Evaluation of Living *Azolla filiculoides* Performance for the Removal of Total Nitrogen, Phosphorus, Sodium, Potassium, COD, BOD, and TDS from Dairy Waste: Full Factorial Design

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ABSTRACT: *In this study, the potential of living Azolla filiculoides was investigated to treat dairy wastewater. The full factorial design was performed to evaluate the effect of contact time, pH, and temperature on the removal of total nitrogen, phosphorus, sodium, potassium, Chemical Oxygen Demand (COD), Biological Ooxygen Demand (BOD), and Total Dissolved Solids (TDS). At the contact time of 6 h, removal efficiencies of 74.67% and 28.78% have been observed for sodium and TDS, respectively. Also, removal efficiency of 59.20% has been obtained for phosphorus at the contact time of 18 h. The results indicated that Azolla filiculoides can be used successfully as an effective adsorbent for sodium and phosphorus removal.*

KEYWORDS: *Azolla filiculoides; Removal; Dairy wastewater; Full factorial design.*

INTRODUCTION

In recent years, industrialization has been a growth factor for countries. However, industrial pollution has attracted researchers' attention. The major effect of contaminants come from the food industry owing to the numerous production of wastewater per unit of production [1]. One of the largest production sources of industrial

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wastes in the dairy industry [2]. Operations of washing, cleaning, loading, discharge, spillage, and leakage can generate dairy effluents [3]. Detergents, sanitizers, and so on for the washing stage as well as the remainder of chemicals during the cleaning process known as dairy wastes. Dairy wastewater can be included proteins, salt, fatty materials, and lactose. The volume of the generated wastewater depends on the amount of processed milk and the product [4,5]. Due to the high organic content of the dairy industry, it can generate wastewater that is characterized by a high Biological Oxygen Demand (BOD), Chemical Oxygen Demand (COD), and nutrient compounds containing nitrogen and phosphorus. Sources of Nitrogen in dairy effluent are organic nitrogen, NH_4^+, NO_2^-, and NO_3^-. On the other hand, inorganic compounds, such as orthophosphates and polyphosphates are sources of phosphorus in milk wastewater. Phosphorus is one of the main factors for increasing algae growth. Furthermore, combined with nitrogen develops eutrophication problems in lakes, rivers, and seas [6-8]. Hence, the removal of them from industrial wastewater is important. There are several methods for treating dairy wastes, such as integrated membrane systems [9], activated sludge [10], coagulation-flocculation [11], anaerobic reactor [12,13], adsorption [14-16] etc. Among them, adsorption is effective, inexpensive, popular, and high selectivity method [17]. Chemistry and surface morphology are important properties of adsorbents, which can affect the adsorption process [18]. Therefore, finding economical, natural, easily available, and strong affinity adsorbents is a serious issue for researchers [19]. One of the types of natural adsorbents is Azolla. Azolla is a small aquatic fern and a species of it is *Azolla filiculoides* [20]. It is a floating aquatic fern, which is widely grown in aquatic environments such as Anzali Lagoon and native areas of Africa, Madagascar, India, Philippines, China, and Japan [21-23]. *Azolla filiculoides* has been used as a low-cost bio-adsorbent to remove heavy metals [24-26], chlorophenol [27], dye [28,29], fluoride [30] and antibiotic [31]. It has a high potential for use in phytoremediation [32].

Optimization of the process factors can be performed using the Design of Experiment (DOE). DOE is an efficient method for arranging experiments in a rational process, which reveals the effective relationship between factors and response. Also, it reduces the number of experiments and causes reliable statistical inferences [33]. A set of statistical techniques and applied mathematics in modeling, the experimental result is Full Factorial Design (FFD). This procedure can be used for surveying the effect of various factors with different levels and their influence on each other. Factorial designs are used primarily for screening significant factors, but can also be used sequentially to model and refine a process [34].

The purpose of this study was to use *Azolla filiculoides* growing in Anzali Lagoon as an efficient and cost-effective adsorbent to remove phosphorus (P), nitrogen (N), potassium (K), and sodium (Na) from dairy waste. Moreover, BOD, COD, and Total Dissolved Solids (TDS) were investigated in dairy wastewater. Furthermore, the FFD was applied to study the effects of contact time, pH, and temperature on the removal of chemical compounds.

