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Full Length Research Paper

Occurrence of Gymnamoebae and physico-chemical parameters in an industrial wastewater treatment system

Elizabeth Ramirez[1*], David P. Ibarra[1], Esperanza S. Robles[1], Maria G. Sainz[1], Maria E. Martinez[1], Reynaldo Ayala[1], Alex Correa-Metrio[2], Victor M. Rivera-Aguilar[3]

[1]Project of Environ Improvement and Conservation. UIICSE. FES Iztacala. National Autonomous University of Mexico

[2]Paleontology Department. Geology Institute. National Autonomous University of Mexico.

[3]Microbiology Laboratory. UBIPRO. FES Iztacala. National Autonomous University of Mexico.

*Corresponding Author's: Email: erf@unam.mx

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The activated sludge system is one of the processes most utilized for both domestic and industrial wastewater treatment. The treatment is carried out by different microbial associations, primarily bacterial, but there are also others, such as gymnamoebae or naked amoebae, which act as regulators of the bacterial populations in the treatment systems. In addition, some gymnamoebae can cause human diseases or be vectors of other pathogens. However, information about the species found in—particularly industrial—wastewater treatment systems is scarce. This work, therefore, aims to determine the occurrence of gymnamoebae and the physico-chemical parameters in an industrial wastewater treatment system. Species belonging to 15 genera of gymnamoebae were found in the treatment system, the genera *Acanthamoeba*, *Vannella* and *Mayorella* being the most represented. Some amoeba species were found throughout the treatment system, while others were found only in one or two zones.

Keywords: Gymnamoebae, activated sludge, textile wastewater

INTRODUCTION

The textile industry uses a complex production chain to make its products; fibres undergo different processes. Wastewater from the two main processes: dying and finishing, typically contains dye residue and a variety of chemicals. The chemical techniques used are determined by the production requirements of the industry, thus generating variations in the concentration of chemicals in wastewater and therefore in the quality of the treated water. The dying process is performed at high temperatures (up to 100°C) to help fix the color, and the wastewater is then emptied into a tank for the temperature to cool. The finishing process consists of washing, rinsing and pressing to make the fabric ready for sale (Dos Santos et al., 2007; Forgacsa et al., 2004).

The use of great amounts of water and diverse chemical compounds in the textile industry produce a wastewater highly pollutant and therefore difficult to treat. For these reason a variety of physicochemical and biological treatments have been used (Ong et al., 2010; Tjasa et al., 2008; Dos Santos et al., 2007; Van der Zee and Villaverde, 2005; Forgacsa et al., 2004; Lin and Peng, 1996).

One of the most important biological treatments, and applied to all types of water, is activated sludge or mud,

Figure 1. Location of the textile industry and the activated sludge treatment plant.

developed in England in the late 20th century (Bitton, 2005). In this treatment, purification is brought about mainly by bacterial populations, which degrade the pollutant organic matter using the principle of bioflocculation and bacterial metabolism. Bioflocculation is the aggregation of organic compounds in colloidal state with specific structures, dimensions and weights called floccule, which constitute the nutritional source for communities of microorganisms, of which 95% are bacteria responsible for the degradation process, and 5% are protozoa and metazoa (Di Marzio, 2004; Vilaseca, 2001). For the biological processes to work depends on the correct operation of an activated sludge plant, through the regulation of physico-chemical parameters which are present in such systems, and which may vary (Bitton, 2005).

The 5% protozoa contain a highly important functional group called gymnamoebae or naked amoebae, which are single-celled eukaryotes of undefined form that emit different cytoplasmic projections called pseudopods (composed of both ectoplasm and endoplasm) used for locomotion and food capture. Gymnamoebae intervene in the regulation of bacterial populations, helping to maintain trophic networks and recirculating nutrients in the system to ensure proper functioning (Bonilla et al., 2004; Finlay and Esteban, 1998); others are a health problem that may cause disease or be vectors of bacteria or virus (Visvesvara, 2014; Thomas et al., 2010; Greub and Raoult, 2004).

