THE INFLUENCE OF ENVIRONMENTAL FACTORS ON THE DIEL VARIATION OF PHYTOPLANKTON IN STABILIZATION PONDS

Elizabeth A. Pastich, Silvia M. Barbosa, Lourdinha Florencio, Savia Gavazza and Mario T. Kato

SUMMARY

Phytoplankton in stabilization ponds is essential for oxygen production required for organic matter degradation. However, in such highly eutrophic environments, the potential toxin producer cyanobacteria may be dominant and ecological studies become important for understanding their role. Our objective was to evaluate relevant factors influencing the variation in the day-night cycle within phytoplankton communities, with emphasis on cyanobacteria. The results obtained in two ponds confirm oxygen oversaturation during the day and anoxia at night; under such variation, only a few species with the ability to obtain energy in the dark can survive. Oscillatoria *was the predominant cyanobacterial genus both at the surface and bottom in the two ponds, with a relative abundance >90% at all times. The species are able to ferment in dark anoxic environments and can grow at a similar rate either under light or dark conditions. Therefore, their presence at high concentrations in the ponds is of great concern.*

Introduction

Stabilization ponds are an important alternative system for treating domestic wastewater in developing countries due to their low construction and operation costs and efficient removal of pollutants. Phytoplankton that grows in ponds has a fundamental role in producing oxygen that is required by aerobic processes for organic matter stabilization. The predominance of several phytoplankton species depends on their ability to adapt to existing environmental conditions. Cyanobacteria are commonly found in highly eutrophic environments, such as stabilization ponds, and deserve attention due to their potential to produce toxins (Kotut *et al.*, 2010; Pastich *et al.*, 2016).

In most cyanobacteria, glycogen, which accumulates during the day as a product of photoautotrophic growth, serves as an energy source during the night and is degraded via the oxidative pentose-phosphate pathway (Stal and Moezelaar, 1997). Metabolic energy is generated by respiration with oxygen, which is the terminal electron acceptor. Most cyanobacterial growth occurs during photosynthesis, and only a small number of species can grow as chemoorganotrophs in the dark. The occurrence and survival of cyanobacteria that live in environments that are permanently anoxic or in environments that become anoxic overnight depend on their ability to generate energy in the dark.

The cyanobacteria *Oscillatoria terebriformis* performs oxygenic photosynthesis during the day and migrates to the hypolimnion regions and ferments at night. In the dark, under anoxic conditions, the glycogen formed during the day is slowly degraded. If the dark conditions are aerobic, the glycogen reserves will be rapidly depleted, and the *Oscillatoria* will die (Richardson and Castenholz, 1987). Species subjected to diel variations from light aerobic to dark anoxic environments require the ability to ferment. Without this ability, the cell can die within 2 to 3h after exposure to dark, anoxic conditions (Stal and Moezelaar, 1997). Although few genera of cyanobacteria are able to grow in the "dark (Post *et al.*, 1985), *Oscillatoria* is able to grow at the same rate in both dark and light conditions. It is important to highlight that *Oscillatoria* is considered a potentially toxic genera (Kaebernick and Neilan, 2001) and is usually reported in stabilization ponds (Pastich *et al.*, 2016; Amengual-Morro *et al.*, 2012; Furtado *et al.*, 2009).

During the day, the dissolved oxygen (DO) concentration established via photosynthesis can reach 300% to 400% supersaturation. At night, oxygen is rapidly consumed by aerobic degradation, resulting in anoxic conditions (Kayombo *et al.*, 2002; Tadesse *et al.*, 2004) and allowing several cyanobacteria genera, such as *Oscillatoria*, to dominate. The goal of this work was to study the diel variation of phytoplankton communities and the physicochemical parameters in two stabilization pond systems used to treat domestic sewage in two regions with different weather conditions.

Material and Methods

This study was performed at two full-scale domestic wastewater treatment plants

KEYWORDS / Cyanobacteria / Cyanophyta / Ecology / Maturation Ponds / *Oscillatoria* sp. /

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Elizabeth A. Pastich. B.Sc. in Biology, M.Sc and Ph.D in Environmental Technology, Universidade Federal de Pernambuco (UFPE), Brazil. Professor, UFPE, Brazil.

Silvia M. Barbosa. B.Sc. in Environmental Science and M.Sc. in Environmental Technology, UFPE, Brazil.

Lourdinha Florencio. Civil Engineer, UFPE, Brazil. M.Sc. in Hydraulics and Sanitation, Universidade de São Paulo (USP), Brazil. Ph.D in Environmental Technology, University of Wageningen, The Netherlands. Professor, UFPE, Brazil.

Savia Gavazza. Civil Engineer, Federal University of Alagoas (UFAL). Ph.D in Hydraulics and Sanitation, USP, Brazil. Professor, UFPE, Brazil. Address: Av. Acadêmico Hélio Ramos, s/n, Cidade Universitária, CEP 50670-901, Recife, PE, Brazil. e-mail: savia@ufpe.br

Mario T. Kato. Civil Engineer, Universidade Federal de Paraná, Brazil. M.Sc in Hydraulics and Sanitation, USP, Brazil. Ph.D in Environmental Technology, at University of Wageningen, The Netherlands. Professor, UFPE, Brazil.

