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Microbiological and Physicochemical Characteristics of an Activated Sludge System Treating Textile Wastewater

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Abstract:

The activated sludge system is biological treatment, which is an intensive treatment and generally quite efficient, but each treatment plant has particular physicochemical and microbiological characteristics that depend on the volume, water type and degree of pollution of the water to be treated. The purpose of this study, therefore, was to determine the microbiological (coliforms, Pseudomonas and free-living amoebae) and physicochemical (BOD_5, COD, total nitrogen, total phosphorus, suspended solids and fats and oils) characteristics of the wastewater from an activated sludge system treating textile wastewater. *Pseudomonas aeruginosa*, total and fecal coliform and the physicochemical parameters were analyzed according to standardized methods (APHA-AWWA-WEF, 1998). Free-living amoebae were determination by culture on NNE medium. The removal percentage of organic matter (BOD_5) was high, reaching 95.5%, followed by COD and total nitrogen with 89.2% and 73.8%, respectively; in contrast, the removal percentages for total phosphorus and suspended solids were relatively low. Total and fecal coliform removal was low with average values below 50%. The *Pseudomonas aeruginosa* bacterium was found on intake and in the sedimentation tank of the treatment system. Free-living amoebae were found in the treatment system throughout the study period; of the 10 amoeba genera found, 6 were detected at both intake and discharge. The presence of microorganisms in the treatment system was important findings due to the characteristics of the wastewater from the textile plant. The activated sludge treatment system is shown to be efficient in the removal of organic matter, but not in the elimination of microorganisms. The physicochemical quality of the treated water complied with the Official Mexican Standard; however, the microbiological quality did not meet the standard.

Keywords: Activated sludge, wastewater, microorganisms, physicochemical parameters

1.0 Introduction:

Water is essential for man's activities, but the irrational and intensive use of this resource has caused it to be polluted, constituting a serious problem worldwide. Wastewater treatment is fundamental to help resolve this issue. There are a great variety of treatments including biological treatments whose aim is to eliminate organic material and pathogenic organisms. These systems are based on the action of microorganisms to decompose organic matter, the most effective method being aerobic, and therefore an oxygen supply is essential to achieve complete mineralization of the organic matter. One biological treatment is the activated sludge method, which is an intensive treatment and generally quite efficient, but each treatment plant has particular physicochemical and microbiological characteristics that depend on the volume, water type and degree of pollution of the water to be treated (Ramahlo, 2003; Russell, 2006). The activated sludge system uses a variety of mechanisms and processes to aerate the wastewater so that the microorganisms can avail of the dissolved oxygen and break down the organic matter. The organisms form a complex community in the activated sludge which is suspended in the water so that when the biologically activated sludge comes in contact with the wastewater the organic materials are oxidized, the particulate matter and colloids tend to coagulate and form a precipitate which settles at the bottom of the tank. This process, in which microorganisms consume the substrate for their own benefit, eliminates the organic matter in the wastewater and transforms it into new cells and innocuous gases that are emitted into the atmosphere, cleaning the water of this contaminant (Tchobanoglous *et al*., 2003).

The activated sludge system is used in the treatment of both domestic and industrial wastewater. This last includes the textile industry which consumes vast amounts of water and energy, generating a huge quantity of wastewater with a variety of chemical products. As a result, its effluent contains high concentrations of organic matter, dyes, refractory organic pollutants, toxic compounds, inhibitory components, surfactants and chlorinated components making it one of the most difficult effluents to treat (Van der Zee and Villaverde, 2005). Wastewater has a large number of microorganisms that help to break down organic matter, but there are also pathogenic microorganisms that can cause illness in humans; the water must therefore be treated before being discharged into the environment to avoid health problems. These microorganisms belong mainly to the following taxonomic groups: bacteria, viruses, fungi and protozoa. Pathogenic microorganisms identified in wastewater are very diverse and are mainly Enterovirus, pathogenic bacteria such as *Salmonella typhi,* which produces typhoid fever; *Salmonella paratyphi,* paratyphoid fever; *Shigella* spp, bacillary dysentery; *Vibrio cholerae,* cholera; *E. coli,* an enteropathogen; *Yersenia enterocolitica* and *Campylobacter jejuni,* gastroenteritis; *Legionella pneumophila,* acute respiratory disease; *Mycobacterium tuberculosis* tuberculosis and *Leptospira*, which causes leptospirosis (Bitton, 2005; Cabral, 2010).

