Journal of Basic and Environmental Sciences

ISSN
Online:2356-6388
Print:2536-9202

Research Paper Open Access

Biological and chemical treatments enhance the quality of wastewater from a potato chips factor

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Abstract

Wastewater discharge contains a high level of contaminants that require sufficient treatment for further reuse and legitimate applications. This study examined the effect of seasonal variations on treatment performance, and data was generated at duration of one year on a monthly basis. Generally, physicochemical and microbial parameters in the wastewater retain better performance in spring and autumn than summer and winter. In this work Pseudomonas putida was isolated, identified, examined for its role in biodegradation of organic matter and wastewater treatment and the isolate showed potential to improve the BOD, COD, TSS. The results indicated that the treatment of wastewater for effluent was at acceptable level according to national regulations. The range of parameters of treated effluent during Autumn and Spring is better than Summer and Winter. The highest BOD after chemical treatment (DAF) was recorded during Spring which also gives the highest ratio after Chemical treatment (DAF) and after biological treatment (Effluent). The highest COD after Chemical treatment (DAF) was recorded during Spring which also gives the highest ratio after Chemical treatment (DAF)/ after biological treatment (Effluent). COD and BOD values were significantly different among Autumn, Spring and Winter as the P value < 0.05. Total Suspended solids (TSS) results indicated that the Effluent highest values were during Summer. While the Effluent lowest values of regional average were scored during Spring. Volatile Suspended Solids (VSS) results indicated that the DAF highest values were during Autumn. While the DAF lowest values were scored during Spring. The Effluent lowest Total phosphorous value was during Autumn. The Effluent lowest Oil & grease values were during Autumn. The Effluent lowest values of Settleable solids were during Spring. The Highest log10 number of total bacterial count and bacterial count by membrane filter methods were recorded in the sequencing batch reactor tank (SBR) (Aeration tank) after chemical treatment and this stage is considered the reactions phase of bacterial biological treatment.

1. Introduction

Wastewater with high levels of organic matter, phosphorus and nitrogen cause several problems such as oxygen consumption and toxicity, when discharged to the environment. It is, therefore, necessary to remove these substances from wastewater to reduce their harm to the environment. (**Borkar et al., 2013**). Chemical treatment of the industry wastewater is a suitable option especially when the treated water is to be utilized for reuse inside the facility or discharged to surface water. There are many kinds of inorganic metal-based coagulants on the chemical market such as aluminum sulfate (alum), ferric chloride and ferric sulfate, poly-aluminum chloride (PACl) and aluminum chlorohydrate (ACH) (**Al-Shaikhli, 2013**). Coagulation-flocculation is widely used for wastewater treatment, as it is efficient and simple to operate .

In addition to chemical treatment, appropriate biological technology is required. Biological treatment of wastewater uses controlled biological population to biodegrade waste from either municipal or industrial wastewater. Basically, these methods use microorganisms to remove organic matters and nutrients present in wastewater which result in a better-quality effluent (**Mustafa et al., 2015**). Combinations of chemical and biological methods are used usually for effective treatment of such industrial wastewater, since it is difficult to obtain satisfactory results by using any one of those methods alone (**Jiaqi et al., 2014).** Therefore, the goal of the current study is to investigate the potential of a combination of chemical and biological treatment of a wastewater from a potato chips factory to improve the physicochemical characteristics of the treated water to the acceptable level. In addition, we assess the role of a Pseudomonas putida strain to biodegrade the organic matter of the wastewater.

2. Material and method:

2.1. Wastewater collection samples:

Three samples of wastewater were collected from industrial food plant producing potato chips company, Giza, Egypt on a monthly base. one sample from influent receiving tank of wastewater which include the wastewater during processing lines, cleaning and sanitizing, second sample collected from the physical/chemical pretreatment and the Dissolved Air Flotation (DAF) system, and the third sample collected from the effluent wastewater treated tank (Manhole Disposal) according to previous work (**Konieczny et al., 2005**).

The purpose of the industrial wastewater treatment plant is the production of treated effluent that is complying with the regulatory standard regulating disposal of industrial wastewater to the public sewage network of the city. This regulatory standard as stated in law 93 for 1962 and its modified amendment by ministerial Decree No. 44 for 2000 is shown in **Table No. (1).**

Bacterial isolates were collected from food industrial Wastewater samples. The same samples are taken to determine physical and chemical parameters of wastewater every month from the three locations (influent receiving tank-physical/chemical pretreatment and the DAF system- effluent wastewater treated tank). These water samples were subjected for microbial analysis according to Standards Methods for the Examination of Water and Wastewater, 23 ed. American Public Health Association, Washington, DC.53–57 (**APHA, 2005**).

Table (1) Egyptian decree 44 for 2000 with the limits of pollutant for disposal of industrial effluent into the public sewage network

Parameter		unit	Limit
Temperature		°C	Not > 43
pH		-	6-9.5
Total suspended solids		Mg/L	800
Settleable solids	At 10 minutes	Ml/L	8
	At 30 minutes	Ml/L	15
Chemical oxygen demand		MgO_2/L	1100
Biochemical oxygen demand		MgO2/L	600
Oil and grease		MgO2/L	100
TKN		MgN/L	100
Total phosphorous		MgP/L	25
Soluble sulphides		MgS/L	10

2.2. Physical parameters.

2.2.1. Temperature (°C):

At the time of sampling, water temperatures were measured directly by using a digital temperature and pH meter.

2.3. Chemical parameters :

2.3.1. Hydrogen ion concentration (pH):

Hydrogen ion concentration of water sample was measured directly by using a digital pH temperature and pH meter. According to Titrimetric method No, 2320, C. **SMWW, (2013).**

2.3.2. Biochemical Oxygen Demand (BOD) mg/L):

BOD determination is an empirical test in which standardized laboratory procedures are used to determine the relative oxygen requirements of wastewater, collected at different periods as described in previous standard methods (APHA, 1995). The test measures the oxygen utilized during a specific incubation period (five days) at 20 **°C** for the biochemical degradation of organic material and the oxygen used to oxidize inorganic material .

2.3.3. Chemical oxygen Demand (COD mg/L):

The COD in water was detected by oxidation of a known volume of water sample by using acidified potassium permanganate as described in previous standard methods (**APHA, 1995**).

2.3.4. Total suspended solids (TSS) (mg/L):

Determination of Total suspended solids (TSS) as described in previous standard methods (**APHA, 1995**). using glass - fiber filter disks and filtration apparatus. Apply Vacuum and wash the disk with three successive 20 mL portions of reagent-grade water. Remove the filter from the filtration apparatus and transfer to an inert aluminum weighing dish. Dry in an oven at 103 to 105°C for 1 hour. Cool the filter in desiccators to balance the temperature. Then weigh the filter and record the weight.

2.3.5. Total Dissolved Solids (mg/L):

Determination of Total Dissolved Solids (TDS) and Total Volatile Dissolved Solids (TVDS) in potable, surface, and saline waters, as well as domestic and industrial wastewaters was performed according to (**APHA, 1995**). “Solids” refer to matter suspended or dissolved in water or wastewater.

2.3.6. Total Kjeldahl nitrogen (mg/L):

Total Kjeldahl nitrogen is defined as the sum of free-ammonia and organic nitrogen compounds which are converted to ammonium sulfate $(NH_4)_2SO_4$ as described in previous standard methods (**APHA, 1995, 4500 NB**).

2.3.7. Total phosphorous (mg/L):

The total phosphorus test measures all the forms of phosphorus in the sample as described in previous standard methods (**APHA, 1995, 4500 PC**).

2.3.8. Settleable solids 10 min and 30 min (ml /l):

Settleable solids in domestic and industrial wastes may be determined and reported on either a volume (mL/L) or a weight (mg/L) basis as described in previous standard methods (**APHA, 1995, 2540 F**).

2.3.9. OIL AND GREASE (ml /l):

Dissolved or emulsified oil and grease is extracted from water and determined as described in previous standard methods (**APHA, 1995, 5520 B. Partition-Gravimetric Method**).

2.4. Bacterial examinations:

2.4.1. Estimation of Total Bacterial Count by Plate Method Technique:

Total Bacterial Count (TBC), formerly known as the standard plate count, was used to determine total bacterial count in the water samples as described in previous standard methods (**APHA ,1998**).

2.4.2 Bacteriological Examination of Waters, Membrane Filtration Protocol:

The membrane filtration technique is used to examine water samples from different sources as described in previous standard methods Membrane Filtration Protocol. (**American Water Works Association ,2016**). Standard Methods for the Examination of Water and Wastewater. (20th Ed).

2.5. Characterization and identification of the bacterial isolates :

A series of preliminary tests was performed to build a phenotypic profile of each isolate. Purification and examination for their systematic position using cultural, morphological, Gram stain.

2.5.1. Morphological and biochemical characterization of bacterial isolates:

2.5.1.1. Morphological and staining characteristics :

The smears were stained with Gram stain (**Hucker and Conn, 1923**). Smear was microscopically examined using oil immersion objectives with magnification of 1000x .

2.5.1.2. Identification of isolated bacteria using VITEK® 2 GN analyzer:

The identity of P. Putida isolates was confirmed using VITEK 2 identification cards for bacteria (**VITEK® 2 GN analyzer, modified version 2018**).

2.6. Effectiveness of P. Putida for biodegradation of wastewater using Experimental flasks:

The effectiveness of biological treatment physically and chemically measured as COD and BOD (**Mujtaba, G et al.,2017**) was investigated by inoculating P. putida in a wastewater sample and incubating for several days.

