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The effects of nutrients and folic acid on the biological treatment of petrochemical wastewater

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ABATRACT

Considering the advantages of biological systems for wastewater treatment in compatibility with the environment, the present study aimed to investigate the effects of different concentrations of folic acid, nitrogen, and phosphorus on the biological treatment of industrial wastewater at Tabriz Petrochemical Company (TPC) in Iran. The Taguchi method with the orthogonal array L9 was used to determine the optimal conditions of microorganism growth. Color, pH, nitrogen, phosphorous, sludge volume reduction, sludge volume index (SVI), and mixed liquor suspended solids (MLSS) were measured. According to the results, the three investigated factors could significantly reduce wastewater. After removing the folic acid color, modulating the pH, and reducing the SVI, the nitrogen factor was considered most effective. Nitrogen also had a significant effect on the removal of output wastewater (62.62%). In addition, the phosphorus factor had the most significant impact on wastewater reduction (65.25%). The optimal conditions were observed with 0.2 ppm of folic acid, 20 ppm of nitrogen, and 4 ppm of phosphorus in the three investigated parameters. Folic acid only significantly affected the increasing of MLSS (90.1%), and the optimal condition of this parameter was with 0.2 ppm of folic acid, 30 ppm of nitrogen, and 4 ppm of phosphorus. Sludge volume reduction was observed in all the reactors. The addition of folic acid, nitrogen, and phosphorous to the TPC wastewater lacking these materials could enhance the output parameters and reduce adverse environmental effects..

Keywords: Folic Acid, Biological Treatment, Nutrients, Petrochemical Wastewater

Introduction

The removal of the existing hazardous combinations from the wastewater output of industries is considered to be of utmost importance in every human community,[1] which highlights the need for extensive research on effective and inexpensive treatment systems. Heavy industries are those producing large volumes of wastewater containing various contaminants, such as petrochemical industries.[2, 3]

For several reasons such as hazardous combinations, hydrological activities, shocks out of organic and vacation loading, and operational breaks, biological wastewater treatment systems lead to breakdowns, which in turn causes reaching the former productivity as a time-consuming process associated with economic losses. For the proper productivity of wastewater treatment, alternative methods have proven efficient, such as the application of adaptable microorganisms to use nutrients and vitamins (e.g., folic acid) and active

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carbon treatment.[4]

Senorer and Barlas are used in the folic acid treatment of domestic wastewater within 30 days and causes the chemical oxygen demand (COD) of the wastewater output to reach the maximum of 60 ppm and average of 30-35 ppm.[4] The COD in wastewater output has been reported to be within the range of 200-1400 ppm. According to a study by Strunkheide, folic acid could reduce the sludge volume index and prevent sludge bulking (bulking phenomenon); the researcher used folic acid (0.2 ppm) in biological wastewater treatment within 15 days, concluding that the total organic carbon reduced by 67%.[5] Parameters such as pH and catalyst dosage also play a key role in degradation rate.[6]

Biological sludge excess of 1.005 with the total solids concentration of 0.5-1.0% is composed of 70-90% organic materials. The rate of the secondary sludge production depends on the applied biological degradation, as well as procedural conditions such as the sludge age, temperature, and organic/hydraulic loading in the biological unit. The annual rate of the secondary sludge produced by activated sludge systems has been estimated at 1.5-2.5 L per person daily.[7, 8] The relatively high production of excess biological sludge is considered to be a major drawback of the aerobic processes involved in wastewater biological treatment. Meanwhile, approximately 40-60% of the investment expenses and more than 50% of the operation and maintenance expenses of activated sludge treatment plants are allocated to the removal of sludge from wastewater treatment plants.[9]

The microorganisms that are involved in the removal of carbon contaminants from wastewater require nitrogen and phosphorous for the formation of protein, nucleic acid, and elements of cellular septum at least for growth and reproduction.[10] In urban wastewaters, nitrogen and phosphorus are often adequate, while in the industrial wastewaters with high volumes of organic materials, nitrogen and phosphorus may not be available and should be added to make wastewater treatable.[11]

The present study aimed to investigate the effects of various concentrations of nitrogen and phosphorus as microorganism nutrients, along with different concentrations of folic acid on increasing the efficacy of the biological wastewater treatment system at Tabriz Petrochemical Company and improve the output parameters of wastewater.

