deviation of triplicated experiments. -: Not applicable. K-W p-value: Statistical significance among the mean rank of participating treatment groups. RE: reduction efficiency.

* Significantly different at prob (p) < F value of 0.05.

respectively. The different sources of pollutants in DWW are the processing of milk-based products and animal excreta (cattle dung, urine, fodder waste, washings). However, the DWW released from the selected study area was openly released in water bodies which may further create various environmental problems. Therefore, DWW should be treated using efficient and eco-friendly methods before discharging an open environment. Previously, several studies have assessed the physicochemical and nutrient characteristics of DWW. Out of which, Lu et al. [33] reported a higher load of TN (283.00 mg/L) and TP (115.90 mg/L) nutrients used for cultivation of microalga i.e. *Chlorella* sp. Besides this, Bortoluzzi and co-workers also assessed the pollution load of DWW and reported higher loads of various physicochemical, nutrients, and heavy metal pollutants [34].

3.2. Pollution load reduction efficiency of *A. pinnata*

After the 14 days of phytoremediation experiments using *A. pinnata*, the pollution load of DWW was significantly ($p < 0.05$) reduced as compared to BWW treatment. As given in Table 1 and Fig. 3, the maximum reduction efficiency of selected physicochemical and nutrients parameters i.e. pH (9.41%), EC (61.42%), TDS (71.56%), TKN (73.25%), and TP (65.37%) was achieved using 75% DWW dilution, respectively. However, the reduction efficiency of selected pollutant parameters gradually increased up to 75% DWW treatment, which again decreased for absolute (100%) treatment. In this, the ANOVA analysis that the initial values (means separated by least significant difference

method, LSD) of pollutant parameters were significantly ($p < 0.05$) different from the finally obtained values which indicated their effective removal. Based on the Kruskal-Wallis post-hoc test, a significant difference between the computed mean ranks of selected treatment groups i.e. BWW to 100% DWW. From these observations, a strong effect of DWW concentration on their removal by *A. pinnata* was noted. Moreover, the reduction of pollutants was more dependent on the supplied DWW concentration. *A. pinnata* is recognized as a prime candidate of wastewater treatment as previously reported by numerous studies. In this study, *A. pinnata* was capable to reduce the pollution load of DWW, significantly. The utilization of different nutrients might be associated with their sequential uptake to assist *A. pinnata* growth. A slowdown in the pollution reduction efficiency using 100% DWW may be due to nutrient stress which did not support *A. pinnata* growth as efficiently as in the case of 75% treatment. N and P as nutrient pollutants in wastewater may act as essential elements which support plant growth and proper cell functioning [10,35]. The level of pollutants present in wastewater strongly determines the performance of plants used for their phytoremediation. However, the adaptability of particular plant species in toxic wastewaters also depends on genetic compatibility which affects the rate of pollutant migration from wastewater to plant [10].

Dairy wastewater is a mixture of various ingredients such as micro, macro, and trace nutrients. The dependency of removal of each parameter depends on the selectivity of the plant to assimilate and uptake them from media. As described earlier, the most common mechanisms of nutrient utilization by plants are passive absorption and active

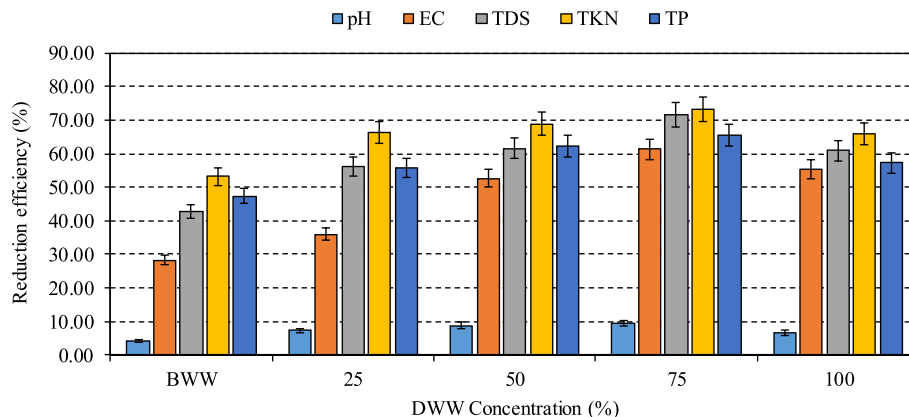


Fig. 3. Pollution load reduction efficiency (%) of *A. pinnata* grown in different concentrations of dairy wastewater.

absorption. The transportation of nutrients takes place in the form of ions through root hairs and then translocating within vegetative parts. In both mechanisms, the pollutant as the form of nutrient travels to the plant root cells where their concentrations are lesser than the media itself. In this, water acts as the most important medium to help ionic dissociation of nutrients through plant enzymes and then making them available to plant roots [35].