EXPERIMENTAL SECTION

Instruments and software

Inductively coupled plasma optical emission spectrometry (ICP-OES) (model VISTA-PRO, Australia), Atomic Absorption Spectroscopy (AAS) (model AA240, Australia) with air-acetylene flame and hollow cathode lamps (HCL), digital Kjeldahl for evaluating total N, HACH-REACTOR and DO meter for measuring COD and BOD respectively, A digital pH meter (model JENWAY 3510) was used to adjust the pH values of the solutions, digital balance (model AJ100), incubator (FG Iran), and oven (model TUTTLINGEN 7200, Germany) were used. Minitab 17, Microsoft Excel 2013, and SPSS v20 were utilized as software.

Chemicals

Sodium chloride (NaCl), potassium chloride (KCl), nitric acid (HNO_3), hydrochloric acid (HCl), n-Hexane, ammonia (NH_3), petroleum ether, magnesium sulfate ($MgSO_4$), iron (III) chloride ($FeCl_3$), ammonium chloride (NH_4Cl), calcium chloride ($CaCl_2$), sodium hydroxide (NaOH), potassium sulfate (K_2SO_4), sulfuric acid (H_2SO_4), mercury (II) oxide (HgO), and sodium thiosulfate pentahydrate ($Na_2S_2O_3.5H_2O$) were purchased with high purity from Merck.

Chemical analysis of dairy wastewater

At first, values of pH, K, Na, P, BOD, COD, and TDS were measured in dairy wastewater before treatment (Table 1).

Table 1: Chemical analysis of dairy wastewater before treatment.

Sample	pH	P	K	Na	N	BOD	COD	TDS
Amount (ppm)	4.29±0.18	2139.00±10.53	980.78±3.69	26503.00±373.62	409.06±2.89	>30000	>50000	1.67±0.01
Amount after dilution (ppm)	6.81±0.34	43.77±0.18	20.22±0.21	549.10±2.91	15.53±0.33	1136.66±37.85	2183.33±20.81	0.03±0.0001

Table 2: Results of chemical analysis of Azolla plant.

Sample	P	K	Na	N	Fat (%)
Amount found (ppm)	4008.00±8.60	2708.66±2.82	428.00±5.67	1.00±0.001	7.67

Harvesting of Azolla filiculoides

Fresh Azolla was collected from the surface of the Anzali Lagoon in the north part of Iran in Gilan province (southwest shores of Caspian Sea) in August, November 2017, and April 2018. The collected Azolla was put in aquarium glass containers containing dechlorinated water and fertilizer solution was added to grow and keep it. Afterward, Azolla was washed with dechlorinated water. Then, 100 g of fresh Azolla was placed in other glass containers containing 2000 mL of water and 40 mL of concentrated dairy waste solution.

Effect of contact time

100 g of fresh Azolla was studied in the presence of dairy waste at 4,6,12,18,24,36,48 and 60 h. Afterward, the solutions were filtered through filter paper, and the amount of Na, K, and P were measured by the AAS, flame photometer, and ICP-OES respectively. Also, the total N was measured by the digital Kjeldahl system. In addition, COD, and BOD were determined by HACH-REACTOR and DO meter respectively. After 18 h, the Azolla plant showed no remarkable performance for eliminating or reducing the factors. Therefore, contact times of 6, 12, and 18 h were selected to complete the process.

Effect of pH

100 g of fresh Azolla at pH of 5, 7, and 9 with different times of 6, 12, and 18 h were evaluated in a certain concentration of dairy wastewater. At the end of the desired time, a certain volume of the solutions was filtered through the filter paper. After that, Na and P were measured by flame atomic absorption and ICP-OES. In addition, TDS of the solutions was determined and evaluated. pH was selected 5 to 9 due to the tolerance conditions of Azolla plant was measured because Azolla did not destroy these pH.

Effect of temperature

100 g of fresh Azolla were placed in contact with a specified concentration of dairy waste at temperatures of 15, 22 (ambient temperature), and 30 °C at different times of 6, 12, and 18 h and pH of 5, 7, and 9. Then, the certain volume of the solutions was filtered and Na, P as well as, TDS of solutions were measured. According to the optimum growth conditions and plant tolerance, as well as saving in energy, these temperatures were selected for use on a semi-industrial and industrial scale.

