Microbiological and Physicochemical Characteristics of a Pilot Plant of Activated Sludge

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Abstract*—The aim of the study was to determine the effectiveness of a pilot plant to remove microorganisms and organic material from domestic wastewater using activated sludge. The bacteria and physicochemical parameters were analyzed according to standardized methods and the amoebae by culturing onto selective media. After the biological treatment there was a 54.8% removal of total coliforms and 60.9% of fecal coliforms; after chlorination the average removal was over 80%. Free-living amoebae presented regularly in the treatment system throughout the study period, decreasing from the influent to the effluent, with the highest number of isolations in the biological reactor. Thirteen genera of amoebae were isolated, the most frequent being *Vermamoeba* and *Vannella* each with 21%, followed by *Acanthamoeba* with 18%. In general, the system presented good efficiency in the removal of FLA. The activated sludge system showed high percentages of organic material removal (BOD$_5$ 99.3%; COD 97%; total nitrogen 98.9% and total suspended solids 96.7%) which confirms the good removal of organic material that is obtained in this kind of system.This electronic document is a "live" template and already defines the components of your paper [title, text, heads, etc.] in its style sheet.

Keywords— Activated sludge; microorganisms; organic matter

I. INTRODUCTION

Mexican cities underwent tremendous urban development and growth during the 20th century, mainly due to the expansion of industrial activities and services and the growing migration of the population into cities, causing them to grow rapidly. This led to the population being concentrated in small areas that require large quantities of water, in turn generating large volumes of wastewater. Mexican cities are facing increasing water supply problems, particularly in low-income populations, in terms of water quality and the continuity of the service. Sewage and sanitation services also face significant challenges, such as wastewater treatment, thus it is important to promote the treatment of wastewater at all population levels [1]. Mexico has 2342 municipal wastewater treatment plants operating formally and it is estimated that treatment has reached an average of 47.5% of all wastewater collected. However, the coverage of wastewater treatment is not evenly distributed throughout the country; there are states where coverage reaches more than 75% and others with much lower treatment rates [2].

Wastewater treatment is quite complicated due to the variety and quantity of pollutants and depends on the characteristics of the water to be treated and the required quality of the effluent. The pollutants contained in wastewater can be eliminated by physical, chemical or biological methods or a combination of these [3, 4]. The most commonly used method as far as treatment plants in Mexico are stabilization ponds, of which there are 732 (31.2% of the total), followed by activated sludge with 698 plants (29.8%). These figures change when the amount of flow treated is analyzed, in which case the most important system becomes activated sludge which treats 46% of collected wastewater, pushing stabilization ponds into second place with 16% [2]. Beside, Mexico has a program to promote the reuse of treated wastewater in those activities where it is a viable and feasible option, such as for agricultural use, irrigation of green areas, industrial cooling processes, and municipal cleaning and secondary services. This would help to stop the over-exploitation of surface and groundwater [1].

The activated sludge biological system is one of the most used treatments due to its intensive operation and because it removes up to 90% of organic material. The system is based on the action of microorganisms to decompose organic material in domestic and industrial wastewater, although in the latter, its composition will depend on the industry of origin. [3, 4].

In the activated sludge system, microorganisms are thoroughly mixed with the organic material of the wastewater to become the food substrate. The mechanical stirring has a double function: 1) thorough mixing, and 2) adding oxygen to the medium so that the process is carried out; under these conditions the microorganisms multiply rapidly oxygenating the different types of organic material in the wastewater and thus breaking it down in the biological treatment. The microorganisms constitute what is called mud or biological sludge; the main microorganisms found in biological treatments include bacteria, protozoa, fungi, algae, rotifers, nematodes and small lower invertebrates. The biochemical activity of the bacteria enables them to metabolize the majority of the organic compounds in the wastewater. An important characteristic of some bacteria is their ability to flocculate; the resulting floc is an aggregate of bacteria and organic and inorganic particles. The formation of this floc causes the sludge to settle better producing a clearer, better quality effluent [5].