Materials and Methods

Apparatus and procedure

This experiment was performed on the industrial wastewater of TPC for 20 days during 24/02/2014-16/03/2014 to determine the optimal conditions for microorganism growth in the biological treatment of wastewater. In total, 10 SBR reactors were used with 12×12×50 centimeters of glass type at the laboratory scale and operated at room temperature (20-25 °C). The available airline in the laboratory system was supplied by compressed air cylinders and employed for aeration. In addition, circular aeration rocks were used at the bottom of the reactors for better aeration. The 24-hour operational cycle of the reactors included 2 min of filling, 22.5 h of aeration (reaction phase), 1 h of sedimentation, 3 min of emptying, and approximately 25 min of the static stage. The reactors were assessed for 20 days, including five days for sludge compatibility and 15 days for the operations of the information recording. Two liters of activated sludge and 2 L of wastewater were used for the operating of the reactors, which were supplied from the wastewater drainage line and inlet wastewater treatment unit at TPC, respectively.

In this experiment, the chemical oxygen demand was set at 600 ppm, and phosphorus, nitrogen and folic acid were selected as the test variables (three factors). In addition, three concentrations were considered for each variable (Table 1). Fig. 1 depicts the wastewater treatment process.

Table 1. Factors and levels exerted in experiment

Factor (ppm)	Level 1	Level 2	Level 3
Folic acid	0.1	0.2	0.4
N	20	30	40
P	4	6	10

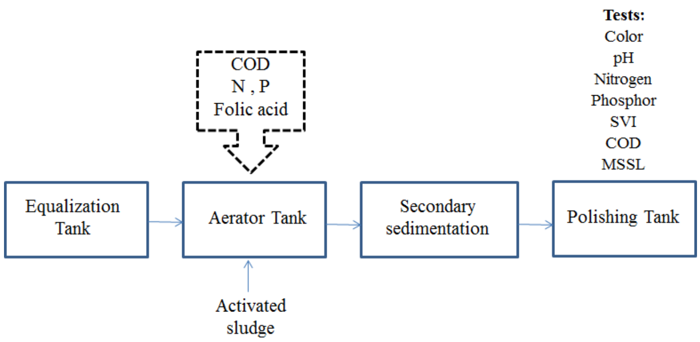

Fig. 1. A schematic diagram of wastewater treatment process in Tabriz Petrochemical Company

Chemicals and solutions

In this study, K_2HPO_4 was obtained from Merck (Germany) with the order number of 105109 and used for exerting various concentrations of phosphorus and NH_4Cl, which were provided by the same company with the order number of A0162745 and used for exerting various concentrations of nitrogen. The pharmaceutical form of folic acid (DSM yellow powder obtained from Switzerland) was used for the preparation of the folic acid solution; to this end, the main solution was initially prepared with the C concentration, and the injection volume was calculated using Eq. (1), as follows:

$$IV = (QV \times D)/C \quad (1)$$

where IV is the injection volume (l/h), Q_v shows the volumetric flow rate (2 l/24 h), D represents the exerted dose to the reactors (mg/L), and C is the concentration prepared for the injection (mg/L).

Initially, the folic acid solutions was prepared with the concentration of 1,000 mg/L (C=1,000 mg/L), and the injection volume was estimated per minute for each of the concentrations and diluted using demineralization water. Each solution was injected to the reactors continuously using a dosing pump. Table 2 shows the dilution amount of each concentration.