Several researchers have investigated the phytoremediation potential of *A. pinnata* using different types of wastewaters. Out of which, Kumar et al. [10] investigated the pollution load reduction efficiency of *A. pinnata* using integrated industrial wastewater. They reported that among different selected concentrations the maximum reduction of pollution load in terms of physicochemical, heavy metals, and microbiological parameters was achieved using 60% wastewater concentration. Another study by Akinbile and coworkers investigated the treatment of domestic wastewater using *A. pinnata*. They observed that *A. pinnata* was capable to reduce significant loads of physicochemical and nutrient parameters of domestic wastewater [39]. These reports are in line with the findings of the present study; therefore, it was recommended that *A. pinnata* might be a promising candidate to eliminate pollutants from DWW.

3.3. Effects on *A. pinnata* growth and chlorophyll contents

Table 2 showed changes in the *A. pinnata* growth and biochemical constituent after 14 days of phytoremediation experiments. In this, the number of *A. pinnata* leaflets was significantly ($p < 0.05$) increased in the case of all DWW treatments. More specifically, the initial number ($n = 5$) of the leaflet was increased to 11, 13, 16, 20, and 16 in the case of BWW, 25, 50, 75, and 100% DWW treatments, respectively. Besides this, the same pattern was observed for the harvested fresh weight of *A. pinnata* i.e. 8.01 ± 0.51 , 10.40 ± 0.30 , 12.82 ± 0.20 , 16.18 ± 0.42 , and 12.91 ± 0.12 g for BWW, 25, 50, 75, and 100% DWW treatments, correspondingly. The dry biomass of *A. pinnata* was also influenced in the same way as in the case of fresh biomass. Moreover, the maximum total chlorophyll content (3.14 ± 0.03 mg/g fwt.) was encountered in the same 75% DWW treatment. The growth performance of *A. pinnata* showed that DWW was a suitable medium for both biomass production and pollutant reduction. The maximum growth performance of *A. pinnata* in 75% DWW concentration might be associated with the presence of optimum levels of different nutrients. However, an absolute (100%) DWW dose was not feasible for *A. pinnata* growth since it showed some toxic effects in terms of a reduced number of leaflets, fresh/dry biomass, and chlorophyll production. The large variation in growth performance of *A. pinnata* under different DWW dilutions might be associated with the assimilation efficiency of the plant which altered its growth and biochemical responses.

A report by Prasad and Singh [36] revealed that the *A. pinnata* may exhibit decreased biochemical response under Cd stressed environments. Abd Kadir et al. [16] also studied the phytoremediation potential of *A. pinnata* and *Lemna minor* plants in the treatment of palm oil mill wastewater. They reported that maximum protein, carbohydrate, and pigment contents were observed using 2.5% dilutions whereas higher

dilution caused a rapid decline in plant growth parameters. Also, Kösesakal and Yıldız [37] studied biochemical profiles of two *Azolla* sp. under controlled environments. They observed that the *A. pinnata* grew at faster rates as compared to *A. caroliniana* with maximum assimilation of biochemical compounds under controlled environments. Hence, it can be concluded the DWW dilutions significantly affected the growth and biochemical parameters of *A. pinnata* in the present study.

