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Distribution of free-living amoebae in a treatment system of textile industrial wastewater

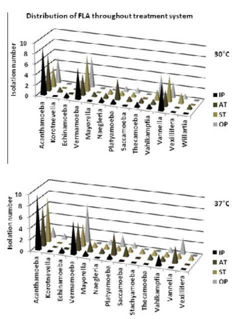
Elizabeth Ramirez *, Esperanza Robles, Blanca Martinez, Reynaldo Ayala, Guadalupe Sainz, Maria Elena Martinez, Maria Elena Gonzalez

Environmental Conservation and Improvement Project, FES Iztacala, National Autonomous University of Mexico, Mexico

HIGHLIGHTS

- Amoebae were present in all stages of the system throughout the study period.
- Cyst and non cyst-forming amoebae were isolated from textile wastewater.
- *Acanthamoeba* and *Vermamoeba* were the amoebae most frequent in the system.
- Studies are needed to understand the adaptation of the amoebae to textile wastewater.

GRAPHICAL ABSTRACT



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ABSTRACT

Free-living amoebae have been found in soil, air and a variety of aquatic environments, but few studies have been conducted on industrial wastewater and none on wastewater from the textile industry. The aim of this study was to determine the presence and distribution of free-living amoebae in a biological treatment system that treats textile industrial wastewater. Samples were taken from input, aeration tank, sedimentation tank and output. Samples were centrifuged at 1200*g* for 15 min, the sediment was seeded on non-nutritive agar with *Enterobacter aerogenes* (NNE) and the plates were incubated at 30 and 37 °C. Free-living amoebae were present in all stages of the treatment system. The highest number of amoebic isolates was found in the aeration tank and no seasonal distribution was observed during the year. A total of 14 amoeba genera were isolated: *Acanthamoeba*, *Echinamoeba*, *Korotnevella*, *Mayorella*, *Naegleria*, *Platyamoeba*, *Saccamoeba*, *Stachyamoeba*, *Thecamoeba*, *Vahlkampfia*, *Vannella*, *Vermamoeba*, *Vexillifera* and *Willaertia*. The most frequently found amoebae were *Acanthamoeba* and *Vermamoeba* which were found in all treatment system stages. The constant presence and diversity of free-living amoebae in the treatment system were important findings due to the characteristics of the wastewater from the textile plant in terms of the residue content from colorants, fixers, carriers, surfactants, etc., used in fabric dyeing and finishing processes. The factors that determined the presence and distribution of amoebae in the activated sludge system were their capacity to form cysts, which allowed them to resist adverse conditions; food availability; an average temperature of 27–33 °C; dissolved oxygen in average concentrations above 2 mg/L, and pH in a range of 5.9–7.1.

1. Introduction

Free-living amoebae have a life cycle consisting of two stages: trophozoite and cyst. The first is the active feeding and reproductive

* Corresponding author. Address: Avenida de los Barrios 1, Los Reyes Iztacala, Tlalnepantla, 54090 Mexico State, Mexico.
E-mail address: erf@unam.mx (E. Ramirez).

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stage, the second a dormant stage. This plasticity allows them to be widely distributed in nature, occupying various habitats throughout the world (Rodriguez-Zaragoza, 1994; Visvesvara et al., 2007). They have been found in soil (Lorenzo-Morales et al., 2005; Sawyer, 1989; Tsvetkova et al., 2004), air (Rivera et al., 1994), air-conditioners (Ithoi et al., 2011; Tanveer et al., 2013) and a variety of aquatic environments including freshwater lakes and ponds, rivers, swimming pools, hydrotherapy tubs, tap water, hot springs, natural thermal water, irrigation channels, artificial lakes, hot effluent from power plants, sea water, bottled mineral water, rain water, groundwater and domestic wastewater (Al-Herrawy et al., 2013; Badirzadeh et al., 2011; Behets et al., 2007; Ettinger et al., 2003; Górnik and Kuzna-Grygiel, 2004; Ithoi et al., 2011; John and Howard, 1995; Kao et al., 2012; Kuiper et al., 2006; Kyle and Noblet, 1987; Moussa et al., 2013; Pérez-Uz et al., 2010; Ramirez et al., 2010, 2006, 2005; Rivera et al., 1993, 1989; Sheehan et al., 2003; Stockman et al., 2011; Tanveer et al., 2013; Tsvetkova et al., 2004; Vesaluoma et al., 1995). However, few studies have been conducted on industrial wastewater and none on wastewater from the textile industry; this water is typically characterized by the presence of residues from colorants and chemicals used in the dyeing and finishing of the fabrics. The treatment of this wastewater is an environmental problem, since many colorants and textile additives are toxic; physical, chemical, and biological treatment processes can be applied to remove wastewater dyes. Biological processes have been considered as effective alternatives for the treatment of colored effluent; one of these processes is activated sludge (Van der Zee and Villaverde, 2005).