Table 2. Dilution amount of each folic acid concentration with demineralization water

Folic acid concentrations (ppm)	0.5*	0.4	0.2	0.1
Dilution amount	200	200	400	850

(*Shocking dose)

At the next stage, nitrogen was prepared from an ammonium chloride solution (800 ppm; C=800 mg/L), and phosphorus was prepared from a dipotassium hydrogen phosphate solution (200 ppm; C=200 mg/L), and the injection volume was calculated for 24 h for various concentrations using Eq. (1). The injections were performed at the outset of each cycle on the reactors.

Test methods and the experimental design

In order to assess microorganism compatibility and growth for five days, phosphorus (6 ppm), nitrogen (30 ppm), and folic acid (0.5 ppm) were exerted to reactors as the shock dose. From day six onwards, 2 L of the new wastewater was added to the reactors, and the operational cycle of the reactors started. The wastewater was sampled once within 48 h, and the parameters of color, pH, nitrogen, phosphorus, SVI, COD, and mixed liquor suspended solids were measured using the test methods presented in Table 3.

In this experiment, the Taguchi method as L_9 orthogonal array was used for the statistical analysis (ANOVA) of the data and determining the optimal conditions of microorganism growth in the biological treatment of wastewater (Table 4), and the Ro was used as the control for the comparison of the results.

In the present study, the wastewater was sampled once within 48 h, and the changes in the COD and MLSS were measured using the MLSS values for the calculation of the Y index and rate of sludge reduction (SR). The criteria for sludge reduction was defined as the decreased Y coefficient. The changes in biomass over time to the consumed substrate (consumed COD) were determine using Eqs. (2) and (3) to determine this index, and SR was estimated using Eq. (4), as follows:[12, 13]

$$dx/dt = Yds/dt \quad (2)$$

where dx/dt is the amount of increased biomass (mg/L), and ds/dt represents the removed substrate (mg/L), which are in line with Eq. (3), as follows:

$$Y = X - X0/S0 - S \quad (3)$$

where S_o and S are the concentration of the primary and final substrates (mg/L), and X_o and X show the concentration of the final and primary biomass (mg/L), respectively.

$$SR\% = Y1 - Y2 * 100/Y1 \quad (4)$$

The analysis of variance (ANOVA) was used to determine the relative significance of the parameters per each outlet. In the ANOVA, the F test indicated the effectiveness or ineffectiveness of the studied parameters at the desired confidence level. Meanwhile, the statistical parameters of the distribution rate contributed to the better understanding of the effects of each parameter and their comparison with other parameters, as well as the induced impact on the outlet. For the parameters with a high distribution rate, even small changes could largely influence the outlet. The relative distribution of the F value and distribution rate were determined using Eqs. (5) and (6), as follows:[14]

$$Fi = MSi/MS_e \quad (5)$$

$$P\,(\%) = (SSi - DFi(MSe))/S_T \quad (6)$$

where SS_i is the sum of the squares of each parameter, MS_i shows the mean sum of the squares for each parameter, DF_i represents the degree of freedom of the factors, and S_T is the sum of the total squares.

Table 3. Number of test methods and equipment used for experiment

Test	Test Method No	Equipment
Color	Standard Method2120ABCD Color - Hach.8025	Spectrophotometer & Vacuum Filter
pH	ASTM.D.1293	pH meter
Nitrogen	ASTM.D.1426	Spectrophotometer
Phosphor	ASTM.D.515	Spectrophotometer & Hiter
SVI	Graduated cylinder	Standard Method 2710D
COD	ASTM D1252	UV/Vis Spectrometer
MSSL	Standard Method 2540B	Crusible & Oven & Furnace &Balance meter

Table 4. Experiment design as Taguchi in L_9 orthogonal array

Reactor	Factor		
	Folic acid	N	P
R_1	1	1	1
R_2	1	2	2
R_3	1	3	3
R_4	2	1	2
R_5	2	2	3
R_6	2	3	1
R_7	3	1	3
R_8	3	2	1
R_9	3	3	2