Chemical analysis of Azolla

The plant was washed with water without chlorine and dried at the laboratory temperature in the shade. After the preparation of dried plant samples, the amount of P, Na, and K were measured using ICP-OES. Also, its fat percentage was evaluated by the Soxhlet method. In addition, the amount of N was measured by the digital Kjeldahl system. Results are reported in Table 2.

RESULTS AND DISCUSSION

Determination of selected factors in the wastewater

The obtained data from the measurement of BOD, COD, TDS, P, K, Na, and N were recorded at 4, 6, 12, 18, 24, 36, 48, and 60 h. Then, data were evaluated in terms of distribution using SPSS software and Kolmogorov Smirnov (K-S) test. It was found that the data for all factors is the normal bell curve at $p < 0.05$ level. Therefore, the change of values of factors at different times was studied using a one-way analysis of variance (ANOVA) test. The results showed that there are no significant differences between BOD, COD, K, and N factors at $p < 0.05$ confidence level. Hence, they were not considered in the next stage. But the Na, P, and TDS factors due to a significant decrease at the $p = 0.03$,

Table 3: Test values and runs according to the removal amount of P, Na, and TDS.

No.	Time	pH	Temp.	P	Na	TDS
1	18	9	30	39.80	1268.73	0.0360
2	18	5	30	19.66	768.63	0.0269
3	12	5	22	30.57	762.00	0.0196
4	18	7	15	36.02	584.70	0.0216
5	12	9	22	38.20	545.80	0.0218
6	12	7	15	24.94	555.63	0.0189
7	12	9	30	34.61	771.86	0.0248
8	12	9	15	33.16	580.03	0.0229
9	18	5	15	32.15	489.46	0.0181
10	18	7	30	16.62	1080.27	0.0217
11	18	9	15	36.57	306.23	0.0235
12	6	7	30	26.13	325.56	0.0178
13	6	9	15	27.69	227.70	0.0222
14	18	9	22	25.47	763.43	0.0218
15	12	5	15	30.60	463.33	0.0205
16	6	7	15	19.42	586.00	0.0175
17	6	5	15	26.14	242.93	0.0216
18	6	7	22	23.58	561.00	0.0191
19	6	5	22	32.74	417.70	0.0174
20	6	5	30	36.83	461.40	0.0226
21	12	7	30	26.03	781.63	0.0186
22	12	7	22	26.91	805.26	0.0190
23	18	5	22	28.86	444.96	0.0219
24	12	5	30	26.12	672.66	0.0225
25	6	9	22	30.60	858.30	0.0227
26	18	7	22	33.95	577.80	0.0168
27	6	9	30	24.91	608.10	0.0230

p = 0.037, and p = 0.046 levels determined as chosen factors respectively at 6-12 h.

Factorial design

Values of Na, P, and TDS at the temperature of 15, 22, and 30 °C, time of 6, 12, and 18 h as well as pH of 5, 7, and 9 were recorded in the Minitab software environment. For this purpose, the Full Factorial (FF) design was used. The temperature, time, and pH factors were investigated at two levels. 27 experimental conditions were designed with 9 runs as the central point (Table 3). All experiments were carried out to reduce the effect of uncontrollable variables with a random pattern. Also, all experiments were performed with three replications and for the central points with 6 repetitions. The total number of experiments was obtained by the FF method according to Eq (1).

$$N = \Pi_i^k = 1_i^L \qquad (1)$$

Table 4:. Estimation of effects and coefficients for phosphorus and sodium elements using the regression approach.