Activated sludge is a suspended-growth process consisting essentially of an aerobic treatment that oxidizes organic matter, where oxygen is provided by mechanical aeration. The microbial cells form flocs (sludge), containing a wide range of prokaryotic and eukaryotic microorganisms, that are allowed to settle in a clarification tank (Bitton, 2005).

Although it is well known that some free-living amoebae can survive in extreme conditions, the question arises as to whether they could resist the prevailing conditions in wastewater from the textile industry. The aim of this study was to determine the presence and distribution of free-living amoebae in a biological treatment system that treats textile industrial wastewater.

2. Materials and methods

2.1. Study site

The treatment system in this study is located in the State of Mexico, Mexico and treats wastewater from a textile industry that manufactures woolen fabrics for men's suits. There are two main processes in this industry: dyeing and finishing, which require a pH between 4 and 7; different chemical substances are used in these two processes, such as organic dyes (azo and anthraquinone), fixers (sodium acetate, formic acid, oxalic acid), carriers (aromatic compounds), surfactants, sodium hydroxide, ammonium, hydrogen peroxide, tetrasodium phosphate, ammonium and sodium sulfate and sodium sulfite.

The treatment system is an activated sludge-type biological process, where the degradation of the organic matter in the wastewater is carried out by microorganisms. The system consists of an aeration tank, where microbial flocs (sludge) are formed, which contains bacteria, protozoa, fungi, and inorganic and organic particles. An important characteristic of the activated sludge process is the recycling of a portion of the biomass; this practice helps maintain a large number of microorganisms that effectively oxidize organic compounds in a relatively short time. After aeration, the wastewater passes to a tank used for the sedimentation of microbial flocs produced during the oxidation phase.

2.2. Sampling

Bimonthly samples were taken from the wastewater treatment system from November 2011 to October 2012 from the following points: input (untreated water) (IP), aeration tank (AT), sedimentation tank (ST) and output (treated water) (OP). Five hundred milliliters of wastewater was collected in sterile containers for the analysis of free-living amoebae. The following physicochemical parameters of the wastewater were measured *in situ*: pH, temperature and dissolved oxygen.

2.3. Culture and identification

An aliquot of 50 mL of each sample was taken and centrifuged at 1200*g* for 15 min, the supernatant was discarded and the sediment was seeded on non-nutritive agar with *Enterobacter aerogenes* (NNE). The plates were incubated at 30 and 37 °C and observed daily with an inverted microscope for 14 days to detect amoebic growth. The amoebae were identified by taking into account the growth at different temperatures, their capacity to transform to a flagellated form and the morphological characteristics of the trophozoite and the cyst (Page, 1988).

3. Results

Free-living amoebae were present in all stages of the treatment system throughout the study period. The highest number of amoebic isolates was found in the aeration tank, although there was little difference with the other stages, of 3 or 4 isolates (Fig. 1).

No seasonal distribution was observed during the year; nor was there any significant difference in the number of isolates obtained at the two incubation temperatures (30 and 37 °C) (Fig. 2).

The isolated amoebae belong to the genera *Acanthamoeba* (Group II), *Echinamoeba*, *Korotnevella*, *Mayorella*, *Naegleria*, *Platyamoeba*, *Saccamoeba*, *Stachyamoeba*, *Thecamoeba*, *Vahlkampfia*, *Vannella*, *Vermamoeba*, *Vexillifera* and *Willaertia* (Table 1, Fig. 3).

Of the total isolated amoebae, 12 genera grew at both incubation temperatures, while *Willaertia* grew only at 30 °C and *Stachyamoeba* at 37 °C. The isolated genera present in all stages of the system were *Acanthamoeba*, *Platyamoeba*, *Vahlkampfia*, *Vannella* and *Vermamoeba*, while *Willaertia* and *Stachyamoeba* presented only in the aeration tank and sedimentation tank, respectively (Table 2).

Acanthamoeba and *Vermamoeba* in addition to presenting in all the treatment system stages were also the genera most frequently isolated at the two incubation temperatures, being slightly higher at 37 °C in both cases. In contrast, 9 genera (*Echinamoeba*,

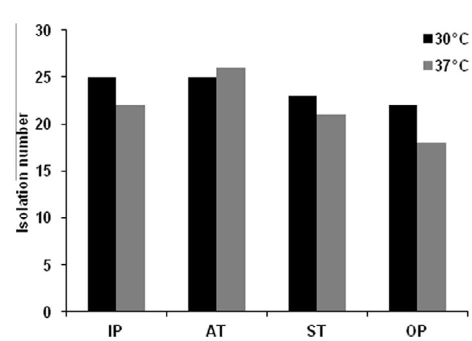

Fig. 1. Spatial distribution of FLA in the treatment system.