2.7. Statistical Data:

For data analysis, SPSS Version 20 (SPSS Inc. Chicago, IL, USA) was applied. The general linear models (LMM) were used to investigate the effects of treatment (influent, Daf, and effluent), seasons (Autumn, Spring, Summer, and winter), and their interaction on the water parameter, where treatment and seasons were considered as fixed effects and samples as random terms. The results are presented as means with standard errors. The statistical model applied Tukey's b multiple comparison test. Significant differences were applied at $P < 0.05$.

3. Result

3.1 Sampling

Collection of different food industrial wastewater samples from potato chips industrial plant, Giza, Egypt for one year on a monthly basis was performed in three locations; one sample from (Before treatment/ Influent), second sample collected from the physical/chemical pretreatment, (DAF system/After chemical treatment) and the third sample collected from (After biological treatment/ Effluent). Physicochemical parameters were determined as described below .

3.2. Physic-chemical parameters:

3.2.1. Temperature (^oC)

The data of recorded temperature ^oC for industrial wastewater from three locations Influent, DAF system and Effluent ranged from 17.33 to 29.33 ,17.67 to 31.67 and 17.33 to 31.00 ^oC respectively .

All the records were represented in **Table (2)** and **Fig (1).** It was clear that the highest temperatures were recorded during summer for Influent, Daf and Effluent.

The lowest temperature was recorded during Winter for Influent, Daf and Effluent and data were 17.33, 17.67 and 17.33 ^oC respectively.

Table (2): Temperature (^{o}C) of industrial wastewater Influent, DAF, and effluent.

Seasons \ Average temp.°C	Influent	DAF	Effluent	SEM	P value
Autumn	**26.67**	**26.33**	**26.67**	**0.882**	**0.954**
Spring	**29.33**	**29.33**	**29.33**	**3.479**	**1.000**
Summer	**29.33**	**31.67**	**31.00**	**0.956**	**0.296**
Winter	**17.33**	**17.67**	**17.33**	**2.158**	**0.992**

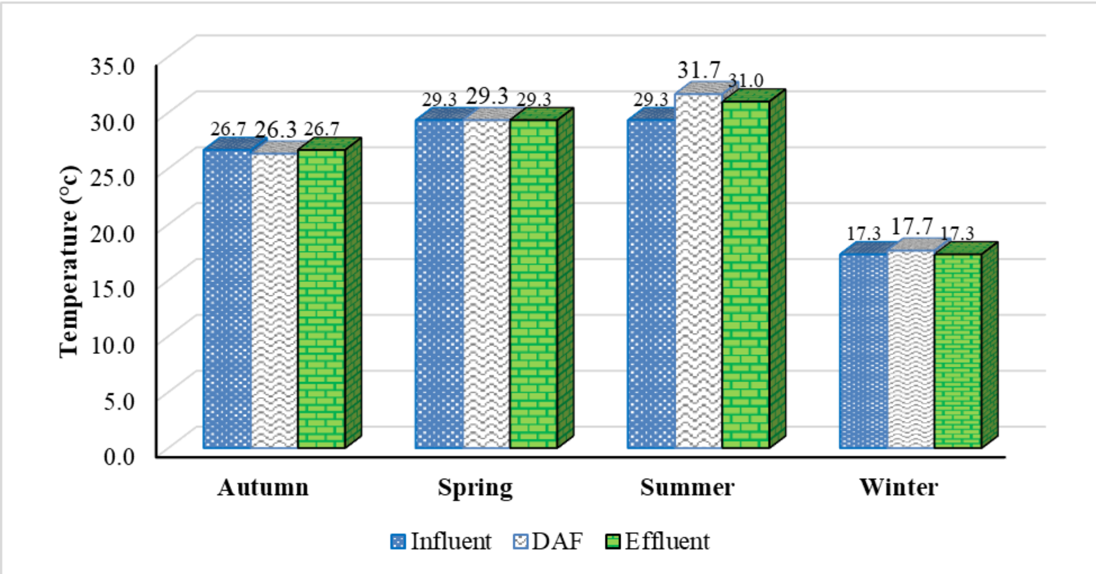


Figure (1): Temperature (^{o}C) of industrial wastewater Influent, DAF, and effluent.

3.2.2. Hydrogen ion concentration pH :

Variations of pH of industrial wastewater (Influent) and after both chemical treatment (DAF) and biological treatment (Effluent) ranged from 6.3 to 8.0, 6.6 to 7.6 and 7.1 to 7.5 respectively. The results indicated that the Effluent highest values were during spring at 7.5. While the Effluent lowest values of regional average were scored during Winter 7.1. The data in **Table (3)** and **Fig (2)** revealed that the pH of examined wastewater were alkaline (reach in summer 8) during all year seasons in Influent before treatment and it was slightly alkaline (above 7 and less than 8) after chemical treatment and biological treatment (DAF and Effluent).

Hydrogen ion concentration pH values were significantly different among Spring, Summer, and Winter as the P value < 0.05.

Table (3): Hydrogen ion concentration value of industrial wastewater Influent, DAF, and effluent.

Average pH / Seasons	Influent	DAF	Effluent	SEM	P value
Autumn	7.4	6.9	7.4	0.27	0.43
Spring	6.3	6.6	7.5	0.17	0.01
Summer	8.0	7.6	7.3	0.17	0.08
Winter	7.5	7.5	7.1	0.06	0.00

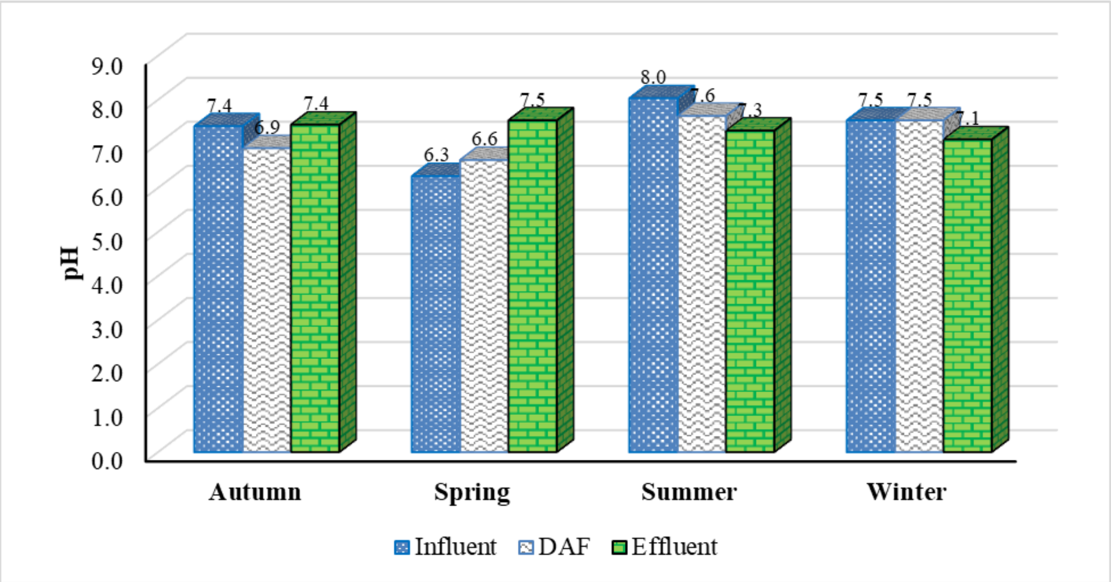


Fig (2): Hydrogen ion concentration value of industrial wastewater Influent, DAF, and effluent.

3.2.3. Biological Oxygen Demands (mgO2/l):

Variations of Biochemical Oxygen Demand (BOD) of industrial wastewater before treatment (Influent) ranged from 1513.3 to 3740.0 mgO2/l. BOD of samples after chemical treatment (DAF) ranged from 1410.0 to 1726.7 mgO2/l. BOD of samples after biological treatment (Effluent) ranged from 133.3 to 243.3 mgO2/l. Data represented in **Table (4)** and **Fig (3**) revealed that the highest BOD after chemical treatment (DAF) was recorded during Spring 1726.7 mgO2/l which also give the highest ratio after Chemical treatment (DAF) and after biological treatment (Effluent).

Biological Oxygen Demands (BOD) values were significantly different among Autumn, Spring and Winter as the P value < 0.05.

Table (4): Biological Oxygen Demand (BOD) industrial wastewater Influent, DAF, and effluent.