	P					Na				
Term	Effect	Coef	SE Coef	T	P	Effect	Coef	SE Coef	T	P
Constant		29.18	0.96	30.40	0.000		614.841	31.45	19.55	0.000
Time	2.18	1.091	1.175	0.93	0.365	227.076	113.538	38.52	2.95	0.008
pH	3.068	1.534	1.175	1.30	0.207	137.149	68.574	38.52	1.78	0.091
Tem.	-1.831	-0.915	1.174	-0.78	0.44	298.151	149.075	38.48	3.87	0.001
Time * pH	5.780	2.890	1.439	2.01	0.059	13.490	6.745	47.18	0.14	0.888
Time *Tem.	-7.142	-3.571	1.438	-2.48	0.023	240.733	120.366	47.12	2.55	0.019
pH *Tem.	1.442	0.721	1.438	0.50	0.622	136.198	68.099	47.12	1.45	0.165
Time * pH *Tem.	7.494	3.747	1.761	2.13	0.047	132.541	66.270	57.72	1.15	0.265

Phosphorus and sodium

Table 4 shows the estimation of the effects and coefficients for phosphorus and sodium based on the experimental design with three independent variables. According to this table and the obtained results for the independent variables, the mathematical model for the P response is given as follows:

$$R_P = 29.180 + 1.091T + 1.534pH 0.915\ Temp + 2.890\ Time \times pH 3.571\ Time \times Temp + 0.721\ pH \times Temp + 3.747\ Time \times pH \times Temp \quad (2)$$

ANOVA was implemented to evaluate the effect of the main parameters and their interaction with the response. The results of P show that the interaction of time×Temp and time×Temp×pH are significant at P=0.023 and P=0.047. According to R-S_q=0.486, the goodness of fit is not significant and indicates that the model is suitable for data of P reduction. The degree of fit is determined by R-S_q, which represents the description of data change using the model. The higher R-Sq, reveals the accuracy of the model, but a low R-S_q does not mean that the model is not valid for understanding the process. Depending on the distance between the levels of the factors, the ratio of the change in the measured reaction may be so small compared to the background error, which causes a decrease in R-S_q, but the model can describe the process. But pH has no significant effect on reducing the amount of Na at P=0.091. Also, the interaction between Time×pH, pH×Temp, and Time×pH×Temp are not significant at P> 0.05. Therefore, the pH variable was eliminated in the optimization process and the regression approach was repeated. The obtained results are presented in Table 5. According to the table, the independent variables in changing the pH value are significantly the time and temperature factors. ANOVA, similar to the regression approach, showed that the two variables of time and temperature, as well as their interaction, were significant at p<0.05. Therefore, the three mentioned parameters in Eq (3) were considered. The mathematical model for the Na response can be defined as:

$$R_{Na} = 614.8 + 113.5 \times Time + 149.1 \times Temp + 120.4\ Time \times Temp \quad (3)$$

R-S_q=0.539 shows that the goodness of fit is not significant and the model is appropriate for data of Na reduction. The goodness of fit of the data is demonstrated in Fig 1. (a) and (b). As shown, mentioned interactions are significant for changes in P.

Also, Fig 2 displays the interaction time×Temp×pH and time×Temp for P and Na respectively. The interaction of time×Temp has a significant effect on Na changes.

In addition, cube plots for the mean of data are presented in Fig 3. Due to the interaction of time, temperature, and pH, the maximum removal rate was at 30 °C and pH=5, and 18 h that the amount of p decreased to 19.663. On the other hand, the highest removal rate of Na was at a temperature of 15 °C and 6 h, which shows a decrease of 352.21.

Also, the relationship between response and independent variables is specified by the contour diagram that is shown in Fig 4. As can be seen, the lowest amount of phosphorus removal occurred in the time range of 15 to 18 min between 15 and 20 °C (blue range). Also,

Table 5. Estimation of effects and coefficients for sodium using the regression approach after elimination of pH

Term	Effect	Coef	SE Coef	T	P
Constant		614.8	33.09	18.58	0.000
Time	227.1	113.5	40.52	2.80	0.010
Tem.	298.2	149.1	40.48	3.68	0.001
Time * Tem.	240.7	120.4	49.57	2.43	0.023