The protozoa include the free-living amoebae (FLA) that inhabit environments such as soil and water, although they are also found in the air, which they use as a means of dispersion [6]. They perform a very important role in aquatic environments by maintaining energy flow and recycling nutrients. Their efficient use of resources makes them a crucial link between disintegrator organisms and those belonging to higher trophic levels [7]. Some FLA species can cause disease in humans [8].

The efficiency of domestic and industrial wastewater treatment using activated sludge depends on a number of factors, among them the microbiological and physicochemical characteristics of the influent, as well as the operating parameters of the treatment plant. Although the activated sludge system is capable of providing adequate treatment for different types of wastewater, problems have been reported concerning microbial stability and load [3, 4]. Therefore, the aim of the study was to determine the efficiency of removing microorganisms and organic material of a pilot domestic wastewater treatment plant treating domestic wastewater.

II. METHODS

A. *Study Zone*

The pilot treatment plant under study uses the activated sludge method to treat domestic wastewater from a housing development located in State of Mexico, Mexico. The treatment system consists of influent (I), biological reactor (BR), settling tank (ST), filters (F) (as tertiary treatment) and effluent (E). The sludge system operates by batches of domestic wastewater of 20 m^3/day and the effluent is used to irrigate the green areas of the housing development.

B. *Field Work*

Eleven monthly samples were taken from February to November, 2013 (M1-M11). The samples were taken from the following points in the treatment plant; influent, biological reactor, settling tank, filters and effluent. The following physicochemical parameters were measured *in situ*: pH with HANNA Instruments HI 8314 potentiometer, dissolved oxygen and temperature with the YSI Model 51-b oximeter. The physicochemical determinations were made by taking two samples at each site in 1.5 L bottles, acidifying one of them and transporting both on ice to the laboratory. Sterile 300 ml vials were used to collect total and fecal coliforms which were transported on ice to the laboratory. Amoebae were determined by taking 500 ml samples in sterile containers which were kept at room temperature until processing.

C. *Laboratory Work*

The following physicochemical parameters were determined in the laboratory: chemical oxygen demand (COD), biochemical oxygen demand (BOD_5), total phosphorus (P), total nitrogen (N), nitrates (NO_3) and suspended solids (SS) according to standard methods. The microbiological parameters: total coliforms (TC) and fecal coliforms (FC) were determined by the MPN technique [9]. Free-living amoebae (FLA) were analyzed by culture; samples were thoroughly stirred and 50 ml aliquots taken and centrifuged at 1200 g for 15 min. The sediment was inoculated onto non-nutritive agar (NNE) culture medium with *Enterobacter aerogenes.* The cultures were incubated at 30°C and 37°C and checked daily for 10 days with an inverted microscope to detect the growth of amoebae. The amoebae were identified by observing the morphological characteristics of the trophozoite and cyst with a phase contrast microscope at 40X and 100X [10].

III. RESULTS AND DISCUSSION

A. *Microbiological conditions*

Total and fecal coliforms presented fluctuations in the influent throughout the study period, the highest values being in M6 and M7 (June and July) (*Fig. 1* and *Fig. 2*). Following the activated sludge system, an average removal of 54.8% was observed for total coliforms and 60.9% for fecal coliforms, which increased slightly on passing through the tertiary treatment (filters) obtaining 56.2% for total coliforms and 63.1% for fecal coliforms. After chlorination, the reduction of bacteria is notable, reaching an average removal of 83.8% for total coliforms and 88.1% for fecal coliforms. In general, fecal coliforms in the effluent reached values permissible by Mexican standard NOM-003-ECOL-1997 for treated wastewater both for direct (240) and indirect (1000) use, with the exception of M9 (September) which exceeded the limit for direct contact use, probably due to deficient chlorination in that month (Table 1) [11].