Due to the complexity of identifying each one of the pathogenic bacteria, pollution indicators are used which solely by their presence indicate that fecal contamination has occurred and the possible existence of pathogenic bacteria. The bacteriological indicators of fecal contamination are total coliform and fecal coliform groups (Lisle *et al*., 2004; Madigan *et al*., 2009). However, these indicators only represent one group of pathogenic microorganisms limited to enteric bacteria; other bacteria must also be taken into account, such as *Pseudomonas aeruginosa,* which causes several types of infections including conjunctivitis, and other microorganisms like pathogenic free-living amoebae, protozoa which cause serious infections to the central nervous system and the eye (Visvesvara *et al*., 2007). The purpose of this study, therefore, was to determine the microbiological (coliforms, Pseudomonas and free-living amoebae) and physicochemical (BOD_5, COD, total nitrogen, total phosphorus, suspended solids and fats and oils) characteristics of the wastewater from an activated sludge treatment system.

2.0 Materials and Methods:

2.1 Sampling

The activated sludge system we studied is located in the State of Mexico, Mexico and treats wastewater from a textile industry producing wool fabrics. The system consists of an activated sludge reactor; a sedimentation tank and sand filter that help to clarify the treated wastewater and so to improve the water quality before discharge. Bimonthly samples were taken from November 2011 to October 2012 on intake (raw wastewater), in the sedimentation tank and on discharge (treated wastewater). Two samples were taken for physicochemical analysis, one in three-liter containers and another in one-liter containers, the latter being fixed with sulphuric acid. The samples for the bacteriological analyses (total coliforms, fecal coliforms and the genus Pseudomonas) were taken in 250 mL sterilized containers. The samples for the free-living amoebae were collected in 500 mL sterilized containers. Some physicochemical parameters (pH, temperature and dissolved oxygen) of the wastewater were measured *in situ*.

2.2 Laboratory

Total coliform and fecal coliform bacteria and the physicochemical parameters biochemical oxygen demand (BOD_5), chemical oxygen demand (COD), total nitrogen, total phosphorus, suspended solids and grease and oil (determined only on discharge) were analyzed according to standardized methods (Table 1) (APHA-AWWA-WEF, 1998). The ANOVA analysis was conducted to detect variations in these parameters in the treatment system.

Table 1. Methods used for the determination of physicochemical parameters.

Parameter	Method
Fecal coliforms	NMP
Total coliforms	NMP
BOD_5	Dilution
COD	Dichromate reflux
Suspended solids	Total nonfiltrable residue dried at 103-105^{0}C
Total phosphorus	Stannous chloride
Total nitrogen	Kjeldahl
Grease and oil	Soxhlet

For *Pseudomonas* the sample was seeded in Asparagine broth and incubated at 37°C, the turbidity of the culture medium and fluorescence observed with UV light was taken as positive proof of growth. The positive tubes were reseeded in Acetamide broth, turbidity or alkalization of the culture medium being positive proof (APHA-AWWA-WEF, 1998). Biochemical oxidase, catalase, citrate, indole and TSI tests were done to confirm genus and species. For the determination of free-living amoebae, an aliquot of 50 mL of each sample was taken and centrifuged at 1200 g for 15 minutes, the supernatant was discarded and the sediment seeded onto non-nutritive agar with *Enterobacter aerogenes* (NNE). The plates were incubated at 30°C and observed daily under an inverted microscope for 14 days to detect amebic growth. The amoebae were identified through their morphological features according to Page (1988).

3.0 Results and Discussion:

The results of physicochemical parameters are showed in the Figures a, b, c, d and e, and the bacteriological parameters in the Figures f and g.