Seasons	Influent	DAF	Effluent	SEM	P value
Autumn	2386.7	1410.0	194.7	182.90	0.00
Spring	3740.0	1726.7	142.7	102.36	0.00
Summer	1513.3	1523.3	133.3	410.46	0.16
Winter	2776.7	1553.3	243.3	194.77	0.00

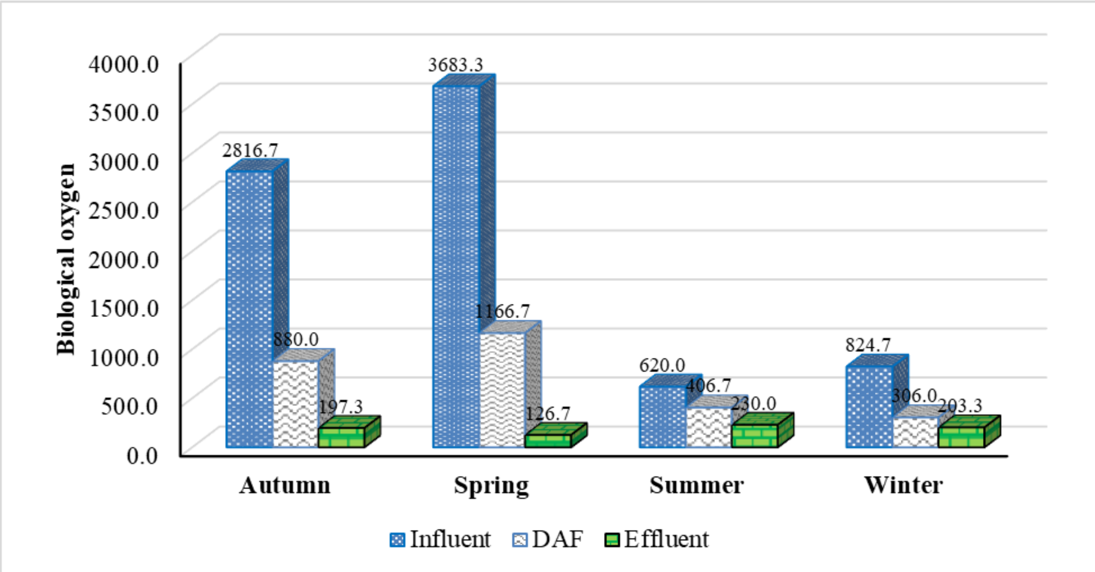


Fig (3): Biological Oxygen Demand (BOD) Influent, DAF, and effluent.

3.2.4. Chemical Oxygen Demands (COD mgO2/l):

Variations of Chemical Oxygen Demand (COD mgO2/l) of industrial wastewater before treatment (Influent)and after both chemical treatment and biological treatment (DAF and Effluent) ranged from 3300.0 to 8066.7, 2976.7 to 3833.3, 306.7 to 646.7 mgO2/l respectively. The results indicated that the Effluent highest values were during Winter. While the Effluent lowest values were scored during Summer and Spring.

Data represented in **Table (5)** and **Fig (4)** revealed that the highest COD after Chemical treatment (DAF) was recorded during Spring 3833.3 mgO2/l which also give the highest ratio after Chemical treatment (DAF)/ after biological treatment (Effluent.(

Chemical Oxygen Demand (COD) values were significantly different among Autumn, Spring and Winter as the P value < 0.05.

Table (5): Chemical Oxygen Demand (COD) industrial wastewater Influent, DAF, and effluent.

Average COD / Seasons	Influent	DAF	Effluent	SEM	P value
Autumn	5066.7	2976.7	493.3	379.62	0.00
Spring	8066.7	3833.3	369.7	101.59	0.00
Summer	3300.0	3106.7	306.7	902.82	0.18
Winter	6050.0	3166.7	646.7	443.59	0.00

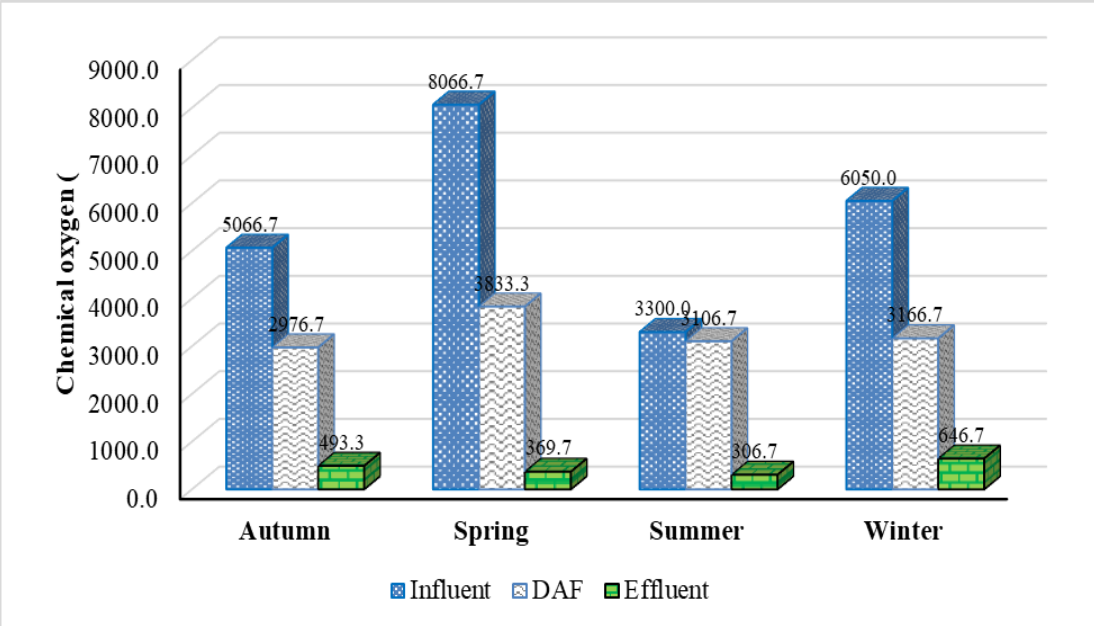


Fig (4): Chemical Oxygen Demand (COD) industrial wastewater Influent, DAF, and effluent.

3.2.5. Total Suspended Solids (TSS mg/L):

Variations of Total Suspended solids (TSS) of industrial wastewater before treatment (Influent)and after both chemical treatment and biological treatment (DAF and Effluent) ranged from 620.0 to 3683.3, 306.0 to 1166.7, 126.7 to 230.0 mg/L, respectively. The results indicated that the Effluent highest values were during Summer 230 mg/l. While the Effluent lowest values of regional average were scored during Spring 126.7 mg/l.

Data represented in **Table (6)** and **Fig (5)** revealed that the highest TSS after Chemical treatment (DAF) was recorded during Spring 1166.7 mg/l.

Total Suspended solids (TSS) values were significantly different among Spring as it has the lowest P value 0.06 < 0.05.

Table (6): Total Suspended Solids (mg/l) (TSS) of industrial wastewater Influent, DAF, and effluent.

Seasons / Average TSS	Influent	DAF	Effluent	SEM	P value
Autumn	2816.7	880.0	197.3	694.46	0.24
Spring	3683.3	1166.7	126.7	581.42	0.06
Summer	620.0	406.7	230.0	135.02	0.23
Winter	824.7	306.0	203.3	139.47	0.10

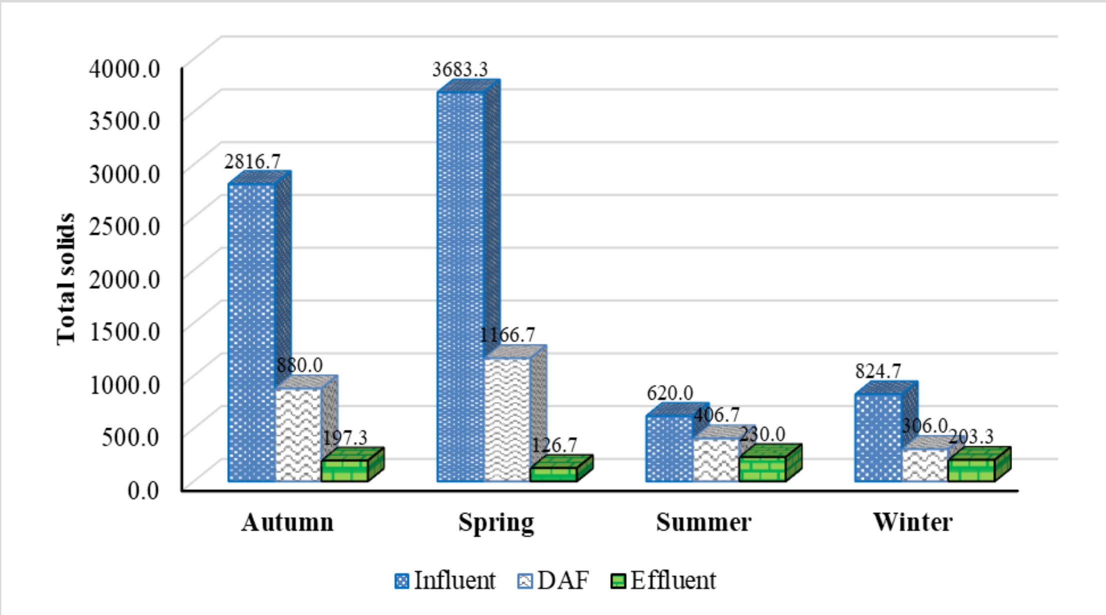


Fig (5): Total Suspended Solids (mg/l) (TSS) of industrial wastewater Influent, DAF, and effluent.

3.2.6. Volatile Suspended Solids (VSS mg/L) and Total Dissolved Solids (TDS) (mg/L):

Variations of Volatile Suspended Solids (VSS) mg/L of industrial wastewater before treatment (Influent) and after chemical treatment (DAF) ranged from 462.7 to 2966.7, 235.0 to 680.0 mg/L respectively. The results indicated that the DAF highest values were during Autumn 680.0 mg/l. While the DAF lowest values of regional average were scored during Spring 235.0 mg/l.

Data represented in **Table (7)** and **Fig (6)** revealed that the highest VSS before treatment (Influent) was recorded during Spring 2966.7 mg/l.

Volatile Suspended Solids (VSS) values were significantly different among Spring as the P value < 0.05.