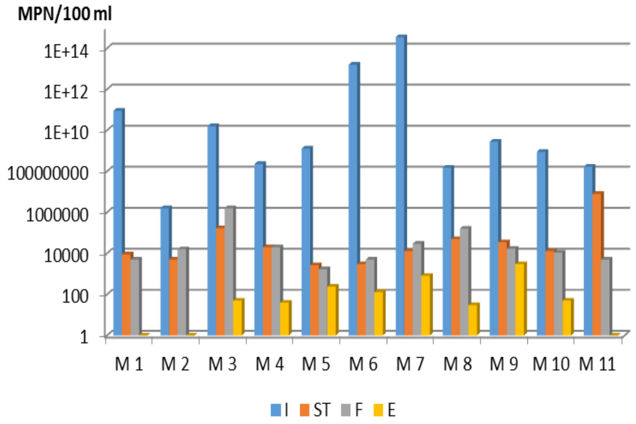

Fig 1. Total Coliforms in different plant processes wastewater treatment.

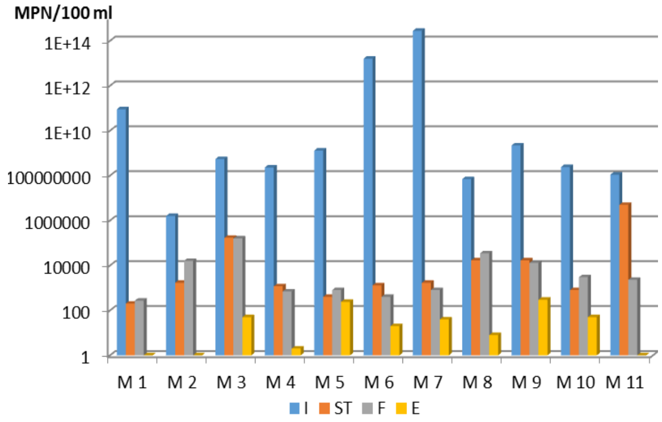

Fig. 2. Fecal Coliforms in different plant processes wastewater treatment.

TABLE I. GEOMETRIC MEANS OF TOTAL AND FECAL COLIFORMS

		Total Coliforms	**Fecal Coliforms**
Influent	Geometric mean	$3.8 x10^{9}$	2.7×10^{9}
	Min.	1.6×10^{6}	1.6×10^{6}
	Max.	3.5×10^{14}	2.8×10^{14}
Sedimentation Tank	Geometric mean	2.5×10^{4}	5.1×10^{3}
	Min.	2700	200
	Max.	8×10^{6}	5×10^{6}
Filters	Geometric mean	1.8×10^{4}	3.2×10^{3}
	Min.	1700	270
	Max.	1.6×10^{6}	1.6×10^{5}
Effluent	Geometric mean	20.1	7.1
	Min.	30	0
	Max.	3000	300

Min: minimum, Max: Maximum

Free-living amoebae presented regularly in the treatment system throughout the study period. Their presence was constant in the influent and in the biological reactor, variable in the settling tank and filter and sporadic in the effluent. Of the 13 genera of amoebae isolated, *Acanthamoeba*, *Korotnevella*, *Naegleria*, *Vahlkampfia*, *Vannella* and *Vermamoeba* were isolated at two incubation temperatures (Table 2). The most frequently found genera were *Vermamoeba* and *Vannella* with 21% each, followed by *Acanthamoeba* with 18%; in contrast, 3 genera presented with the lowest frequency of 0.5% (*Deuteramoeba*, *Guttulinopsis* and *Mayorella* (*Fig. 3*).