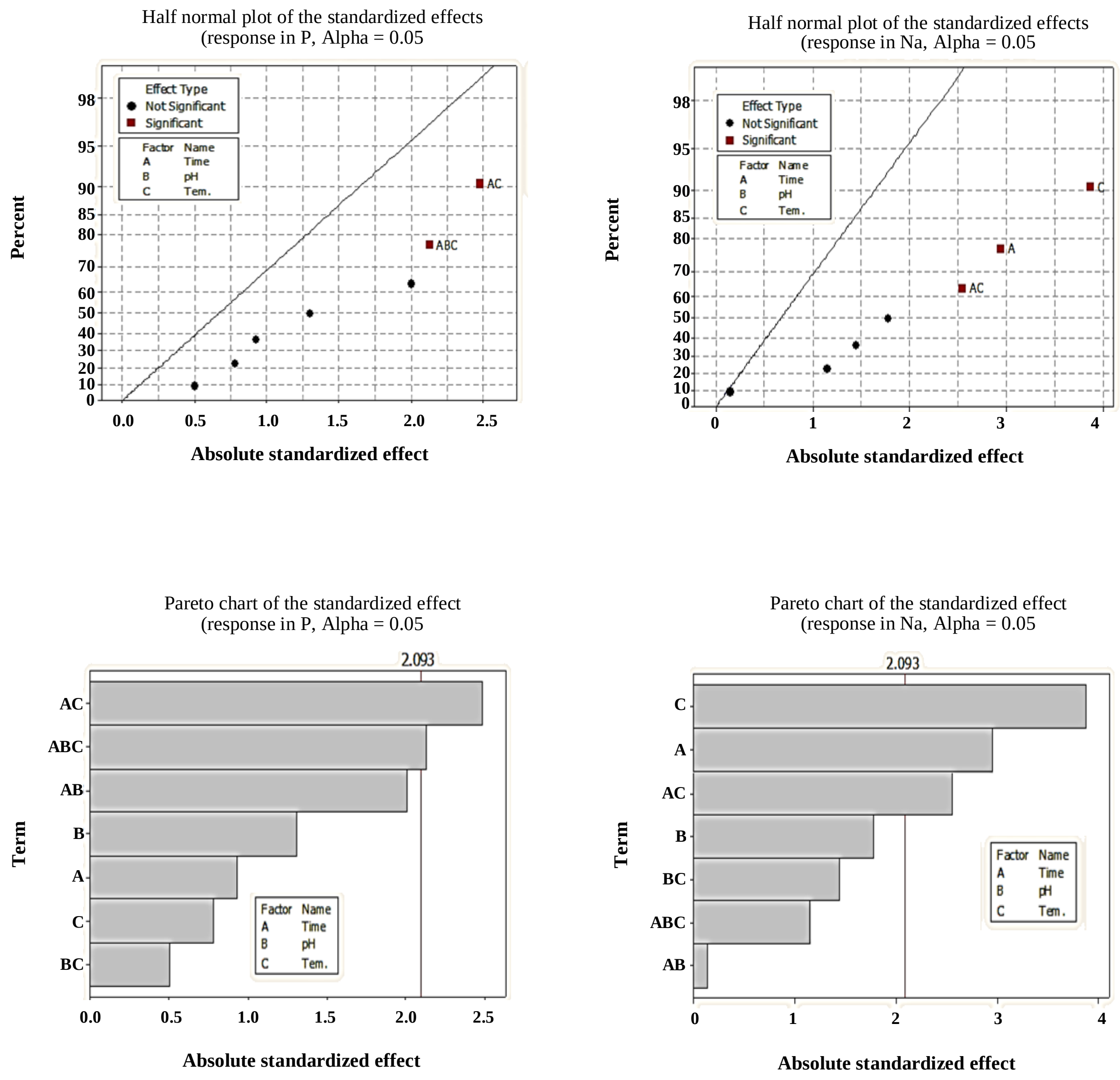

Fig. 1: Half normal plot and Pareto chart for P and Na.