3.4. Feasibility of image recognition and prediction models techniques in leaflet growth simulation

This study integrated the image processing technique to recognize *A. pinnata* leaflets under laboratory-scale phytoremediation experiments. In this, the frequently taken images of *A. pinnata* were analyzed using MATLAB software to identify and count the leaflet boundaries. Fig. 4 showed the initial and final views of *A. pinnata* leaflets grown in different treatments of DWW. The first image was unprocessed while the second image was threshold processed. Similarly, the third image showed the defined boundary levels of leaflets used to count their numbers. As given in Fig. 4 and Table 2, it was observed that the technique was helpful to count the actual number of leaflets from the captured images. Based on the obtained data, the growth pattern of *A. pinnata* was simulated using two different prediction models i.e. logistic and modified Gompertz, separately. The results showed that the time-series number of leaflet production as a function of the sigmoid model gave identical results. Out of the two models, the logistic function was more appropriate to precisely predict the time-series leaflet growth as compared to modified Gompertz. Data provided in Table 3 showed that the logistic model performed better in terms of predicting the total number of leaflets (y), coefficient of determination (COD), and maximum specific leaflet production potentials. The prediction errors were also lesser in the case of the logistic model. Moreover, Fig. 5 showed the time-series trend fitness of leaflet growth using two selected models. It can be seen from the trend analysis that the logistic model had robust trend simulation i.e. near to the values obtained experimentally.

Logistic and modified Gompertz models are a form of sigmoid function which enables understanding the exponential growth patterns of plants and microbes [29]. In this, logistic and modified Gompertz models are used to predict the time-series response of plant/microbe growth processes which may include their biomass or specific constituent formation and reduction. However, the performance of both models varies according to the processes. Both models are well applied to avoid the additive errors while adjusting the variance criterion and the model having minimum error could be considered to model the processes. On the other hand, image processing and deep learning techniques have received great attention from the scientific community in different areas.

Numerous researchers have been successfully implemented these techniques in agriculture and environment-related aspects. Recently, Kharuf-Gutierrez et al. [27] used multispectral aerial images and processed them using image processing and deep learning techniques to obtain important crop vegetation indices. In their study, Fu et al. [23] developed an image processing-based kiwi fruit sorting system. The

Table 2

Leaflet growth, biomass production, and chlorophyll content of *A. pinnata* grown in different concentrations of dairy wastewater.

Treatment	Leaflet number (n)		Fresh biomass (g)		Dry biomass (g)		Chlorophyll content (mg/g fwt.)	
	Initial	Final	Initial	Final	Initial	Final	Initial	Final
BWW	5	11	4.00 ± 0.02	8.01 ± 0.51	0.36 ± 0.01	0.79 ± 0.05	2.87 ± 0.02	2.91 ± 0.03
25%		13		$10.40 \pm 0.30^*$		$0.94 \pm 0.02^*$		2.98 ± 0.04 ns
50%		16		$12.82 \pm 0.20^*$		$1.15 \pm 0.07^*$		$3.08 \pm 0.05^*$
75%		20		$16.18 \pm 0.42^*$		$1.47 \pm 0.04^*$		$3.14 \pm 0.03^*$
100%		16		$12.91 \pm 0.12^*$		$1.16 \pm 0.02^*$		$3.02 \pm 0.03^*$

Data presented as mean followed by the standard deviation of triplicated experiments. * and ns: significantly different and not different at prob (p) < F value of 0.05, respectively.