INFLUENCIA DE FACTORES AMBIENTALES EN EL CICLO DIURNO DEL FITOPLANCTON EN LAGUNAS DE ESTABILIZACIÓN
Elizabeth A. Pastich, Silvia M. Barbosa, Lourdinha Florencio, Savia Gavazza y Mario T. Kato

RESUMEN

En lagunas de estabilización el fitoplancton es esencial para la producción del oxígeno necesario para degradar la materia orgánica presente en el agua. Sin embargo, en ambientes hipereutróficos con presencia de cianobacterias, la producción de cianotoxinas puede llegar a ser dominante en el ambiente acuático, tornando entonces muy importantes los estudios ecológicos para la comprensión de su función. Nuestro objetivo principal fue evaluar los factores relevantes que influyen en el ciclo diurno de la comunidad fitoplanctónica, con énfasis en las cianobacterias. Los resultados obtenidos en dos lagunas estudiadas confirman que, tanto en la condición diurna de sobresaturación de oxígeno, como en la condición nocturna de anoxia, pocas especies tienen la capacidad de obtener la energía necesaria para garantizar su sobrevivencia. El género de cianobacteria Oscillatoria *predominó tanto en la superficie de las lagunas como en el fondo de las mismas, con una abundancia relativa >90% durante el estudio. Los resultados obtenidos muestran que las especies de ese género son capaces de fermentar en condiciones anóxicas y crecer con tasas similares en condiciones de luz o de oscuridad. En consecuencia, su presencia en alta densidad en lagunas de estabilización es una preocupación válida.*

INFLUÊNCIA DOS FATORES AMBIENTAIS NO CICLO NICTEMERAL DO FITOPLÂNCTON EM LAGOAS DE ESTABILIZAÇÃO
Elizabeth A. Pastich, Silvia M. Barbosa, Lourdinha Florencio, Savia Gavazza e Mario T. Kato

RESUMO

Em lagoas de estabilização o fitoplâncton é essencial para a produção de oxigênio requerido para degradação da matéria orgânica. No entanto, em ambientes hipereutróficos, cianobactérias potencialmente produtoras de cianotoxinas podem se tornar dominantes, então, estudos ecológicos são importantes para a compreensão do seu papel. Nosso objetivo foi avaliar relevantes fatores no ciclo nictemeral que influenciam a comunidade fitoplanctônica, com ênfase nas cianobactérias. Os resultados obtidos nas duas lagoas confirmam que, sob condição de supersaturação de oxigênio durante o dia e a anoxia durante a noite, poucas espécies possuem habilidade de obter energia no escuro para sobreviver. O gênero de cianobactéria Oscillatoria *foi predominante tanto na superfície, quanto no fundo de duas lagoas de estabilização, com abundância relativa maior 90% durante o estudo. Essas espécies são capazes de fermentar em condições anóxicas no escuro e podem crescer com taxa similar em ambas as condições de luz e escuro e por isso, sua presença em elevada densidade em lagoas de estabilização é uma grande preocupação.*

(WWTP) in Pernambuco State, Brazil. The first WWTP is located in the city of Rio Formoso, on the Atlantic coast (08°39'50''S and 35°09'32''W). The system consists of a UASB reactor with an 8h hydraulic retention time (HRT), a polishing pond and, a coarse rock filter to remove suspended solids and phytoplankton. The second WWTP is located in the city of Petrolândia, located in a semiarid region ~600km from Rio Formoso (8°58'28''S and 38°12'52''W). This system consists of a facultative pond and two maturation ponds in series with HRTs of 10, 6, and 6 days, respectively. Rio Formoso has a humid climate with an average annual rainfall of 2788mm; in contrast, Petrolândia has a dry climate with an average annual rainfall of less than 450mm. In this study, the polishing pond and the last maturation pond of the first and second WWTP, respectively, were sampled. Some design and operational characteristics of these two ponds are shown in Table I.

Sampling of the diel variations was conducted over a full day in the Rio Formoso polishing pond, and in the third pond of Petrolândia (maturation pond). Samples were taken from the influent and effluent and at the center of each pond. At the center, a Van Dorn bottle was used to collect samples at 10cm from the water surface and at 40cm from the bottom. The pH, temperature, and DO were measured over a 24h cycle at 2h intervals. Samples for phytoplankton and nutrients were taken every 6h in the pond of Rio Formoso and every 4h in that of Petrolândia. The physicochemical parameters were determined according to standard methods (APHA, 2012). The total ammonia nitrogen ($N\text{-}NH_3$) was estimated based on the procedure of Emerson *et al.* (1975). The identification and classification of the phytoplankton were based on Komarék and Anagnostidis (1986) for Chroococcales; Anagnostidis and Komarék (1988) for Oscillatoriales; and Bourrelly (1972) for Euglenophyta, Cryptophyta and Chlorophyta. Phytoplankton was counted using an inverted microscope, according to the procedure of Utermohl as described by Lund *et al.* (1958).