Table (7): Volatile Suspended Solids (mg/l) (VSS) of industrial wastewater Influent and DAF.

Average VSS / Seasons	Influent	DAF	SEM	P value
Autumn	2175.7	680.0	770.08	0.32
Spring	2966.7	235.0	257.36	0.00
Summer	462.7	288.7	106.67	0.34
Winter	615.0	236.0	155.45	0.19

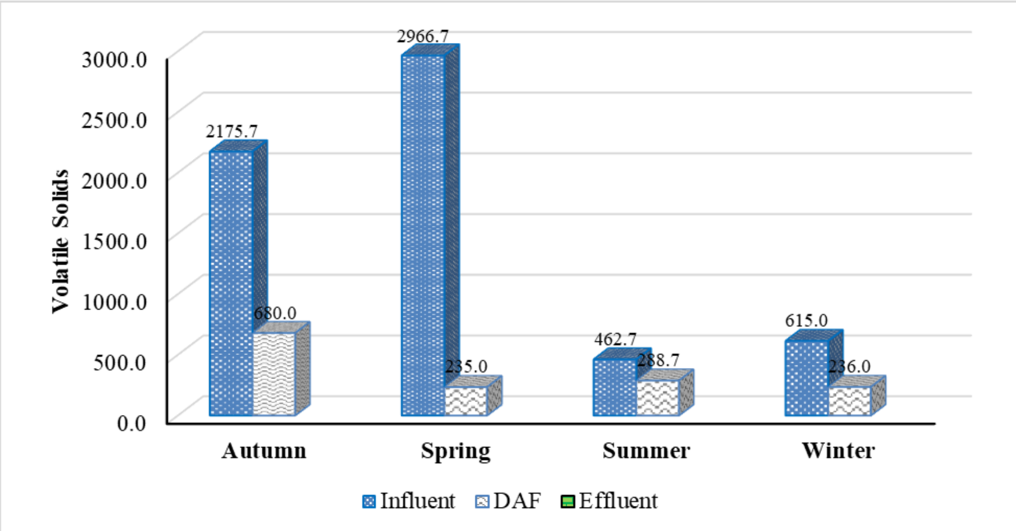


Fig (6): Volatile Suspended Solids (mg/l) (VSS) of industrial wastewater Influent and DAF.

Variations of Total Dissolved Solids (TDS) (mg/L) of industrial wastewater before treatment (Influent) and after both chemical treatment and biological treatment (DAF and Effluent) ranged from 2250.0 to 5066.7, 2033.3 to 3140.0, 1452.7 to 2040.0 mg/L respectively. The results indicated that the Influent highest values were during Autumn 5066.7 mg/l. While the Effluent lowest values of regional average were scored during Spring 1452.7mg/l.

Data represented in **Table (8)** and **Fig (7)** revealed that the highest TDS before treatment (Influent) was recorded during Autumn 5066.7 mg/l.

The second highest ratio of before treatment (Influent) / after biological treatment (Effluent) happened during spring (3800.0 / 1452.7) giving 2.6.

Total Dissolved Solids (TDS) values were significantly different among Spring, Autumn, and winter as the P value < 0.05.

Table (8): Total Dissolved Solids (mg/l) (TDS) of industrial wastewater Influent, DAF, and effluent.

Seasons \ Average TDS	Influent	DAF	Effluent	SEM	P value
Autumn	5066.7	3106.7	1851.3	607.17	0.10
Spring	3800.0	3026.7	1452.7	73.76	0.00
Summer	2250.0	2033.3	2040.0	579.74	0.96
Winter	4056.7	3140.0	1786.7	163.14	0.00

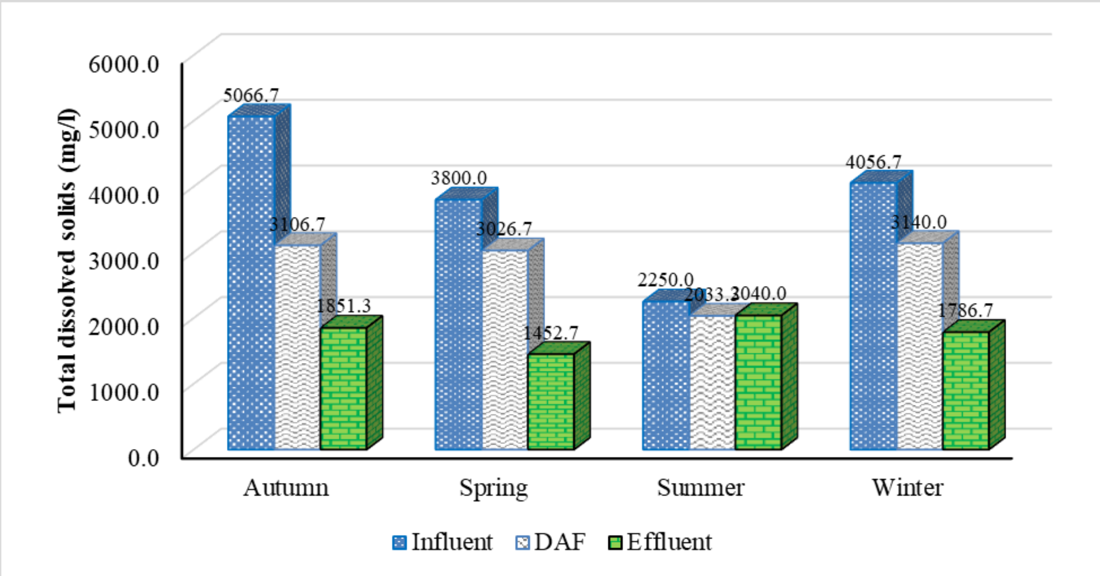


Fig (7): Total Dissolved Solids (mg/l) (TDS) of industrial wastewater Influent, DAF, and effluent.

3.2.7. Total Kjeldahl nitrogen mg/L:

Variations of Total Kjeldahl nitrogen (mg/L) of industrial wastewater before treatment (Influent) and after both chemical treatment and biological treatment (DAF and Effluent) ranged from (90.7 to 247.7) (58.7 to 163.3) (47.0 to 65.3) mg/L respectively. The results indicated that the Effluent highest values were during Winter 65.3 mg/l. While the Effluent lowest values of regional average were scored during Summer 47.0 mg/l.

Data represented in **Table (9)** and **Fig (8)** revealed that the highest Average Kjeldahl nitrogen after Chemical treatment (DAF) was recorded during Spring 163.3 mg/l.

Total Kjeldahl nitrogen values were significantly different among Autumn, Spring and Winter as the P value < 0.05.

Table (9): Total Kjeldahl nitrogen (mg/l) of industrial wastewater Influent, DAF, and effluent.

Average Kjeldahl nitrogen / Seasons	Influent	DAF	Effluent	SEM	P value
Autumn	**195.7**	**109.3**	**54.3**	**6.61**	**0.00**
Spring	**247.7**	**163.3**	**60.3**	**19.79**	**0.00**
Summer	**90.7**	**58.7**	**47.0**	**18.67**	**0.44**
Winter	**180.0**	**98.3**	**65.3**	**8.08**	**0.00**

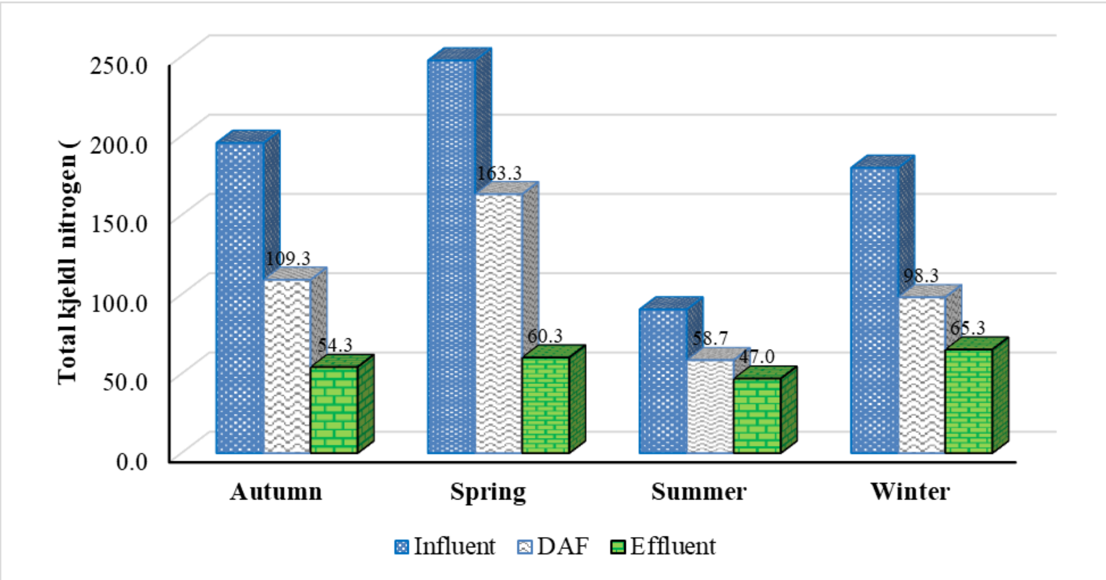


Fig (8): Total Kjeldahl nitrogen (mg/l) of industrial wastewater Influent, DAF, and effluent.