TABLE II. FREE-LIVING AMOEBAE ISOLATED FROM TREATMENT SYSTEM

Genus	Incubation temperature	
	30 °C	37 °C
Acanthamoeba	X	X
Deuteramoeba		X
Guttulinopsis		X
Hartmannella		X
Korotnevella	X	X
Mayorella		X
Naegleria	X	X
Platyamoeba		X
Vahlkampfia	X	X
Vannella	X	X
Vermamoeba	X	X
Vexillifera	X	
Thecamoeba	X	

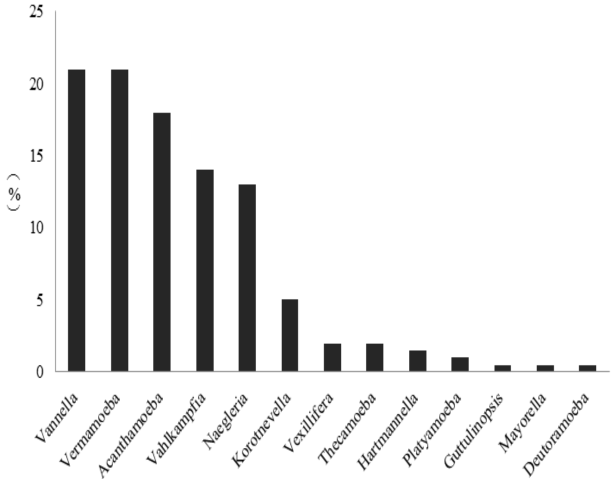

Fig. 3. Frequency of FLA occurrence in the treatment system.

In general, *Vermamoeba* had been reported with high frequency in water with a low concentration of organic material, such as surface and groundwater [12, 13], therefore the fact that it was found with high frequency in this study suggests that it can also withstand high concentrations of organic material. This amoeba has been associated with some eye infections and a brain infection, but its role as causing these infections has not been verified ([14, 15]. Meanwhile, *Vannella* has been isolated in a patient with keratitis [16].

The high frequency with which amoebae from *Acanthamoeba* genus was found is not unusual, since it has been reported in water with high concentrations of organic material from diverse environments [17-20]. The ability of this genus to be found in a variety of environments is due to the resistance of its cyst, whose main characteristic is that it is formed by a double wall: an outer layer (exocyst) composed of phosphoproteins, and an inner layer (endocyst) composed of cellulose [10]. Some species of genus *Acanthamoeba* can cause infection of the central nervous system, lung, skin, eyes and ears [8].

A reduction of amoebae was observed from the influent to the effluent of the treatment plant, with the highest number of isolations in the biological reactor (*Fig. 4*). This may be because this stage of the treatment is where the degradation of the organic material in domestic wastewater takes place and therefore the bacteria (which are food for the amoebae) proliferate [3-5]. Amoebae of genera *Acanthamoeba* and *Naegleria* were isolated in the effluent of the treatment plant and, although only in one month, special attention must be paid to chlorination and the future use of the treated water, since species belonging to these genera have been reported to cause diseases in humans [8]. A higher number of amoebae isolations presented from June to September (M6-M9) (*Fig. 5*); this behavior is similar to the seasonal pattern known for these protozoa in natural waters, where the highest growth occurs in the warmer months [7].

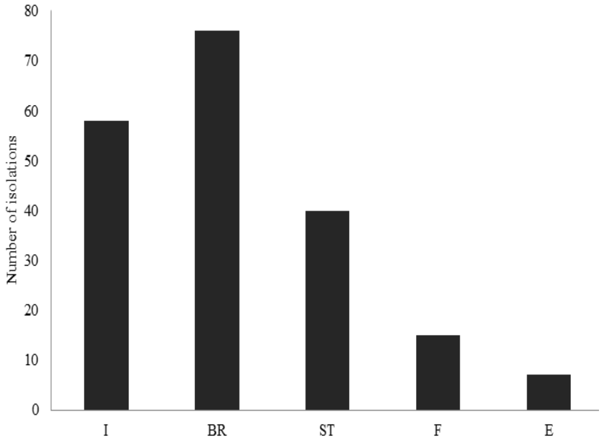

Fig. 4. Spatial distribution of FLA in the treatment system.