Despite the importance of the gymnamoebae in the wastewater treatment systems, few studies have been performed, which have focused mainly on domestic wastewater (Muchesa et al., 2014; Garcia et al., 2011; Ramirez et al., 2015, 2005, 1993; Rivera et al., 1993). This work, therefore, aims to determine the occurrence of Gymnamoebae and the physico-chemical parameters in an industrial wastewater treatment system.

METHODS

Study Zone

The activated sludge treatment system where this research was conducted is located in a wool textile industry in the municipality of Cuautitlán Izcalli in the northeast part of the State of Mexico, coordinates 19°40’50”N and 99°12’25”W (Figure 1). The climate is typified as temperate subhumid with summer rains, mean humidity of 30.6% on the surface and temperate subhumid with summer rains of lower humidity C (w0) of 69.4%, with an average temperature typical of temperate subhumid climate, ranging from 27.8°C maximum to 5°C minimum. The mean annual temperature is 16°C.

Description of the treatment plant

Wastewater from the dying processes is collected together in a tank and, where necessary, stabilized at pH 7. The wastewater is then taken to the activated sludge plant for treatment. The treatment plant begins with two sieves to remove large solids, followed by a homogenizer tank to mix the chemical compounds and remove sedimentary particles. The water then passes to an aeration tank where the organic matter is decomposed by the microbial consortia. A secondary settler then separates the water

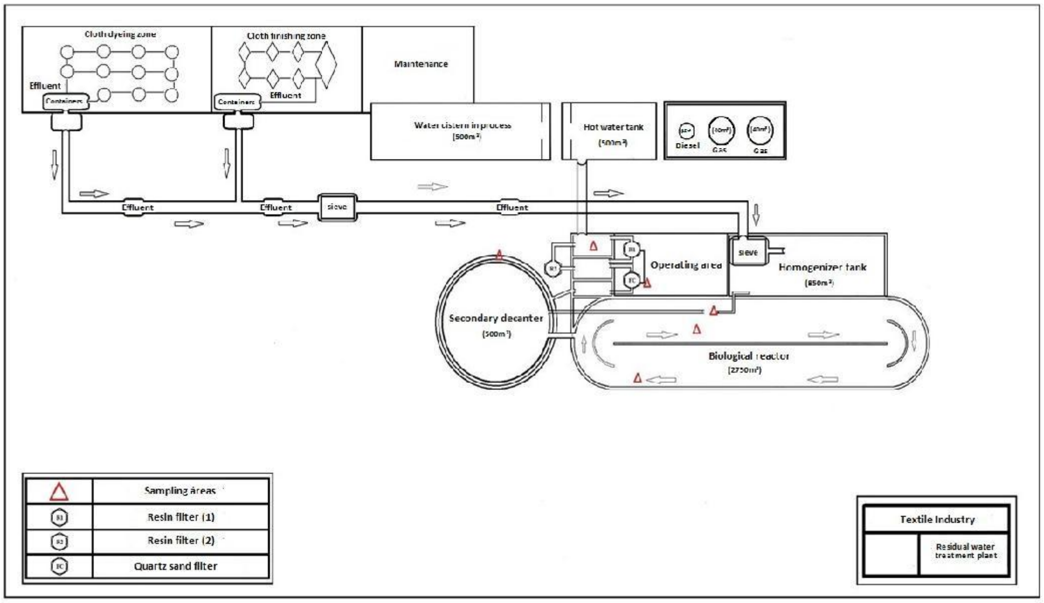

Figure 2. Diagram of the treatment plant of activated sludge.

from the activated sludge formed in the aeration tank by sedimentation; part of the biomass is recycled to the aeration tank to maintain the microorganism populations. After being treated in the activated sludge system, the water then passes through a quartz sand filter and two resin filters (Figure 2).

Sample collection

Eleven monthly samples were taken during one year. The samples to determine gymnamoebae were collected in 1000 ml plastic containers previously sterilized from different zones of the treatment system: input (IP), aeration tank (AT), sedimentation tank (ST), filters (F) and output (OP). The samples were kept at room temperature until analysis.