Results and Discussion

Color

Among the experimental factors, only folic acid had a significant effect on the wastewater color (5% probability). The reduction of color due to folic acid use (71.748%) was higher compared to the use of nitrogen (19.116%) and phosphorous (4.677%) (Table 5). At the concentrations of 0.2, 20, and 6 ppm, folic acid, nitrogen, and phosphorous provided the optimal conditions for the removal of color form wastewater, respectively (Table 6).

pH

The pH of the environment is considered to be an important influential environmental factor in microorganism growth.[15] According to the results of the present study, folic acid, nitrogen, and phosphorous had a significant effect on the pH of wastewater. The highest and lowest reduction of pH was recorded for folic acid (74.663%) and phosphorus (3.985%), respectively (Table 5). In addition, the optimal pH of the biological area was within the range of 6.5-8, and any deviation from this range resulted in the death of microorganisms; in this experiment, the optimal conditions were defined within this range. During the experiment, pH remained in the mentioned range, and the optimal conditions for this parameter were obtained at 0.1 ppm of folic acid, 30 ppm of nitrogen, and 4 ppm of phosphorus (Table 6).

Table 5. Results of variance analysis for color, pH, nitrogen, phosphors, SVI, COD, and MLSS

		Factor			
		Folic acid	N	P	Error
df		2	2	2	2
Color	Variance (V)	16.339*	4.5636	1.289	0.249
	Effect (%)	71.748	19.116	4.677	4.459
pH	Variance (V)	0.089**	0.025**	0.004*	0.001
	Effect (%)	74.663	20.896	3.985	0.456
Nitrogen	Variance (V)	0.035	0.081*	0.003	0.003
	Effect (%)	25.5	62.25	0.316	11.56
Phosphor	Variance (V)	0.003	0.022	0.09	0.008
	Effect (%)	0	11.382	65.255	23.363
SVI	Variance (V)	4.9*	28.09**	10.44**	0.241
	Effect (%)	10.67	63.753	23.36	2.22
COD	Variance (V)	785.95	373.18	320.33	8.57
	Effect (%)	52.24	24.50	20.95	2.31
MLSS	Variance (V)	1581.49*	4.75	29.54	65.86
	Effect (%)	90.13	0	0	9.87

Table 6. Optimum condition for removal of traits

		Factor		
		Folic acid	N	P
Color	Level description	0.2	20	6
	Level	2	1	2
pH	Level description	0.1	30	4
	Level	1	2	1
Nitrogen	Level description	0.2	20	4
	Level	2	1	1
Phosphor	Level description	0.4	20	6
	Level	3	1	2
SVI	Level description	0.2	30	4
	Level	2	2	1
COD	Level description	0.2	30	6
	Level	2	2	2
MLSS	Level description	0.1	30	4
	Level	1	2	1

Nitrogen and Phosphorus

The results of ANOVA indicated the significant effect of nitrogen on the removal of nitrogen from wastewater. However, folic acid and phosphorus had no significant effects in this regard (Table 5). The optimal conditions for the removal of nitrogen in the biological treatment were obtained at 0.2, 20, and 4 ppm of folic acid, nitrogen, and phosphorus, respectively (Table 6). None of the experimental factors had a significant effect on the phosphorus content. Phosphorus (65.25%) was observed to be the most effective factor in this parameter, while folic acid had no effect on the removal of phosphorus from wastewater, and the effect of nitrogen was estimated at 11.382%. In addition, the optimal condition for the removal of phosphorus was obtained at 0.4 ppm of folic acid, 20 ppm of nitrogen, and 6 ppm of phosphorus (Table 6).