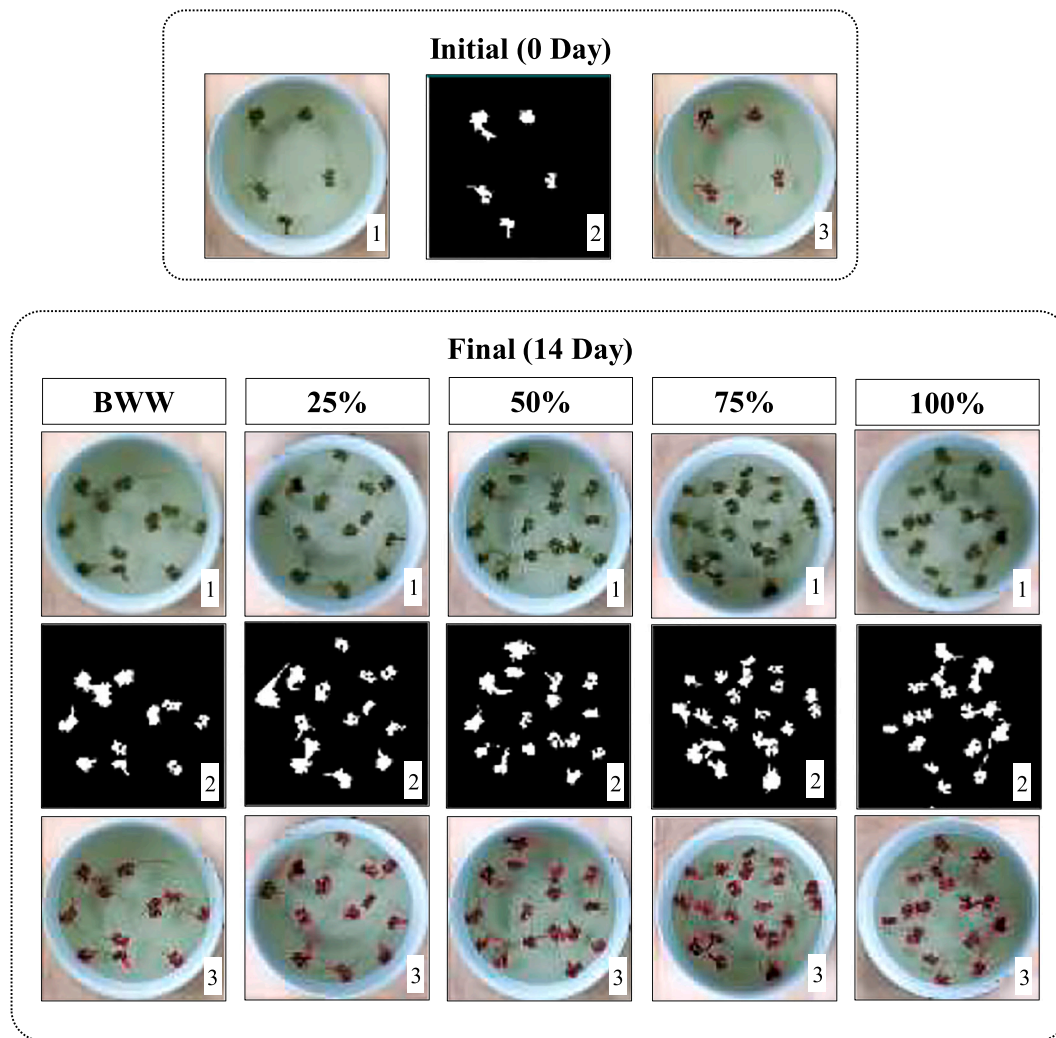


Fig. 4. Initial and final view of *A. pinnata* leaflets grown in different treatments of dairy wastewater (1: actual, 2: threshold processed, and 3: boundary defined for leaflet counting).

Table 3

Model variables of *A. pinnata* grown in different concentrations of dairy wastewater.

Model	Variables	Treatment				
		Control	25%	50%	75%	100%
Experimental	n	11	13	16	20	16
Logistic	y	11.15	13.26	16.47	20.43	16.16
	y_0	4.89	4.88	5.01	4.83	4.92
	P	11.95	14.64	18.84	25.28	16.66
	x_0	7.98	7.82	8.06	8.85	6.76
	x	3.63	3.08	2.85	2.54	4.25
Modified Gompertz	R^2	0.9889	0.9909	0.9956	0.9962	0.9972
	y	11.68	13.79	17.00	21.01	17.09
	P	34.76	23.88	26.33	33.40	22.39
	μ_m	15.83	6.90	6.20	7.17	4.67
	λ	0.04	0.08	0.10	0.11	0.14
	R^2	0.9533	0.9663	0.9794	0.98526	0.9556

n: measured final number of leaflets, y: predicted final number of leaflets, y_0 : initial number of leaflets, x and x_0 : interpolated and center points of the growth phase, P: maximum number of leaflets production, μ_m : maximum specific leaflet growth rate, λ : lag phase; R^2 : coefficient of determination.

algorithm developed by the was helpful to work in both day and night visions with 97% accuracy. Recently, Mullins et al. [38] developed an image processing-based wastewater treatment system and evaluated turbidity removal. Previously, no study has been done for the identification of *A. pinnata* leaflets using image processing techniques, therefore, the results of this study showed the comprehensive application of

this technique in plant growth monitoring in phytoremediation systems.