The pH (with a field potentiometer), temperature (T) and dissolved oxygen (DO) (with a YS1 oxymeter, model 51B) were measured *in situ.* The samples for the physico-chemical parameters were taken in 2000 ml containers from each zone of the treatment system and kept under refrigeration until analysis.

Culture and identification of gymnamoebae

The wastewater samples collected from each zone of the treatment system were inoculated onto non-nutrient agar seeded with *Enterobacter aerogenes (NNE).* The cultures were incubated at 30°C and observed daily for 14 days with an inverted microscope to detect the growth of gymnamoebae.

The morphological identification of the amoebae consisted of observing fresh preparations using phase contrast microscopy at 40 x and 100 x, observing the characteristics of the phases of their life cycle (Page, 1988).

Physico-chemical parameters

The following physico-chemical parameters were analyzed: biochemical oxygen demand (BOD) by the dilution method; nitrates (NO_3) by the brucine method; nitrites (NO_2) by the diazotization method; conductivity (C); total phosphates (TP) by the stannous chloride method, suspended solids (SusS) and sedimentation solids (SedS) by gravimetric methods (APHA-AWWA-WEF, 2012).

Statistical analysis

A maximum distance cluster analysis was used to establish the fluctuation of variables and group the zones of the treatment system in terms of similarities between the species and physico-chemical parameters. This method also helped to determine the existence of any batch production or if there were any differences in the water treated during the sampling period. The above mentioned analyses were performed using free R software (R project, version 3.0.2 updated 2013-09-25) standardizing the measurement scales for correct application, using the Vegan, Cluster and Biodiversity R statistics packages.

Table 1. Gymnamoeba isolated from the activated sludge treatment of the textile industry (ADL, et al., 2012).

Supergroup	Class	Order	Family	Genus	Species
AMOEBOZOA	Flabellinea	Dactylopodida	Vexilliferidae	Vexillifera	*Vexillifera bacillipedes*
			Paramoebidae	Korotnevella	*Korotnevella stella*
				Mayorella	*Mayorella cultura*
					Mayorella penardi
					Mayorella
		Thecamoebida	Thecamoebidae	Thecamoeba	*Thecamoeba similis*
					Thecamoeba striata
		Himatismenida	Cochliopodiidae	Cochliopodium	*Cochliopodium minus*
		Vannellida	Vannellidae	Vannella	*Vannella lata*
					Vannella platypodia
					Vannella simplex
	Variosea	Acanthopodida	Acanthamoebidae	Acanthamoeba	*Acanthamoeba astronyxis*
					Acanthamoeba hatchetti,
					Acanthamoeba polyphaga
		Varipodida	Filamoebidae	Filamoeba	*Filamoeba nolandi*
	Tubulinea	Euamoebida	Hartmannellidae	Hartmannella	*Hartmannella cantabrigiensis*
				Saccamoeba	*Saccamoeba stagnicola*
		Echinamoebida	Echinamoebidae	Vermamoeba	*Vermamoeba vermiformis*
				Echinamoeba	*Echinamoeba silvestris*
EXCAVATA	Heterolobosea	Acrasida	Guttulinopsidae	Rusculus	*Rosculus ithacus*
		Schizopyrenida	Vahlkampfiidae	Naegleria	*Naegleria sp*
				Vahlkampfia	*Vahlkampfia avara*
				Paravahlkampfia	*Paravahlkampfia ustiana*

RESULTS AND DISCUSSION

Gymnamoebae

Twenty three species of gymnamoebae were found belonging to 15 genera, *Acanthamoeba*, *Vannella* and *Mayorella* having the most species. According to Adl et al. (2012), amoebae belonged to the super groups Amoebozoa and Excavata, Amoebozoa being the most represented with the highest number of species (Table 1).

The amoebae found in this textile wastewater treatment system have been reported in domestic wastewater treatment systems (Ramirez et al., 2015, 2005, 1993; Rivera et al., 1993), which is associated with the high concentrations of organic matter that increase the bacterial populations and other microbial populations that serve as a food source for the amoebae (Loret et al., 2010; Thomas et al., 2010; Ramirez et al., 2005; Ramirez et al., 1993). The presence of gymnamoebae in different conditions is an indication of their adaptability to different environmental conditions, resisting the chemical pollutants in the textile wastewater.