3.2.8 Total phosphorous mg/L:

Variations of Total phosphorous (mg/L) of industrial wastewater before treatment (Influent) and after both chemical treatment and biological treatment (DAF and Effluent) ranged from (8.1 to 12.3) (6.3 to 9.1) (3.2 to 5.1) mg/L respectively. The results indicated that the Effluent highest values were during Spring 5.1 mg/l. While the Effluent lowest values of regional average were scored during Autumn and Summer 3.2 and 3.3 mg/l respectively.

Data represented in **Table (10)** and **Fig (9)** Revealed that the highest Total phosphorous after Chemical treatment (DAF) was during Autumn 9.1 mg/l. Effluent lowest Total phosphorous value was during Autumn 3.2 mg/l confirming the conclusion.

Table (10): Total phosphorous (mg/l) of industrial wastewater Influent, DAF, and effluent.

Seasons \ Total phosphorous	Influent	DAF	Effluent	SEM	P value
Autumn	12.3	9.1	3.2	0.40	0.00
Spring	9.5	7.4	5.1	1.39	0.17
Summer	8.1	6.3	3.3	1.24	0.11
Winter	9.8	7.1	3.6	0.46	0.00

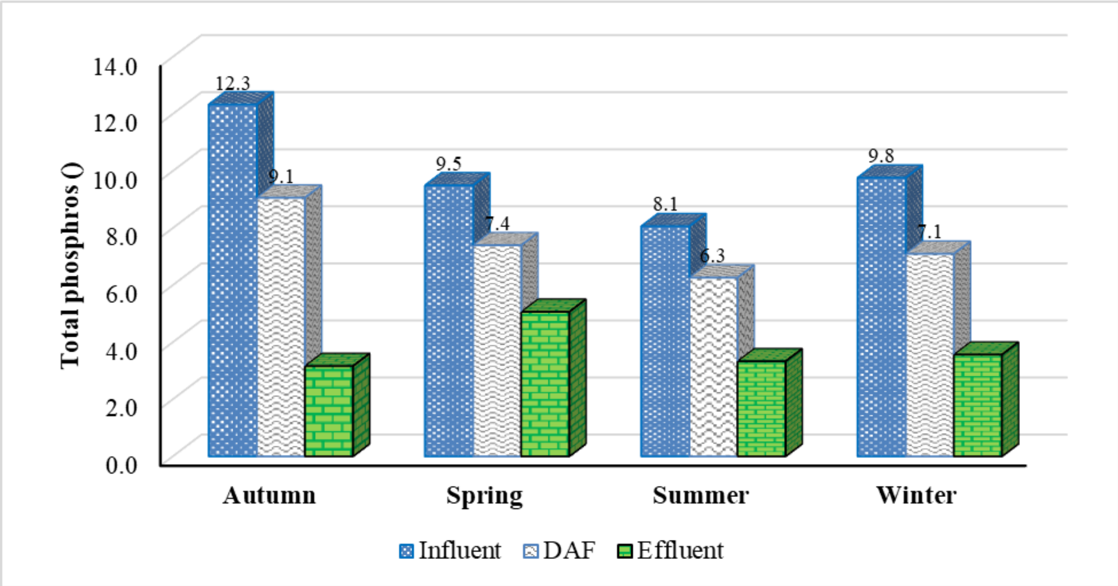


Fig (9): Total phosphorous (mg/l) of industrial wastewater Influent, DAF, and effluent.

3.2.9. Oil & grease mg/L:

Variations of Oil & grease (mg/L) of industrial wastewater before treatment (Influent) and after both chemical treatment and biological treatment (DAF and Effluent) ranged from 113.0 to 180.7, 63.7 to 94.3, 19.7 to 33.0 mg/L respectively. The results indicated that the Effluent highest values were during Spring 33.0 mg/l. While the Effluent lowest values of regional average were scored during Autumn 19.7 mg/l.

Data represented in **Table (11)** and **Fig (10)** revealed that the highest Oil & grease after Chemical treatment (DAF) was recorded during Spring 94.3 mg/l .

Oil & grease values were significantly different among Autumn, Spring and Winter as the P value < 0.05.

Table (11): Oil & grease (mg/l) of industrial wastewater Influent, DAF, and effluent.

Oil & grease. / Seasons	Influent	DAF	Effluent	SEM	P value
Autumn	**149.3**	**83.0**	**19.7**	**3.96**	**0.00**
Spring	**180.7**	**94.3**	**33.0**	**5.98**	**0.00**
Summer	**113.0**	**63.7**	**22.0**	**23.45**	**0.18**
Winter	**155.7**	**92.3**	**24.0**	**1.77**	**0.00**

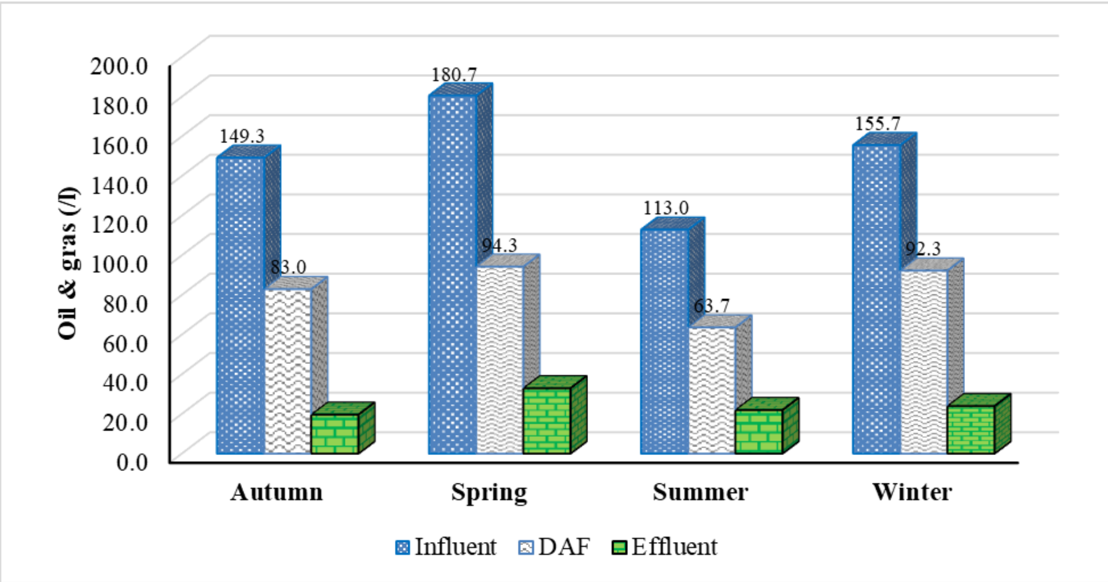


Fig (10): Oil & grease (mg/l) of industrial wastewater Influent, DAF, and effluent.

3.2.10 Settleable solids (10 min, ml /l) and (30 min, ml /l):

Variations of Settleable solids (ml /l) of industrial wastewater after biological treatment (Effluent) for both tests Settleable solids (10 min, ml /l) and (30 min, ml /l) ranged from 0.10 to 0.37, 0.17 to 0.80 ml /l respectively. The results indicated that the Effluent highest values for Settleable solids were during Summer 0.37 and 0.80 mg/l respectively. While the Effluent lowest values of regional average for Settleable solids were scored during Spring 0.1 and 0.2 mg/l and during Winter 0.1 and 0.17 mg/l respectively.

Data represented in **Table (12)** and **Fig (11)** revealed that the highest Settleable solids (10 min, ml /l) and (30 min, ml /l) after biological treatment (Effluent) was recorded during Summer 0.37 and 0.80 mg/l respectively .

Also, there is a high ratio of Settleable solids (30 min, ml /l)/ Settleable solids (10 min, ml /l) during Spring 2.0.

Table (12): Settleable solids (10 min, ml /l) and (30 min, ml /l) of industrial wastewater after biological treatment (Effluent.(

Settleable solids \ Seasons	Autumn	Spring	Summer	Winter	SEM	P value
Settleable solids (10 min, ml /l)	0.13	0.10	0.37	0.10	0.08	0.47
Settleable solids (30 min, ml /l)	0.20	0.20	0.80	0.17	0.18	0.43

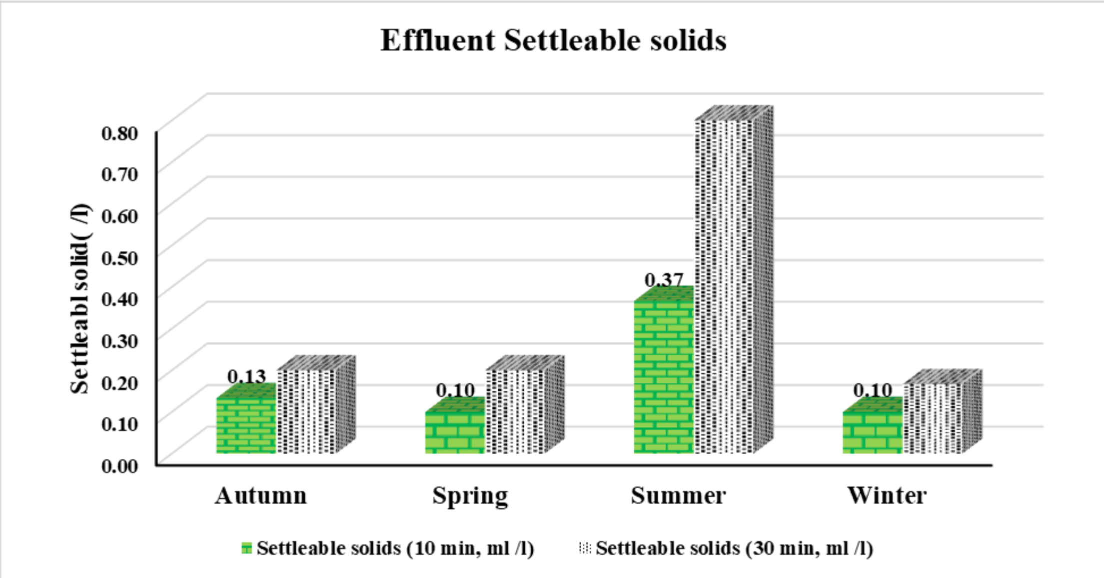


Fig (11): Settleable solids (10 min, ml /l) and (30 min, ml /l) of industrial wastewater after biological treatment (Effluent.(

3.3. Microbiological assessment of water samples.

Assessment of microbiological characteristics of the industrial wastewater from samples from two locations aeration tank (sequencing batch reactor - SBR) after chemical treatment and effluent tank after biological treatment, was performed.