4. Conclusion

The findings of this study concluded that *A. pinnata* maximally reduced the pollution load of DWW using 75% concentration. The

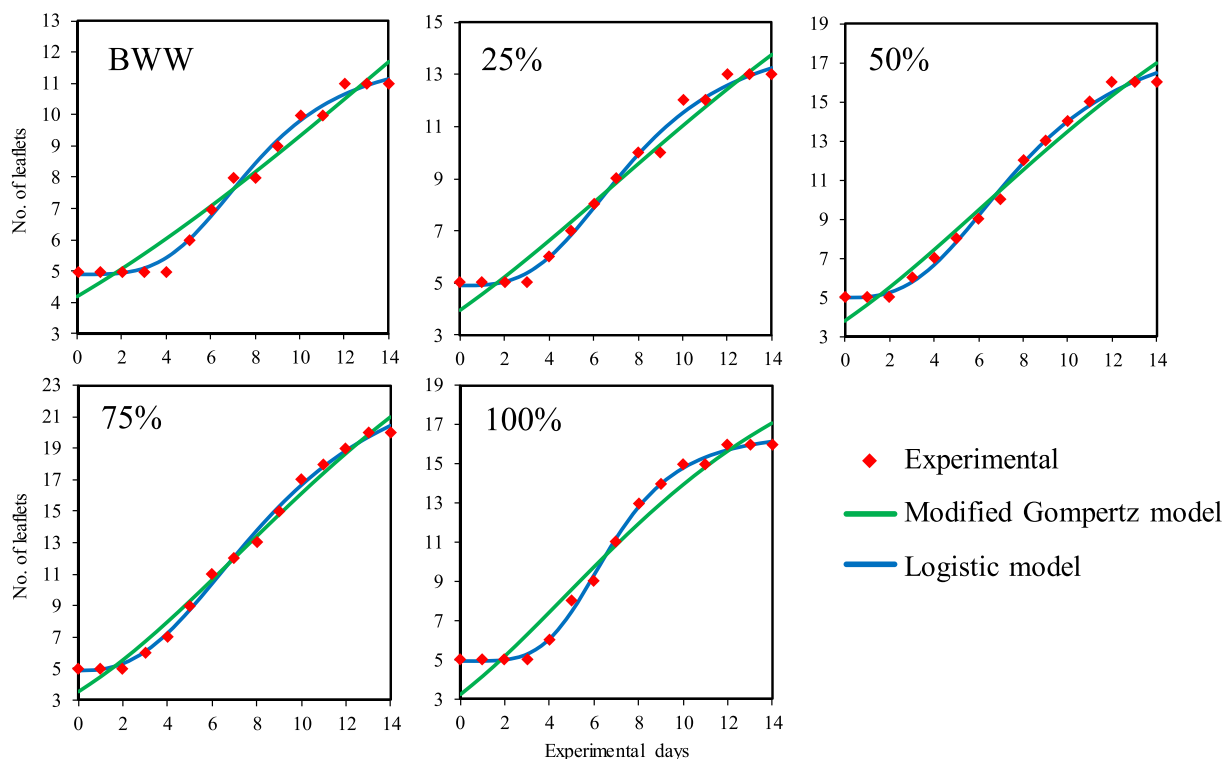


Fig. 5. Comparison of logistic and modified Gompertz models for prediction of *A. pinnata* leaflets grown in different treatments of dairy wastewater.

maximum plant growth and biochemical parameters of *A. pinnata* were observed using the same treatment. Besides this, the image processing technique was helpful to precisely count the number of leaflets, accurately. Out of the two tested sigmoid functions, the logistic model gave more precise results to predict the time-series leaflet production in a 14 days of phytoremediation experiment. The findings of this study present an effective method of DWW treatment using abundantly available *A. pinnata* while using image processing techniques to monitor their growth. Further studies on the treatment of DWW using other aquatic plants along with the development of plant-specific image recognition algorithms are highly recommended.

CRedit authorship contribution statement

Madhumita Goala: Supervision, Conceptualization, Methodology, Software, Writing-Original draft preparation. **Krishna Kumar Yadav:** Conceptualization, Methodology, Software, Writing-Original draft preparation. **Javed Alam:** Validation, Writing-Reviewing and editing. **Bashir Adelodun:** Data curation, Investigation Software, Writing-Original draft preparation. **Kyung Sook Choi:** Validation, Writing-Reviewing and editing. **Marina M.S. Cabral-Pinto:** Validation, Writing-Reviewing and editing. **Ali Awadh Hamid:** Validation, Writing-Reviewing and editing. **Mansour Alhoshan:** Validation, Writing-Reviewing and editing. **Fekri Abdulraqeb Ahmed Ali:** Validation, Writing-Reviewing and editing. **Arun Kumar Shukla:** Validation, Writing-Reviewing and editing.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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