Of all the species found, *Acanthamoeba hatchetti* (Ah), *Acanthamoeba polyphaga* (Ap), *Korotnevella stella* (Ks), *Vermamoeba vermiformis* (Vv), *Mayorella cultura* (Mc), *Vannella platypodia* (Vp) and *Vexillifera bacillipedes* (Vb), were broadly distributed, being found in 5 zones of the system. *Cochliopodium minus* (Cm), *Acanthamoeba astronyxis* (Aa), *Rosculus ithacus* (Ri), *Saccamoeba stagnicola* (Ss, *Vannella simplex* (Vs), *Mayorella penardi* (Mp), *Vahkampfia avara* (Va) and *Vannella lata* (Vl) were present in some zones of the system. *Filamoeba nolandi* (F), *Mayorella vespertiloides* (Mv), *Naegleria* sp. (N), *Tecamoeba similis* (Ts), *Tecamoeba striata* (Tst), *Paravahlkampfia ustiana* (Pu), *Echinamoeba silvestris* (Es) and *Hartmannella cantabrigiensis* (Hc) were present only in zones 1 and 2 (Table 2). The distribution of the amoeba species in the different zones of the treatment system suggests that their presence depends on the biotic and abiotic conditions in each zone of the treatment system.

Amoebae of the genera *Mayorella*, *Vannella*, *Vexillifera*, *Korotnevella* and *Thecamoeba* do not present cysts, but are capable of taking full advantage of the food resources

Table 2. Distribution of gymnamoeba species in the treatment system.

Species	Ah	Ap	Ks	Vv	Mc	Vp	Vb	Ss	Aa	Ri	Vs	Cm	Va	Vl	Mp	Hc	Es	F	Pu	Mv	Ts	Tst	N	Total ssp
IP	1	1	1	1	1	1	1	1	1	1	0	0	1	0	0	1	0	1	1	0	0	0	0	14
AT	1	1	1	1	1	1	1	1	1	1	1	0	0	1	1	1	1	0	0	1	1	1	0	19
ST	1	1	1	1	1	1	1	1	1	1	1	1	1	0	0	0	1	0	0	0	0	0	1	15
F	1	1	1	1	1	1	1	0	0	0	1	1	1	0	1	0	0	0	0	0	0	0	0	11
OP	1	1	1	1	1	1	1	0	0	0	1	1	1	1	0	0	0	0	0	0	0	0	0	10

Binary system: presence "1" and absence "0"
IP: Input, AT: Aeration tank, ST: Sedimentation tank, F: Filters: OP: Output
ssp: species

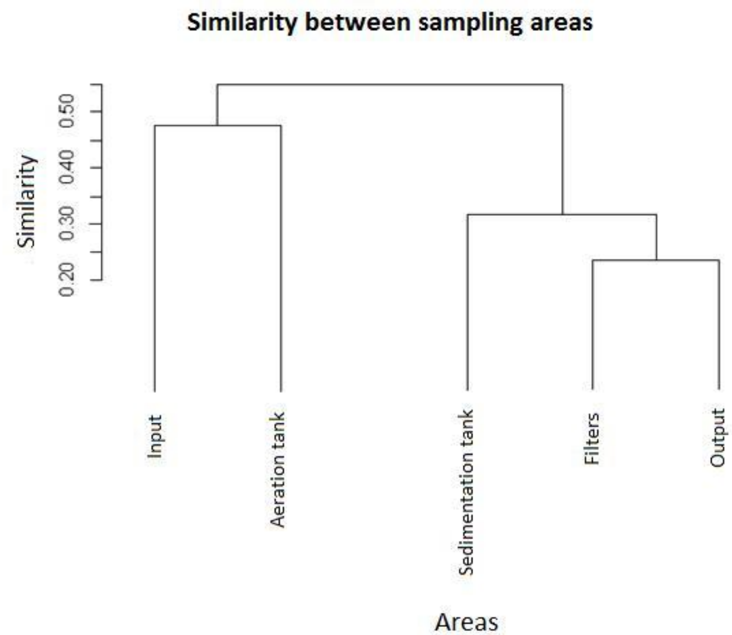

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Figure 3. Similarity of treatment system areas in according of the presence-absence of gymnamoeba species.

in natural environments to increase the number of individuals in their populations, and in the case of *Mayorella*, *Vannella* and *Vexillifera* also may use the strategy of presenting small specimens (Rodriguez-Zaragoza, 1994).