3.3.1. Total Bacterial Count by Plate Method Technique at 37 °C ± 1 for 2 days:

The log10 number of total bacterial count at 37°C in industrial wastewater in the aeration tank (SBR) after chemical treatment ranged from 9 to 9.08 CFU/mL. The second location from effluent tank after biological treatment ranged from 8.93 to 8.95 CFU/mL .

The Highest value was recorded in the sequencing batch reactor tank (SBR) (Aeration tank) after chemical treatment and this stage is considered the reactions phase of bacterial biological treatment .The data in **Table (13)** and **Fig (12)** revealed that, the bacterial count in industrial wastewater increased in sequencing batch reactor tank (SBR) after chemical treatment to degrade the organic matter of the waste water .The lowest value was recorded in the effluent tank after finishing the biological treatment.

This mean that degradation of organic matter and wastewater treatment take place during SBR, and the bacterial count decreased after the finalizing the wastewater treatment and depleting the organic matter content which support bacteria to live and regenerate.

Table (13): The log10 numbers for Total Bacterial Count at 37 °C CFU /mL of industrial wastewater from two locations Effluent and Aeration tank.

Plates \ Log_{10} No. of bacterial count	Units	Effluent tank	Aeration tank	Analysis methodology
1st plate	Log_{10} (CFU/ml)	8.93	9	APHA ,1998
2nd plate	Log_{10} (CFU/ml)	8.95	9.08	APHA ,1998

Table (14): Data analysis for both tests (Total Bacterial Count at 37 °C CFU /mL) of industrial wastewater from two locations Effluent and Aeration tank.

Location \ Test method.	Total Bacterial Count (Log_{10} CFU/mL)
Effluent tank	**8.94**
Aeration	**9.04**

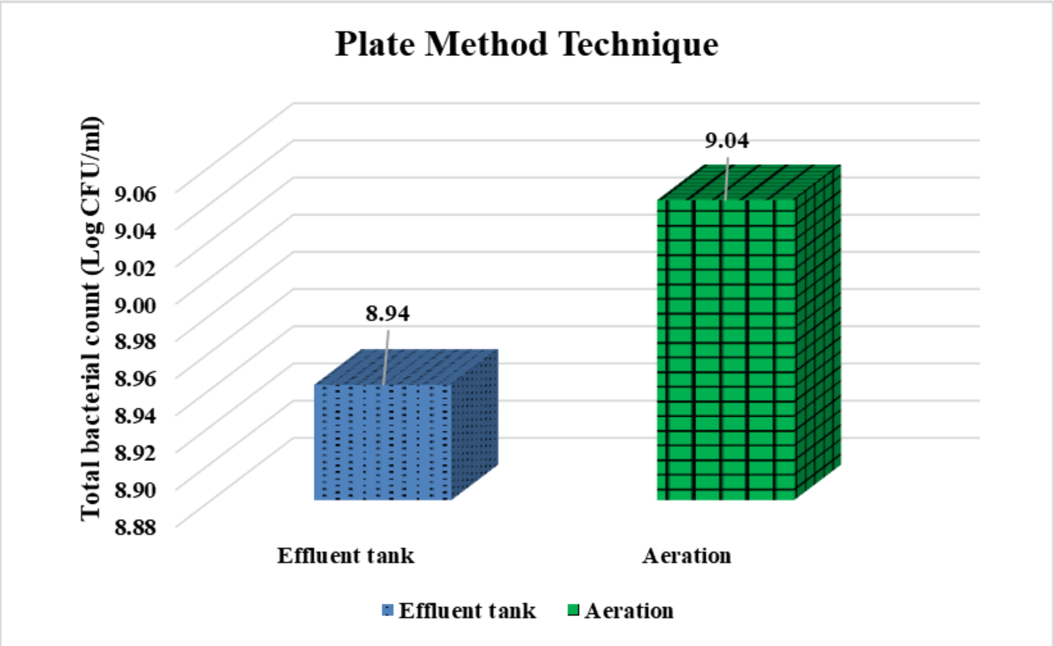


Fig (12): data analysis for (Total Bacterial Count at 37 °C) of industrial wastewater from two locations Effluent and Aeration tank.

3.3.2. Bacteriological Examination of Waters: Membrane Filtration Protocol using R2A agar plates at 35 ± 0.5 °C for 22 – 24 hours.

The Log10 number of bacterial count at 35 ± 0.5 **°C** in industrial wastewater in two locations (SBR and after chemical treatment) ranged from 6.18 to 6.34 CFU/mL. The second location from effluent tank after biological treatment ranged from 6.08 to 6.2 CFU/mL.

The Highest value was recorded in sequencing batch reactor tank (SBR) (Aeration tank) after chemical treatment **Table (15)** and **Fig (14).**

Bacteriological Examination by Membrane Filtration Protocol using R2A agar plates.

Effluent tank Aeration tank

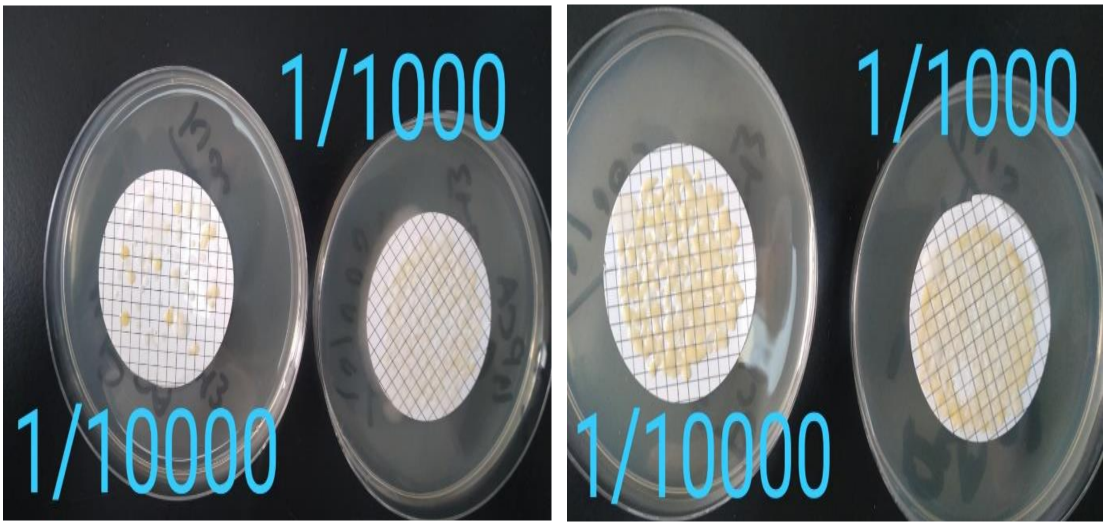


Fig (13) Bacteriological Examination by Membrane Filtration Protocol using R2A agar plates.

Table (15): The log numbers for Bacteriological Examination by Membrane Filtration Protocol at 35 ± 0.5 °C CFU /mL of industrial wastewater from two locations Effluent and Aeration tank.

Log_{10} No. of bacterial count / Plates	Units	Effluent tank	Aeration tank	Analysis methodology
1st plate	Log_{10} (CFU/ml)	6.08	6.18	American Water Works Association ,2016
2nd plate	Log_{10} (CFU/ml)	6.2	6.34	American Water Works Association ,2016

Table (16): data analysis for both tests (Membrane Filtration Protocol at 35 ± 0.5 °C CFU /mL of industrial wastewater from two locations Effluent and Aeration tank.