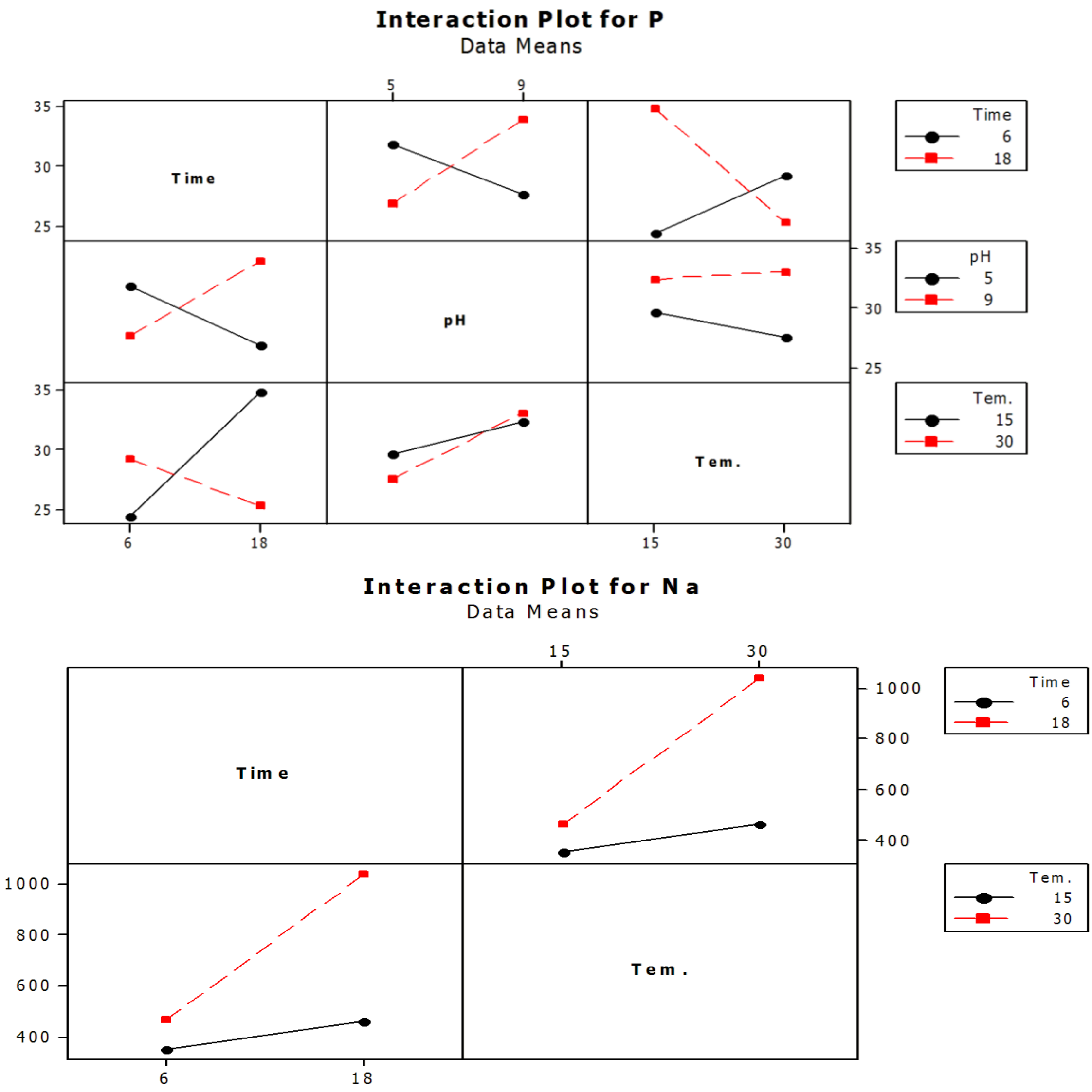

Fig. 2: Interaction of time×temp×pH for P and time×temp for Na.