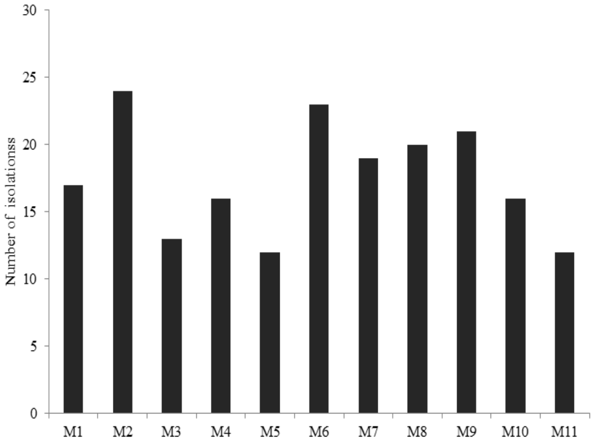

Fig. 5. Seasonal distribution of FLA throughout the period of sampling.

B. *Physicochemical conditions*

The physicochemical parameters DQO, DBO_5, suspended solids and total phosphorus in general reflect a decrease of the values found in the influent with respect to other points in the treatment system, as a result of the degradation of organic material that is carried out in the biological reactor. There was a high concentration of total nitrogen in the effluent, which was oxidized into nitrates, thus decreasing total nitrogen and increasing nitrates in the settling tank. There was good removal of suspended solids after biological treatment, with further removal in the filters (Table 3).

The activated sludge system showed high removal percentages of organic material ($BOD_{5:}$ 99.3%; COD: 97%; total nitrogen: 98.9%, and total suspended solids: 96.7%), these values being higher than those reported. Only 37.7% of total phosphorus was removed, which can be normal in this type of treatment system [3, 4].

The literature suggests a ratio of C-N-P = 100- 5-1 for the good degradation of organic material by microorganisms in domestic wastewater [3, 4, 21]; in this study we found a ratio of C-N-P = 100-13-2 in the influent, which indicates that in the treatment system studied there are more nutrients than necessary, causing high concentrations of phosphorus in the effluent and therefore less removal of this nutrient.

TABLE III. VALUES OF THE PHYSICOCHEMICAL PARAMETERS

		I	**ST**	**F**	**E**
pH	Mean	7.2	7.4	7.1	7.5
	Max.	8.3	7.9	7.7	8.5
	Min.	5.6	7.1	6.3	6.9
BOD (mg/L)	Mean	408	2.88	2.55	< 2
	Max.	888	8.6	8.3	5.78
	Min.	165	< 2	< 2	< 2
COD (mg/L)	Mean	1253	36	25.4	20.7
	Max.	3700	48	38	30
	Min.	595	25	18	6
Total Nitrogen (mg/L)	Mean	52.75	0.567	0.415	0.314
	Max.	104	0.896	1.43	0.812
	Min.	17.5	0.238	0.106	0.053
Nitrate (mg/L)	Mean	0.673	9.31	5.8	5.39
	Max.	2.26	21.1	19.97	10.3
	Min.	0.078	2.23	0.22	0.82
Total Phosphorus (mg/L)	Mean	8.52	5.3	5.6	5
	Max.	14.34	12.45	18.96	11.95
	Min.	5.3	0.774	0.43	0
Total Suspended Solids (mg/L)	Mean	303	10.1	5.42	8.75
	Max.	78 8	18	11	22
	Min.	95	2	1	1

Max: Maximum, Min: Minimum

IV. CONCLUSIONS

In general, a reduction of amoebae was observed from the influent to the effluent of the treatment plant, which indicates good efficiency in the removal of these microorganisms. However, the presence of amoebae of genera *Acanthamoeba* and *Vermamoeba* in the effluent, although on only one occasion, is a warning not to neglect tertiary treatment and the disinfection given to treated wastewater.

The concentrations of total and fecal coliforms and the physicochemical parameters in the influent were variable throughout the study period; however, this did not affect the efficiency of the biological process in decreasing those parameters.

The average values of fecal coliforms, DBO_5 and suspended solids in the effluent were below the maximum permissible limit for treated wastewater according to Mexican standard NOM-003-ECOL-1997, which confirms the good removal of organic material obtained with this type of system. The treatment system studied was suitable for the management of domestic wastewater from small communities.

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