Location \ Test method.	Membrane Filtration Protocol (Log_{10} CFU/mL)
Effluent tank	6.17
Aeration	6.26

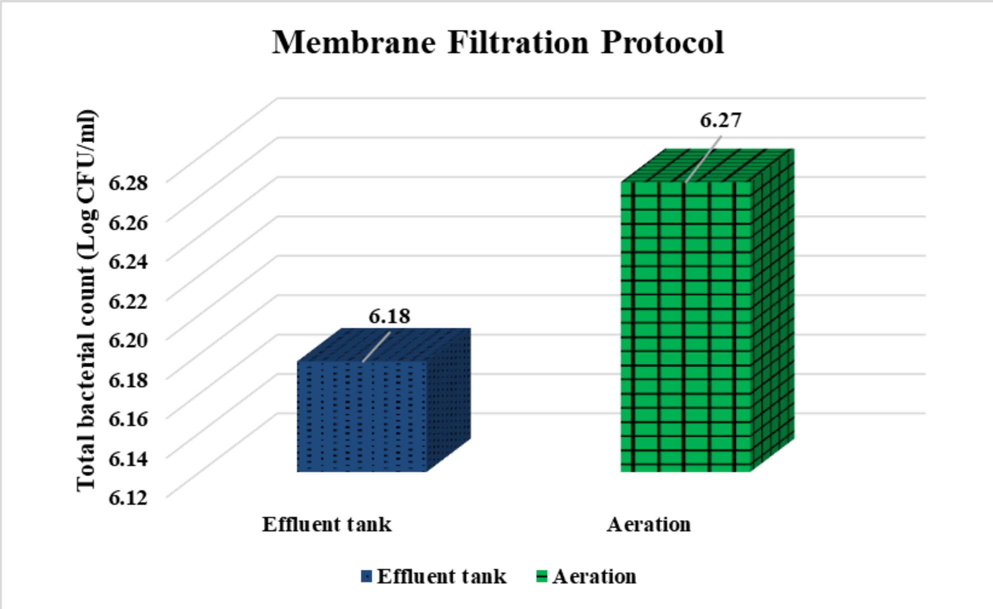


Fig (14): Data analysis for both tests using Membrane Filtration Protocol at 35 ± 0.5 °C of industrial wastewater from two locations Effluent and Aeration tank.

3.3.3. Characterization and identification of the bacterial isolates

The morphology of colonies in Effluent tank appeared to be Off white color **Fig (15).**

Bacterial colonies separately were picked and transferred to agar plate medium then purified by streaking several times on agar medium, until pure single colonies were obtained then subculture in the same medium and stored at 4 **°C** for further studies .

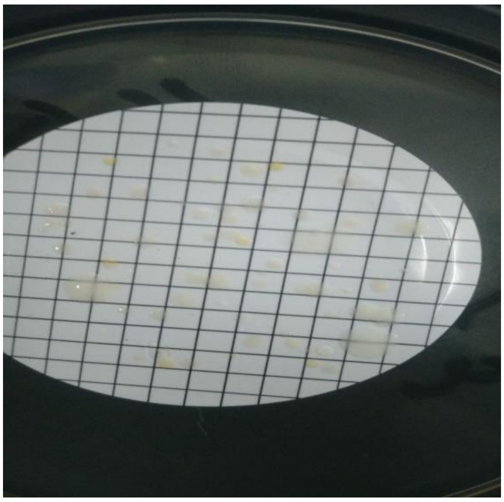


Fig (15): Off white color Morphology of colonies.

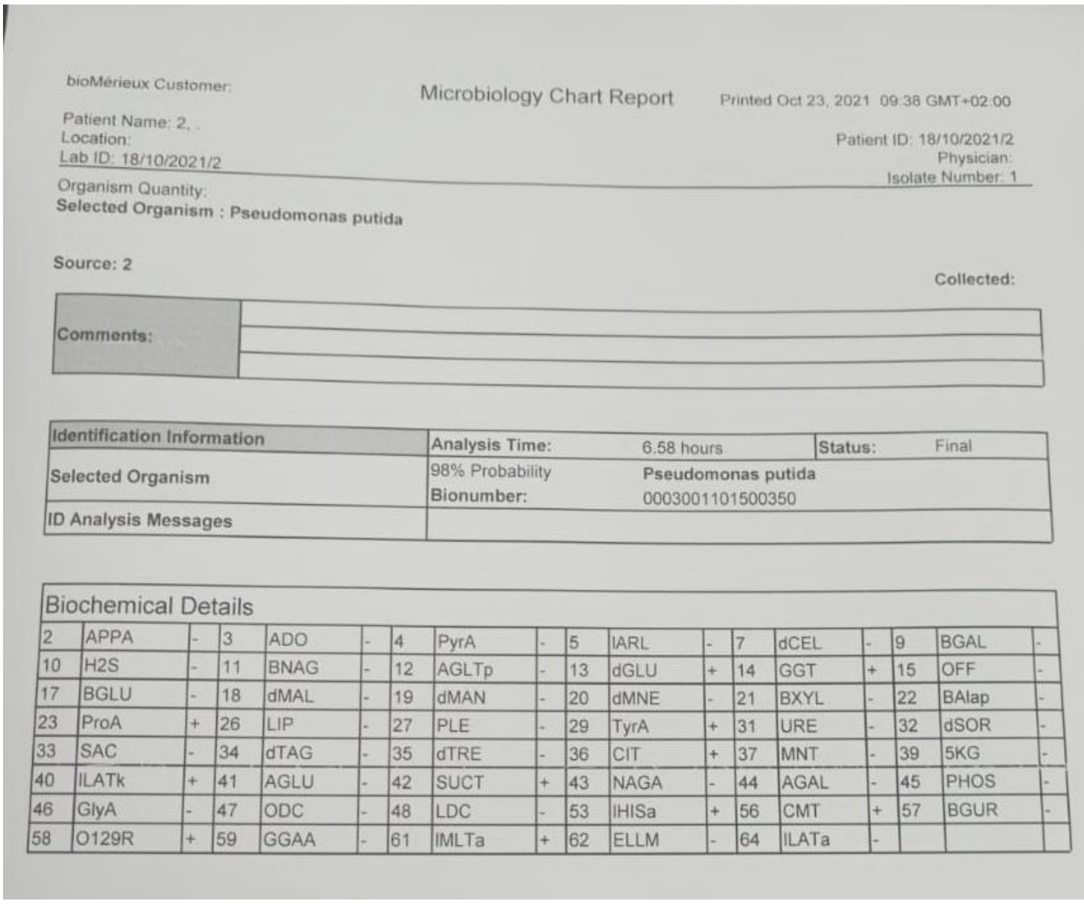
Fig (16): Three-phase streak for ensuring purification of the P. putida colony.

3.3.4. Collected isolates Gram's stain:

In this experiment the most predominant cells from the membrane filter technique gram negative bacterial isolates. These results indicated that the Gram-negative, rod-shaped bacterial isolates were the most predominant in industrial wastewater samples which indicate that isolate is more likely to be P. Putida.

3.3.5. Identification of isolated bacteria using VITEK® 2 GN analyzer:

The identity of P. Putida isolates was confirmed using VITEK 2 identification cards for bacteria. The biochemical profile of the isolates matched accurately with 98% similarity as shown in **Scheme (17).**



bioMérieux Customer:

Microbiology Chart Report

Printed Oct 23, 2021 09:38 GMT+02:00

Patient Name: 2, .
Location:
Lab ID: 18/10/2021/2

Patient ID: 18/10/2021/2
Physician:
Isolate Number: 1

Organism Quantity:
Selected Organism : Pseudomonas putida

Source: 2

Collected:

Comments:

Identification Information	Analysis Time:	6.58 hours	Status:	Final
Selected Organism	98% Probability	Pseudomonas putida		
	Bionumber:	0003001101500350		
ID Analysis Messages				

Biochemical Details

2	APPA	-	3	ADO	-	4	PyrA	-	5	IARL	-	7	dCEL	-	9	BGAL	-
10	H2S	-	11	BNAG	-	12	AGLTp	-	13	dGLU	+	14	GGT	+	15	OFF	-
17	BGLU	-	18	dMAL	-	19	dMAN	-	20	dMNE	-	21	BXYL	-	22	BAlap	-
23	ProA	+	26	LIP	-	27	PLE	-	29	TyrA	+	31	URE	-	32	dSOR	-
33	SAC	-	34	dTAG	-	35	dTRE	-	36	CIT	+	37	MNT	-	39	5KG	-
40	ILATk	+	41	AGLU	-	42	SUCT	+	43	NAGA	-	44	AGAL	-	45	PHOS	-
46	GlyA	-	47	ODC	-	48	LDC	-	53	IHISa	+	56	CMT	+	57	BGUR	-
58	O129R	+	59	GGAA	-	61	IMLTa	+	62	ELLM	-	64	ILATa	-			

Scheme (17): The biochemical profile of P. Putida using VITEK® 2 GN analyzer.

3.3.6. Effectiveness of wastewater treatment by P. Putida using Experimental flasks.

P. Putida was inoculated into 250-mL flasks containing 200 mL working volume of wastewater from Aeration tank as a test and another control sample without organism inoculation (**Fig. 18-20**), The temperature was controlled at 25°C. External aeration or CO_2 was not supplied into the reactors; the mixing was solely driven by flask-shaking. The effectiveness of biological treatment was assessed by determining COD .

Fig (18): Two samples from the aeration tank :1- pure culture of suspended P. putida (Sample), and 2- negative control culture of wastewater (Control) on 1st Day of incubation.

Fig (19): Two samples from the aeration tank :1- pure culture of suspended P. putida (Sample), and 2- negative control culture of wastewater (Control) on 4th Day of incubation.

Fig (20): Comparison between the pure culture of suspended P. putida (Sample) in the 1st day of incubation Vs. the 4th Day of incubation.

COD is measured in 1st day of incubation for both water samples with pure culture of suspended P. putida (Sample), and without the bacterium (Control),was equal 5430 mgO2/l. COD decreased for the P. putida-treated Sample after the 4th Day of incubation to be 476 mgO2/l and the sample became more clear than control which indicate the effectiveness of the added P. Putida in degradation of organic matter and waste water treatment .