Of the amoeba genera found throughout the system, *Acanthamoeba* has been reported to have a wide distribution in the environment due to the presence of cellulose in its cyst wall, giving it the ability to resist extreme environmental conditions (Visvesvara, 2014; Bonilla et al., 2004). In terms of health, species of *Acanthamoeba* have been reported as pathogens, and they may present a health risk when found in the final stages of the water treatment system. Therefore is important to disinfect the wastewater treated in the system (Ramirez et al., 2015; Loret et al., 2008). In addition, amoebae of the genera *Acanthamoeba*, *Vermamoeba* and *Vannella* have been reported as vectors of bacterial pathogens and certain viruses (Visvesvara, 2014; Thomas et al., 2010; Greub and Raoult, 2004).

The type and number of gymnamoebae species represented in each of the zones of the treatment system enabled the zones to be grouped into two groups: one formed by the input and aeration tank and another formed by the filters and output, this last having a certain relationship with the settling tank (Figure 3). The similarity

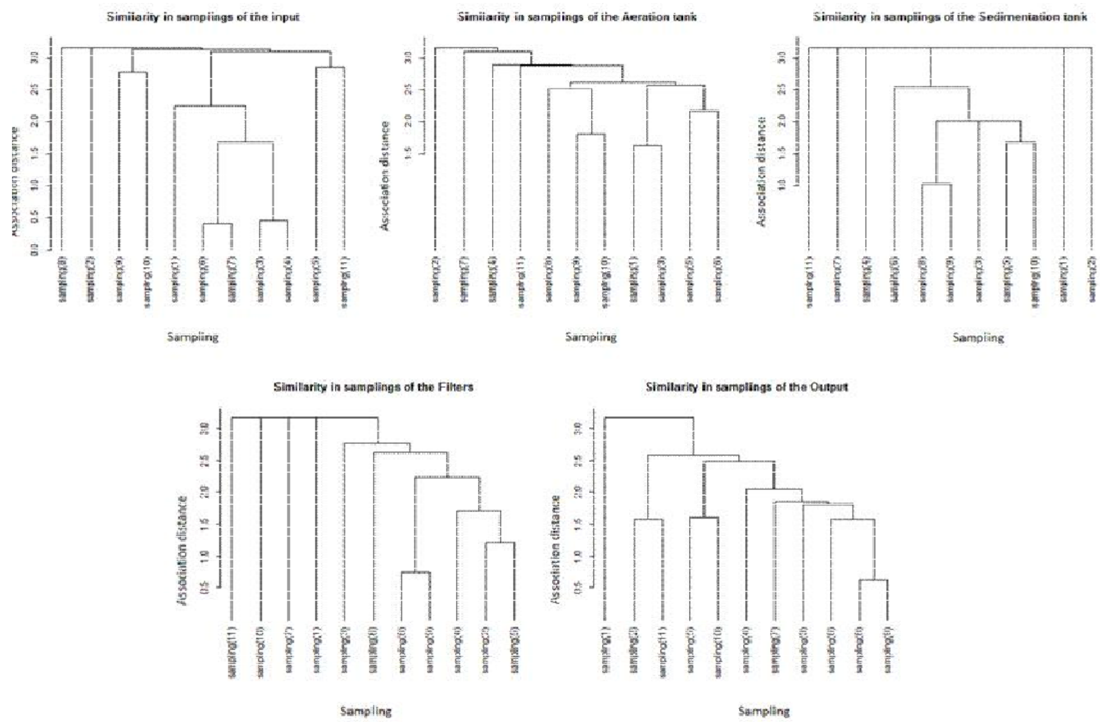

Figure 4. Similarity of sampling in according of the physicochemical parameters

Table 3. Minimum and maximum values of the physicochemical parameters of treatment system.