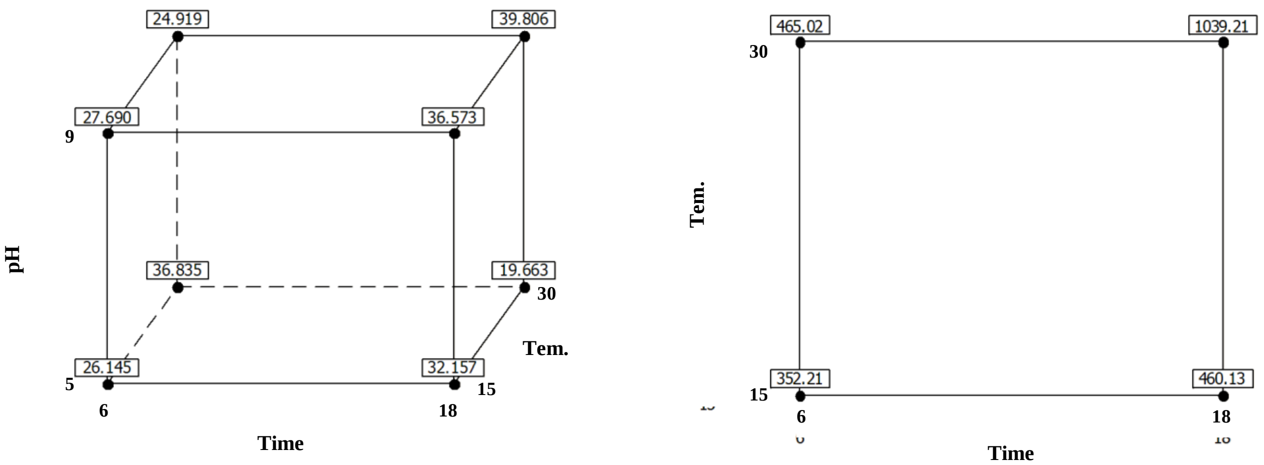

Fig. 3: Cube plots for P and Na.

Table 6. Estimation of effects and coefficients for TDS using the regression approach

Term	Effect	Coef	SE Coef	T	P
Constant		0.0215	0.0006	33.26	0.000
Time	0.0027	0.0013	0.0007	1.75	0.095
pH	0.0030	0.0015	0.0007	1.95	0.067
Tem.	0.0031	0.0015	0.0007	1.96	0.064
Time * pH	0.0013	0.0006	0.0009	0.70	0.492
Time *Tem.	0.0032	0.0016	0.0009	1.68	0.109
pH *Tem.	0.0005	0.0002	0.0009	0.29	0.777
Time * pH *Tem.	0.0011	0.0005	0.0011	0.47	0.647

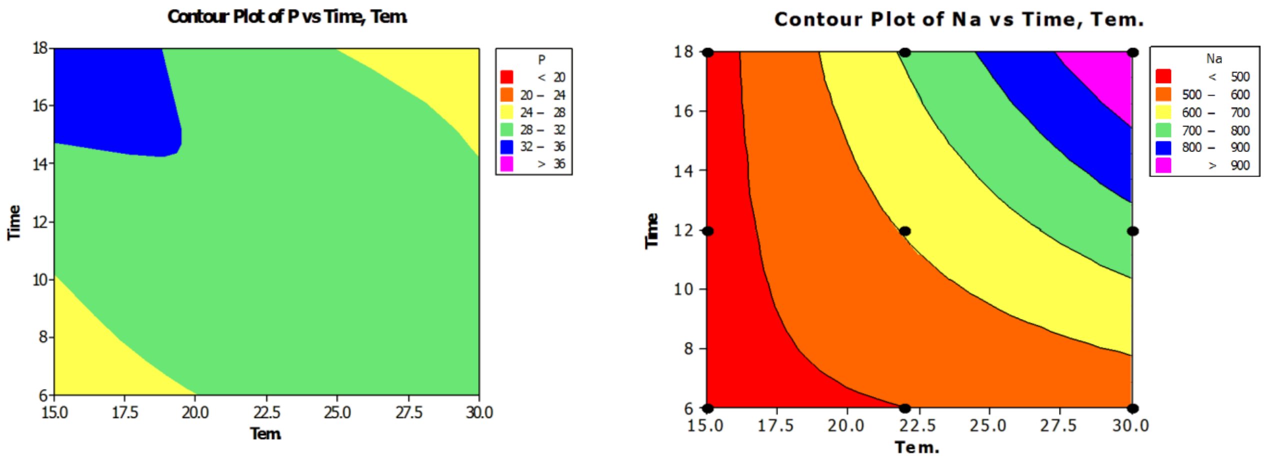

Fig. 4: Contour plots for P and Na.

the highest removal rate of Na is seen in the red area, where the temperature and time were about 15 to 22 °C and 6 to 18 h, respectively.

Total dissolved solids

According to the estimated effects and coefficients for total dissolved solids, the experimental design with three independent variables was obtained in Table 6. With respect to the results of Table 6, none of the independent variables have significant effects on TDS changes ($P > 0.05$). TDS was less removed than P and Na.