4. Discussion

Industrial wastewater treatment methods commonly use chemical and biological methods. Among these methods, biological treatment is currently widely used in industrial wastewater treatment, which is the most economical and environmentally friendly. (**Fernando, 2011**). The present work represented both the physiochemical and bacteriological characteristics of food industrial Wastewater samples contaminated with P. Putida for one year monthly.

In the present study the data recorded temperature **^{o}C** for industrial wastewater from three locations, one sample from Influent tank, second sample from DAF system and the third sample collected from the Effluent tank. In this study the temperatures of wastewater ranged from 17.33 to 31.67 0C. It is Revealed that the highest BOD ratio of Before treatment (Influent) / after biological treatment (Effluent) are 26.2 for spring and 12.25 autumn respectively, which indicated a high rate of biodegradation during optimum temperature of Spring and Autumn. These results match with (**Alsulaili, A.et al.,2020**) Average values of physicochemical parameters in the effluent temperature (°C), parameters during different seasons (Summer Winter Spring and Autumn) are (32.4 ,23.4, 28.2 and 30.53) respectively. Results match with (**Abdo, 2005**) The recorded temperatures of water ranged from 19 to 34 0C.

In the present study the hydrogen ion concentration (pH) values of Influent, DAF and Effluent ranged from 6.3 to 8.0, 6.6 to 7.6 and 7.1 to 7.5 respectively. The result matched with (**Daboor, 2006**) the mean values of hydrogen ion concentration (pH) for Rosetta branch at the soil drain (agricultural area) ranged from 7.2 to 8.2. The average pH values were in line with the permissible standard limits 7 to 8.5, it was 7.56 in west, 7.67 in central and 7.59 in east Delta (**ESER,2008**).

In the present study the data Revealed that the highest BOD after Chemical treatment (DAF) was recorded during Spring 1726.7 mgO2/l which also give the highest ratio of after Chemical treatment (DAF)/ after biological treatment (Effluent) 12.1 which indicated a high rate of biodegradation. These results are matching with (**Priya et al., 2015**) who reported that the treated wastewater instead of untreated wastewater improves the physic – chemical properties of water BOD before treatment and after treatment changes from 300 to 60mg/l. Similarly (**Sonune et al.,2015**) reported that the BOD is an indication of the organic load of domestic wastewater.

The results Revealed that the highest COD after Chemical treatment (DAF) was recorded during Spring 3833.3 mgO2/l which also give the highest ratio of after Chemical treatment (DAF)/ after biological treatment (Effluent) 10.36 which indicate the depend on chemical treatment and concentration of coagulation and flocculation. The maximum limits of (COD) were recorded in the following sites of the River Nile, Rostta and Damietta branch respectively (18–26–20 mg/L) (**ESER, 2008**). The results matched with (**Sonune et al., 2015**) reported that the COD is another parameter used to characterize the organic strength of domestic wastewater. The minimum COD value was recorded as 180±1.82 mg/L and the maximum of 300±2.5 mg/L was observed .

The variations of Total Suspended solids (TSS) of Influent, Daf and Effluent ranged from 620.0 to 3683.3, 306.0 to 1166.7, 126.7 to 230.0 mg/L, respectively. The results indicated that the highest TSS after Chemical treatment (DAF) was recorded during Spring 1166.7 mg/l which also gives the highest ratio of after Chemical treatment (DAF)/ after biological treatment (Effluent) 9.2. These results match with (**Alsulaili, A.et al.,2020**) Average values of physicochemical parameters in the effluent TSS, (mg/L) parameter during different seasons (Summer Winter Spring and Autumn) are 5.95, 5.8, 5.9 and 4.75respectively.

The variations of Volatile Suspended Solids (VSS) of Influent and Daf ranged from 462.7 to 2966.7, 235.0 to 680.0 respectively. The data revealed that the highest VSS before treatment (Influent) was recorded during Spring 2966.7 mg/l which also gives the highest ratio of before treatment (Influent) / after Chemical treatment (DAF) 12.6. These results match with (**Mirbagheri, S. A.et al.,2015**) the VSS values were 1636 mg/L at the aeration time of 6 h. indicated that VSS directly affect removal of organic pollutants.

The Variations of Total Dissolved Solids (TDS) (mg/L) of Influent, Daf and Effluent ranged from 2250.0 to 5066.7, 2033.3 to 3140.0, 1452.7 to 2040.0 respectively. These results match (**Albaggar, 2021**) in spring season the TDS value where 212 to 301 mg/l in the terminal.

The Variations of Total Kjeldahl nitrogen of Influent, Daf and Effluent ranged from 90.7 to 247.7, 58.7 to 163.3, 47.0 to 65.3 respectively. The data Revealed that the highest Average Kjeldahl nitrogen after Chemical treatment (DAF) was recorded during Spring 163.3 mg/l which also give the highest ratio of after Chemical treatment (DAF)/ after biological treatment (Effluent) 2.7. The results are matched with (**Manu, D. S., & Thalla, A. K., 2017**) it can be noticed that the effluent TKN at the time (t) is strongly correlated with the influent total Kjeldahl Nitrogen concentration.

The Variations of Total phosphorous of Influent, Daf and Effluent ranged from 8.1 to 12.3, 6.3 to 9.1, 3.2 to 5.1 respectively. The Data in this study revealed that the highest Total phosphorous after Chemical treatment (DAF) was recorded during Autumn 9.1 mg/l. The results are matched with (**Tikariha, A., & Sahu, O., 2014**) Total phosphate value had a variation in values from 18.00-26.42 mg/L in wastewater.

The Variations of Oil & grease of Influent, Daf and Effluent ranged from 113.0 to 180.7, 63.7 to 94.3 ,19.7 to 33.0 respectively. The data Revealed that the highest Oil & grease after Chemical treatment (DAF) was recorded during Spring 94.3 mg/l - also it is noticed that during Autumn the highest ratio of after Chemical treatment (DAF)/ after biological treatment (Effluent) 4.2. These results are matching with (**Alsulaili, A.et al.,2020**) Average values of physicochemical parameters in the effluent O&G, (mg/L) parameter during different seasons (Summer Winter Spring and Autumn) are (0.99, 1.56, 1.18 and 1.0) respectively.

In this study the Variations of Settleable solids of industrial wastewater after biological treatment (Effluent) for both tests Settleable solids (10 min, ml /l) and (30 min, ml /l) were ranged from 0.10 to 0.37, 0.17 to 0.80 respectively. These results match with (**de Oliveira Ramos, R.et al., 2022**) the monitoring of total suspended (TSS) and settleable (SetS) solids in wastewater is essential to maintain the quality parameters.

In this study the log number of **Total Bacterial Count by Plate Method Technique at 37 ^{o}C ± 1 for 2 days** in industrial wastewater in two locations the first location from aeration tank (SBR)after chemical treatment and the second location from effluent tank after biological treatment. The Highest value was recorded in sequencing batch reactor tank (SBR) (Aeration tank) after chemical treatment and this stage is considered the reactions phase of bacterial biological treatment. Bacteriological quality of water is usually controlled by certain parameters like bacterial density in terms of plate count at 22 ^{o}C and 37 ^{o}C (**Sabae ,1999**). This result is matched with (**Elahcene et al. 2019**) who found TVBC count at 37°C from raw water (Ain Zada Dam) was 40 CFU/mL. This result is matched with (**Tariq, M. et al. ,2020**) The microbiological results of wastewater samples collected from different discharge points of industries of Kala Shah Kaku are higher levels of total viable count (TVC) load was observed.

In this study the log number of bacterial count (**Bacteriological Examination by Membrane Filtration Protocol using R2A agar plates**) at 35 ± 0.5 ^{o}C in industrial wastewater in two locations the first location from aeration tank

(SBR)after chemical treatment and the second location from effluent tank after biological treatment. The Highest value was recorded in sequencing batch reactor tank (SBR) (Aeration tank) after chemical treatment and this stage is considered the reactions phase of bacterial biological treatment. These results match with (**Ghayeni, S. S.et al. ,1999**) total count results, removal of bacteria was 100% with all membranes when assessed by culture able counts, i.e. numbers of bacterial colonies recovered on R2A agar plates. These results match with (**Vergine, P. et al. ,2017**) For the enumeration of bacteria.

In this study the identity of P. Putida isolates was confirmed using VITEK 2 identification cards for bacteria (VITEK® 2 GN analyzer, modified version 2018). The biochemical profile of the isolates matched accurately with 98% similarity. These results matched with (**Vélez, J. M. B et al. ,2021**) Identification of the isolates of Pseudomonas spp. using the VITEK® 2.

In this study the separate growth of P. Putida inoculated into 250-mL flasks containing 200 mL working volume of wastewater from Aeration tank as a test and another control sample without organism inoculation, then check the effectiveness of biological treatment physically and chemically using COD test. These results match the (**Mujtaba, G et al., 2017**) Effectiveness of P. Putida using Experimental flasks.

5. Conclusion

Generally, the physicochemical and microbial parameters in the wastewater retain better performance in spring and autumn than summer and winter. The examined wastewater samples were subjected to both biological and chemical treatment. It is recommended the use of biological and chemical treatment and using the normal flora P. Putida, will clarify the water to acceptable levels and degrade the organic matter of the wastewater. The lowest count of P. Putida was recorded in the effluent tank after finishing the biological treatment, this mean that degradation of organic matter took place after the chemical treatment, and the bacterial count decreased in the effluent after the finalizing the wastewater treatment and depleting the organic matter content.

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