The industrial wastewater of TPC lacks nitrogen and phosphorus, and microorganisms need these elements for the formation of protein, nucleic acid, and parts of the cell wall, as well as their growth and reproduction.[16] Since the extra nitrogen and phosphorus in the output wastewater of treatment plants are considered as pollutants, the amounts of these elements in the output wastewater were measured in the present study. Although the effect of phosphorus on the removal of this element from the wastewater was not considered significant, the element had the most significant effect compared to the factors. In other words, similar to the findings regarding nitrogen, the addition of phosphorus to the wastewater without nutrients could improve microorganism growth, with the most significant effect observed on the removal of this element from the wastewater output. In another study, Akerboom *et al.* evaluated the nutritional needs of microorganisms in the biological treatment of industrial waste resulted from the production of olive oil in both aerobic and anaerobic conditions, concluding that 900:5:1.7 and 170:5:1.5 of COD:N:P in the anaerobic and aerobic conditions reduced COD by 80% and 75%, respectively.[17]

MLSS

The results of ANOVA (Table 5) indicated that folic acid had a significant effect on the MLSS content at the rate of 90.1%. However, nitrogen and phosphorus had no effects on this parameter. The optimal condition for the MLSS concentration was observed with 0.1 ppm of folic acid, 30 ppm of nitrogen, and 4 ppm of phosphorus (Table 6).

The MLSS indicates the amount of biomass, resulting in the increase of this parameter and suggesting the increased volume of microorganisms. The MLSS content in the biological pond of TPC at the sludge harvest time to fill the experimental reactors was estimated at 1,100 ppm, which was below the standard rate. After five days (shock dose and timing compatibility microorganisms), the MLSS content did not increase significantly, which was considered to be standard.

SR

According to the information in Table 7, the SR reduced in all the treatments, which was mostly observed in reactor nine, while reactor three had the least significant reduction. In addition, the first concentration of folic acid (0.1 ppm) affected the SR, and the higher concentration increased the SR.

SVI

According to the results of ANOVA (Table 5), folic acid, nitrogen, and phosphorus had significant effects on the reduction of SVI. The Taguchi method was used to determine the optimal conditions for the reduction of SVI, which were observed with 0.2 ppm of folic acid, 30 ppm of nitrogen, and 4 ppm of phosphorus (Table 6). On the other hand, 0.1 ppm of folic acid had the lowest effect on the SR, and the sludge volume increased at the higher concentrations.

According to the findings of the current research, folic acid was the most significant influential factor in the removal of color and pH adjustment, while nitrogen was the most important factor in the reduction of SVI. In biological areas, the bulking phenomenon and growth of *Filamentous* bacteria lead to improper sedimentation and increase the SVI. During the experiment in the present study, no bulking phenomenon was observed in the reactors, and the reduction of SVI was recorded in all the reactors. According to the study by Strunkheide, the use of folic acid in more than 60 urban and industrial wastewater treatment plants in the United States during 1995-2005 resulted in the higher efficacy of the biological treatment system, while the SVI reduced to 50%.[5] Furthermore, the results obtained by Akerboom *et al*. indicated that the SVI of wastewater reduced to 23% in the biological wastewater treatment using folic acid.[17]

COD

According to the results of ANOVA (Table 5), the effects of all the three studied factors on the COD removal were significant (P<0.05). Folic acid (52.653%) was the most effective factor in the removal of COD, while the efficiency of nitrogen and phosphorus in the removal of COD was estimated at 25.24% and 20.572%, respectively. In addition, the highest efficacy in the removal of COD was observed with 0.2 ppm of folic acid, 30 ppm of nitrogen, and 6 ppm of phosphorus (Table 6).

The balance of nutrients and vitamins in industrial wastewater, which mostly lack these materials, could improve the conditions of the microorganisms involved in the biological treatment of wastewater. According to the results of the present study, the effects of the three studied factors on COD removal were significant (Table 5). As for the optimal conditions and S/N (Tables 5 and 6), the highest COD removal rate was obtained in the second level of the three factors (i.e., 0.2 ppm of folic acid, 30 ppm of nitrogen, and 6 ppm of phosphorus).

In the current research, sludge volume reduction was observed in all the treatments, with the most significant reduction observed with 0.4 ppm of folic acid, 40 ppm of nitrogen, and 10 ppm of phosphorus (Table 6). Folic acid increases microorganism growth and metabolism and nitrogen and phosphorus are essential elements for microorganisms as basic elements in enzymes and nucleic acid.[18] In this experiment, the function of microorganisms increased with the addition of these materials to the biological treatment of wastewater, thereby improving the effluent parameters.