	pH	T (°C)	DO (mg/L)	BOD (mg/L)	NO_3 (mg/L)	NO_2 (mg/L)	C (µS/cm)	TP (mg/L)	SedS (mg/L)	SusS (mg/L)
IP	5.4-7.6	28-37	1.0-4.0	152-350	0.04-1.84	0.152-0.508	694-1235	0.48-2.13	0.08-1.60	61-129
AT	5.7-6.8	25-33	0.2-1.4	1.9-2.7	1.22-33.89	0.078-0.349	571-1198	0.45-3.0	0.08-0.10	4730-7990
ST	5.7-6.8	25-33	0.3-3.8	1.9-2.7	1.22-33.89	0.078-0.349	571-1198	0 .45-3.0	0.08-0.10	12.50-38
F	6.2-7.4	24-32	1.4-5.0	1.7-12.7	5.29-30.70	0.026-1.43	562-1158	0.13-2.83	0.08-0.20	9.5-44.20
OP	3.8-8.1	22-30	2.2-6.0	1.9-16.7	3.34-33.68	0.062-0.392	558-1238	0.17-2.77	0.10-0.20	6.5-21.50

IP: Input, AT: Aeration tank, ST: Sedimentation tank, F: Filters, OP: Output

between the input and the aeration tank was due to these being the first stages of the treatment system and where the pollutants were found in their highest concentrations; unlike the group formed by the filters and output, which, being the final stages of the treatment, received the water which had already undergone the decomposition process in the aeration tank.

Physico-chemical parameters

The physico-chemical parameters of textile wastewater are dependent on the production requirements of the factory, therefore the physico-chemical parameters measured showed variations during the sampling period. Some samples presented similarities between them in the form of clusters (Figure 4). At input, samplings 9 and 10; 6 and 7; 3 and 4; 5 and 11, showed similarities between them, suggesting that in each of these sampling pairs chemical substances were used in similar concentrations and therefore the physico-chemical parameters were similar. Meanwhile, samplings 8 and 2 did not belong to any cluster, suggesting that the physico-chemical conditions were different from the rest of the samplings. The similarities between the samplings that presented in the remaining zones of the system also depended on the processes that take place in each one.

The average values of the physico-chemical parameters are shown in Table 3. In general, biochemical oxygen

Table 4. Average values of physicochemical parameters of input and output.

Parameter	Input	Output
pH	6.7	6.8
T (°C)	33.4	22
DO (mg/l)	2.0	4.6
BOD(mg/L)	263	5.1
NO_3 (mg/L)	0.84	13.88
NO_2 (mg/L)	0.27	0.11
C (µS/cm)	999.4	978
TP (mg/L)	1.44	1.60
SedS (mg/L).	0.38	0.09
SusS (mg/L)	99.3	13.6

demand, sedimentation solids and suspended solids were observed to decrease from the input to the output of the system. Average pH values were close to neutrality, and dissolved oxygen and nitrates values increased. The temperature at input was above 30°C, but decreased on the way through the treatment system, reaching 22°C at output. Conductivity values were high, indicating a large concentration of salts, due to the salts utilized in the dying and finishing processes performed in the factory and to the high temperature at which these processes are carried out, which enhances the solubility of the salts in water. Solids represent the biological and non-biological matter found in the wastewater, which decreased from input to output following biological treatment and filtration (99.3 to 13.6 mg/l) (Table 4).

The efficiency of the treatment system in relation to the physico-chemical parameters was high. The amount of organic matter decreased as follows: BOD_5 by 90%, nitrates by 60%, sedimentation solids by 70% and suspended solids by 80%.

CONCLUSIONS

The diversity and frequency of gymnamoebae in the treatment system indicates the adaptability of these amoebae to different environmental conditions, especially the genera that presented throughout the system.

The textile industry treatment system was efficient since it removed up to 90% of pollutants; however, the purification process is affected by the lack of control in the production processes of the textile company, which is reflected in the variation in the physico-chemical parameters.

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