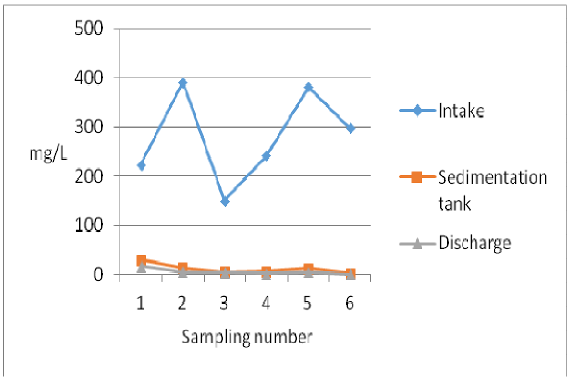

Figure a. Values of the BOD_5

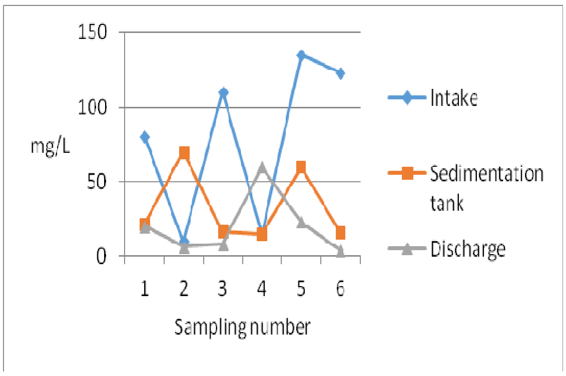

Figure b. Values of Suspended solids

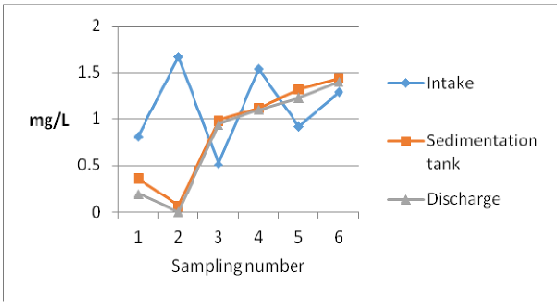

Figure c. Values of total phosphorus

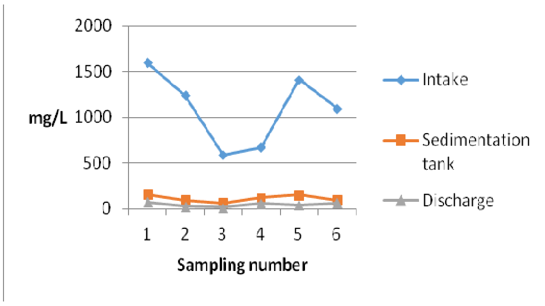

Figure d. Values of COD

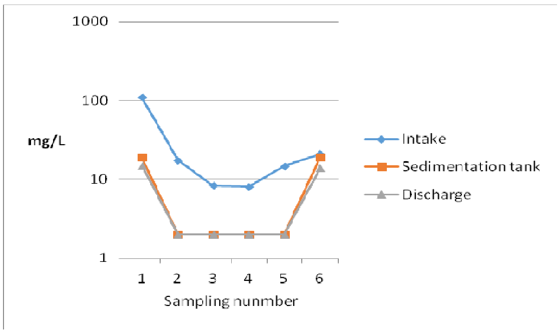

Figure e. Values of total nitrogen

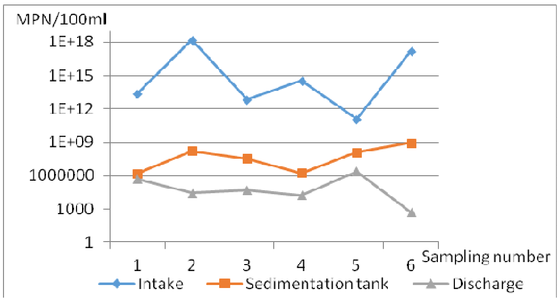

Figure f. Values of Total coliform

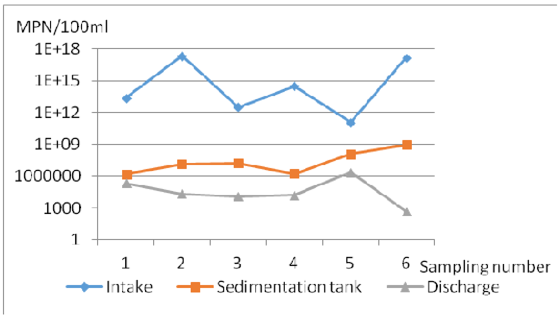

Figure g. Values of Fecal coliform

The average, maximum and minimum values of the bacteriological and the physicochemical parameters are given in Table 2, which shows that the pH of the water acidified slightly towards discharge; the temperature decreased and dissolved oxygen increased. The parameters in the intake showed different values, probably due to changes in processes.