In the study by Akerboom *et al*., folic acid was used in the biological treatment of effluent and improved the efficacy of the system in the secondary clarifier at the second date of application.[17] After one week, the number of the rotifer bacteria was estimated at 1-5 in all the slides with normal sludge, reaching 30 in each slide using folic acid. In such case, the number of the bacteria was reported to be 54. In the mentioned research, increased folic acid reduced the COD by 62% on average and 50% in the end. In addition, total suspended solids reduced to 54% in the outlet, while the SVI reduced to 23%.

In another research, Lanzrath investigated the effect of folic acid on urban wastewater, reporting that COD and sludge volume reduced with the use of folic acid in the biological treatment of wastewater, so that the sludge in the effluent would reduce by 30±15% due to the type of the wastewater, thereby stabilizing the process in the biological treatment unit.[19]

SR and Y and COD reduction rate

According to the results of the present study, COD reduced from 600 to 182 mg/L with Ro and without nitrogen, phosphorus or folic acid, which indicated that in these conditions, the efficiency of COD removal was 69.7%. The reduction of COD in the other reactors was more significant compared to Ro, with the most significant COD reduction obtained at R_2 (87.5%) (Table 7). In addition, the reduction of Y coefficient and sludge were observed in all the reactors, with the highest and lowest reduction recorded in reactors nine and three, respectively.

Table 7. Values of Y reduction, SR (n%), and COD reduction (%)

Trial	Y (mg Biomass/mg COD)	SR %	COD reduction %
R_0	0.40	----	69.7
R_1	0.35	12.5	80.9
R_2	0.30	25	87.5
R_3	0.39	5	84.9
R_4	0.23	42.5	86.5
R_5	0.21	45	83.3
R_6	0.24	40	85.3
R_7	0.20	50	83.0
R_8	0.17	57.5	86.7
R_9	0.15	62.5	81.5

The results of the experiments conducted by Ferrer-polonio *et al*. indicated the reduction of the excess sludge by approximately 40% with the use of folic acid in the biological treatment of wastewater.[20] Furthermore, Akerboom *et al.* evaluated the dietary needs of microorganisms in the biological treatment of industrial wastewater resulted from the production olive oil in aerobic and anaerobic conditions, concluding that 900/5/1.7 and 170/5/1.5 of COD/N/P reduced COD by 80 and 75% in the aerobic and anaerobic conditions, respectively.[17]

Conclusion

In the present study, the concentrations of 0.1, 0.2, and 0.4 ppm of folic acid were assessed in one, three, and one parameters to create the optimal conditions. The nitrogen concentrations of 20 and 30 ppm showed the optimal conditions in three and two parameters, respectively, while the phosphorus concentrations of 4 and 6 ppm created the optimal conditions in three and two parameters, respectively. On the other hand, the nitrogen concentrations of 40 ppm and phosphorus concentration of 10 ppm did not lead to the optimal conditions in any of the parameters.

The addition of folic acid, nitrogen, and phosphorus to the biological treatment system of wastewater at TPC, which lacked these materials, increased microorganism growth and function, thereby improving the parameters of effluent and reducing the sludge volume. An ideal approach to solving the waste sludge problem would be the reduction of excess sludge production in the water line of the wastewater treatment plant. The treatment should be cost-effective with no impact on the effluent quality and settling properties. According to our findings, folic acid, nitrogen, and phosphorus could effectively reduce sludge growth, with the sludge reduction efficiencies within the range of 5-62.5%.

In conclusion, the results of this experiment demonstrated that the addition of nitrogen, phosphorus, and folic acid vitamin nutrients to the biological area of TPC industrial wastewater could enhance the growth and reproduction of wastewater microorganisms, which in turn improved the parameters of output wastewater and reduced the possible environmental risks.

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