Effect of time, pH, and temperature on adsorption

As shown in Fig 5 related to Na, the adsorption percentages have increased at 6 h, but at 12 and 18 h, there is a decreasing trend in adsorption, which could be said that the desorption step has occurred. In the case of P, the positive adsorption is observed at the mentioned times. In TDS, the adsorption is relatively high at 6 and 12 h, but negative adsorption has been observed at 18 h which is probably related to desorption. Eventually, the highest adsorption percentage was 74.67% for Na at 6 h, 15°C, and pH=9. Also, P has the maximum adsorption (59.20%) at 18 h, 30°C, and pH= 7. Furthermore, the best adsorption was 28.78 % at 6 h, 22°C, and pH=5 for TDS. It can be concluded that the highest adsorption is related to sodium.

CONCLUSIONS

Azolla filiculoides is a non-native and unnecessary plant in Anzali Lagoon. In this study, the performance of this plant was investigated for the adsorption of dairy wastewater. The present study showed that Azolla can be effectively used as an adsorbent for the treatment of dairy wastewater. The effect of different parameters, including contact time, pH, and temperature were studied on the removal of total nitrogen, phosphorus, sodium, potassium,

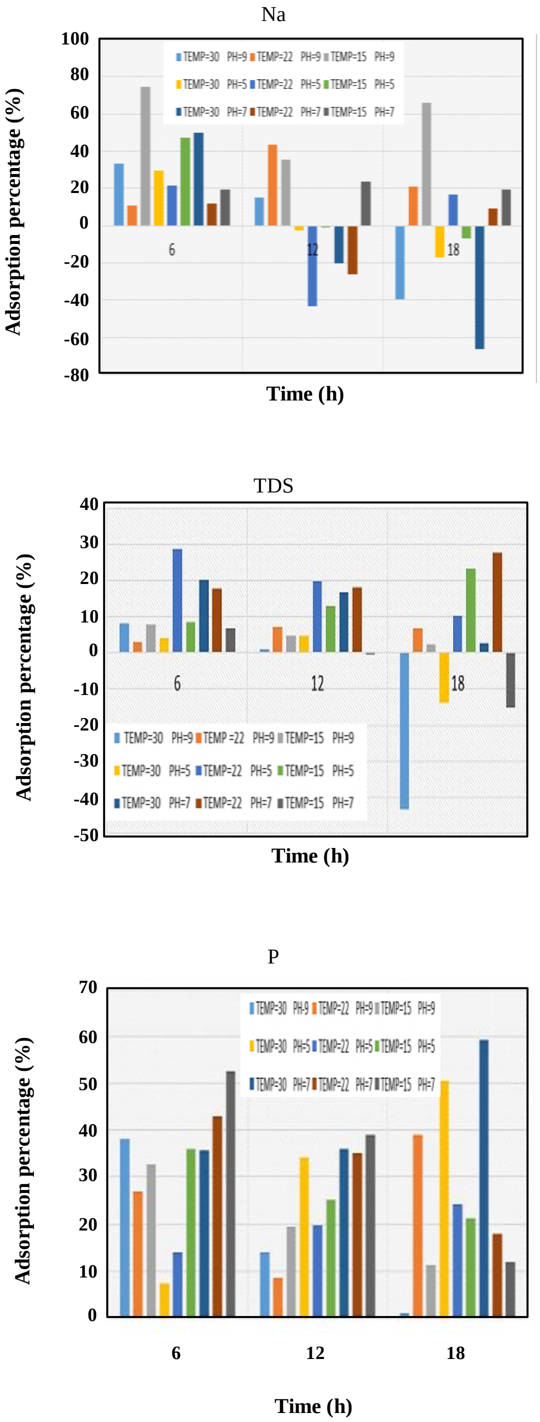

Fig 5:Effect of contact time, pH, and temperature on removal efficiencies for Na, P, and TDS

COD, BOD, and TDS. Among them, the highest percentage of adsorption was observed for sodium and phosphorus. According to the experimental design method, the optimum time of 12 and 18 h, pH=9 and 7, as well as ambient temperature were obtained for Na and P. Regarding the short contact time, the feasibility of good adsorption, and reduction of expenses, Azolla can use as a proper adsorbent for sodium and phosphorus removal. Even in the future, the adsorption efficiency of this plant can be improved with other compounds and used as a composite to remove various contaminants.

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