Table 2. Average, maximum and minimum values of physicochemical and bacteriological parameters.

Parameter		Intake	Sedimentation tank	Discharge
BOD_5 mg/L	Average	281	11.6	5.88
	Max. val.	391	30	16.9
	Min. val.	150	< 2	1.19
pH	Average	7.1	6.1	5.8
	Max. val.	8.1	6.6	6.8
	Min. val.	6.1	5.6	3.8
Temp. ^0C	Average	33.2	29.6	27.5
	Max. val.	37	32	31
	Min. val.	32	27	25
OD mg/L	Average	2.8	3.3	5.2
	Max. val.	5.5	5	7.8
	Min. val.	0.2	1.7	2.4
COD mg/L	Average	1102	113	44.2
	Max. val.	1600	158	70
	Min. val.	588	63	14.7
Suspended solids mg/L	Average	78.8	33.2	20.3
	Max. val.	135	70	60
	Min. val.	10	15	4
Total phosphorus mg/L	Average	1.13	0.883	0.809
	Max. val.	1.67	1.44	1.39
	Min. val.	0.516	0.069	0
Total coliforms NMP/100 ml	Average	8.0E+17	204383333	498917
	Max. val.	1.6E+18	900000000	2400000
	Min. val.	1.2E+11	1600000	500
Fecal coliforms NMP/100 ml	Average	1.1E+17	177383333	443750
	Max. val.	2.2E+17	900000000	2400000
	Min. value	1.2E+11	1600000	500

Table 3. Average removal percentages of physicochemical parameters and coliform bacteria.

Parameter	Sedimentation Tank	Discharge
BOD_5[1]	95.5	97.6
COD[1]	89.2	95.7
Total nitrogen[1] (Kjeldahl)	73.8	81.8
Total phosphorus[1]	59.5	68.2
Suspended solids[1]	60.1	76.1
Fecal coliforms	47.7	66.4
Total coliforms	46.9	65.7

The removal percentage of organic matter (BOD_5) from wastewater in the water treatment system was high, reaching 95.5%, followed by COD and total nitrogen with 89.2% and 73.8%, respectively. In contrast, the removal percentages for total phosphorus and suspended solids were relatively low. An increased removal of the parameters was observed in the treated wastewater (on discharge) after passing through the filter. Total and fecal coliform removal was low with average values below 50% and increased removal was also observed in the discharge after filtering (Table 3).

The ANOVA analysis showed significant differences ($p < 0.05$) between the different stages of the process in BOD^5, COD, total coliforms and fecal coliforms, but the analysis showed no significant differences ($p < 0.05$) in these parameters through time. The *Pseudomonas aeruginosa* bacterium was found on intake and in the sedimentation tank of the treatment system in June and August 2012; while other species of *Pseudomonas* were found in other months. Free-living amoebae were found in the treatment system throughout the study period. Of the 10 amoeba genera found, 6 were detected at both intake and discharge; the rest were found at either intake or discharge only (Table 4). Amoebae of the *Acanthamoeba* (26%) and *Hartmannella* (24%) genera were those most frequently isolated; *Echinamoeba*, *Naegleria*, *Saccamoeba*, *Thecamoeba* and *Vexillifera* were isolated less frequently (1 to 5%).

Table 4: Free-living amoebae isolated from the activated sludge treatment system.

	Intake	Discharge
Acanthamoeba	+	+
Echinamoeba	+	+
Hartmannella	+	+
Naegleria	+	-
Platyamoeba	+	+
Saccamoeba	-	+
Thecamoeba	+	-
Vahlkampfia	+	+
Vannella	+	+
Vexillifera	-	+

Table 5 shows that the values of BOD_5, fats and oils of the treated wastewater were below the limits permitted by the Official Mexican Standard (NOM-003-ECOL-1997), while suspended solids were slightly above the permitted limit for use in public services with direct contact, although still permitted for use in services with indirect or occasional contact. Fecal coliform values were well above the limits for use in services with both direct contact and indirect contact.

The treatment system proved to be efficient in the removal of organic matter and total nitrogen, but not in the removal of suspended solids and phosphorus. Neither was it efficient in the removal of coliforms. This may be due to fluctuations in the functioning of the system due to variations in the quality and quantity of wastewater produced in the plant (Lisle *et* al., 2004; Vilanova *et al*., 2004). The parameters presented substantial variations between the different stages of the treatment system due to the characteristics of each one. On intake, the raw wastewater is still untreated; the activated sludge tank is where organic matter is broken down by microorganisms and in the sedimentation tank the suspended solids fall as sediment to the bottom helping to eliminate them from the wastewater (Tchobanoglous *et al*., 2003). Meanwhile, no seasonal variance was observed since the functioning of the system depends on variations in the processes used in this kind of industry and not on ambient temperature. Physicochemical parameters fell below the values permitted by the Official Mexican Standard for use in public services with direct and indirect contact, although fecal coliforms far exceeded the permissible value of the standard, so care must be taken in the use of treated water.

Table 5. Comparison of average values of the physicochemical and bacteriological parameters with the Official Mexican Standard (NOM-003-ECOL-1997)

Parameter	Average value	MPL[1] Direct contact	MPL[1] Indirect or occasional contact
BOD (mg/L)	5.88	20	30
Suspended solids (mg/L)	20.3	20	30
Fats and oils (mg/L)	Mld[2]	15	15
Fecal coliforms (MPN/100 ml)	498917[3]	240	1000

1: Maximum permissible levels for public service; 2: Below the detection limit of the technique (5 mg/L); 3: Geometric mean.

The presence of *Pseudomonas* aeruginosa in the treated wastewater can be explained due to the presence of a dense layer of polysaccharides, which establishes a barrier not only physics but also chemistry can protect the bacteria from the environmental conditions (Wolf- Rainer; 2011; Marchand, 2013). Free-living amoebae are widely distributed in nature and have been reported from different environs (John y Howard, 1995; Ettinger *et al*., 2003; Sheehan *et al*., 2003; Tsvetkova *et al*., 2004; Visvesvara *et al*., 2007; Badirzadeh *et al*., 2011; Kao *et al*., 2012; Al-Herrawy *et al*., 2013), including in man-made environments (Rivera *et al*., 1993; Górnik and Kuzna-Grygiel, 2004; Ramirez *et al*., 2005; Ramirez *et al*., 2010; Stockman *et al*., 2011; Tanveer *et* al., 2013;), but the presence of these amoebae and the diversity found in the treatment system was an important finding, since wastewater from the textile plant contains a variety of toxic chemical compounds used in dyeing and finishing processes. The factors that determined the presence of amoebae in the system were their capacity to form cysts (Barret and Alexander, 1977), which enables them to withstand adverse conditions; the availability of food; an average temperature between 27°C and 33°C; the presence of dissolved oxygen in average concentrations above 2 mg/L, and close to neutral pH (Visvesvara *et al*., 2007). Amoebae of the *Acanthamoeba* and *Hartmannella* genera were present constantly in the treatment system (including discharge) and presented frequently. Some species of *Acanthamoeba* have been reported as pathogenic and can cause infections of the central nervous system and the eye. Although *Hartmannella* has not been reported as pathogenic, is has been found in relation to these infections. Besides amoebae are carriers of pathogenic bacteria and niches for their survival (Visvesvara *et al*., 2007; Ramirez et al., 2010).

4.0 Conclusions:

The activated sludge treatment system is shown to be efficient in the removal of organic matter, but not in the elimination of microorganisms. The physicochemical quality of the treated water complied with the Official Mexican Standard; however, the microbiological quality did not meet the standard since coliforms were above permitted levels and in some months the *Pseudomonas aeruginosa* bacteria was found as well as amoebae of the *Acanthamoeba* genus in treated wastewater. In light of the above, it is recommended that water from the treatment system be disinfected before use to avoid health risks.

5.0 Acknowledgments:

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