Results and Discussion

Figure 1 shows the average chemical oxygen demand (COD), $N\text{-}NH_3$ and total phosphorus (in terms of $P\text{-}PO_4^{-2}$) levels in the influent and effluent of the studied ponds. The COD removal efficiency of the Rio Formoso pond was 60% with an effluent COD of $74mg{\cdot}l^{-1}$ (Figure 1a); the efficiency of the Petrolândia pond was greater, ~70%, with an effluent COD of $<50mg{\cdot}l^{-1}$ (Figure 1b). This lower level of COD at Petrolândia pond effluent is probably a consequence of the higher temperatures achieved in that pond (Figure 2f), associated to the fact of being the last pond in the series of three.

The removal efficiency of total ammonia nitrogen (Figures 1c, d) in the ponds was 30 and 20% for Rio Formoso and Petrolândia, respectively. There was no phosphorus removal in either pond

TABLE I
DESIGN AND OPERATIONAL CHARACTERISTICS OF THE TWO SAMPLING PONDS

WWTP characteristics	Rio Formoso (polishing pond)	Petrolândia (maturation pond)
Flowrate ($L.s^{-1}$)	40	16
Depth (m)	1.50	1.45
Hydraulic retention time (d)	8	6

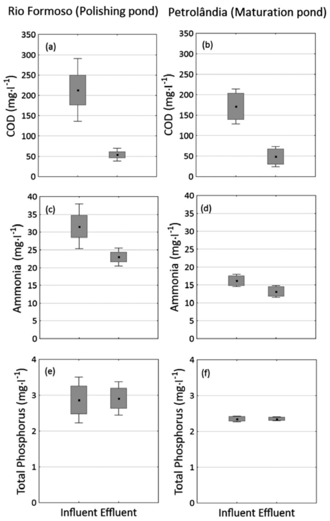

Figure 1. Chemical oxygen demand, total ammonia nitrogen ($N\text{-}NH_3$) and total phosphorus ($P\text{-}PO_4^{-2}$) concentrations in the influent and effluent of the ponds of Rio Formoso (a, c, e) and Petrolândia (b, d, f). Dot: mean, gray box: ±SE (standard error), bar: ±SD (standard deviation).

(Figures 1e, f). This similar behavior in both ponds can be related with the pH levels found; maximum values were 9.1 and 8.7, detected between 14 and 16h at the surface samples of Rio Formoso and Petrolândia ponds, respectively. Phosphorus could have precipitated as struvite crystals if higher pH values would have been found in the pounds (Cavalcanti *et al.*, 2002).

The changes in cumulative solar radiation, DO, water temperature, and $N\text{-}NH_3$ in both ponds during the 24h period are shown in Figure 2. The cumulative solar radiation started to increase after 9h, followed a normal distribution, with maxima around 15 and 17h for Rio Formoso and Petrolândia, respectively (Figures 2a, b). However, the DO concentration at the surface (Figures 2c, d) for both ponds reached a maximum of $22mg{\cdot}l^{-1}$, ~2h prior to the maximum cumulative solar radiation. This result can be explained by the fact that many species of phytoplankton migrate to the deeper layers to avoid high-intensity light (Garcia-Pichel *et al.*, 1994). During the day, the air temperature increases, and the water surface temperatures are higher than those at the bottom, which results in the pond becoming either chemically or thermally stratified. During the night, the air temperature decreases, which cools the surface layer sand eliminates the stratification. Without the photosynthetic activity of the phytoplankton, all of the oxygen produced during the day is consumed by heterotrophic organisms and, the ponds become anoxic.

The phytoplankton taxa found in the ponds are listed in Table II. The phytoplankton community was represented by eight taxa in the Rio Formoso pond and 18 taxa in the Petrolândia pond, which were classified within the Cyanophyta, Chlorophyta, and Euglenophyta divisions. All of the species found have also been reported in previous studies on stabilization ponds (Vasconcelos and Pereira, 2001; Furtado *et al.*, 2009; Kotut *et al.,* 2010). Among the detected cyanobacteria species, three of the taxa have been reported to be toxin producers: *Cylindrospermopsis raciborskii*, *Microcystis* sp. and *Oscillatoria* sp. (Kaebernick and Neilan, 2001). Some authors highlight the risk associated with discharges in water bodies of effluents containing species of cyanobacteria that are potential producers of toxin (Kotut *et al.*, 2010; Vasconcelos and Pereira, 2001). In some cases, the solution adopted for the treatment of wastewaters, aiming environmental protection, can became a public health issue.

The relative abundance of the taxa detected is shown in Figure 3; however, only the taxa present at densities >100 cell/ml are considered. Members of the *Oscillatoria* genus were the most abundant taxa in both ponds for all the samples collected at all times and either at the surface or the bottom of the pond. The average relative abundance was 96 and 95% of the total phytoplankton for the Rio Formoso and Petrolândia ponds, respectively. *Oscillatoria* belong to a cyanobacteria filamentous and colony-forming group that is commonly found in shallow lakes with high turbidity (Scheffer *et al.,*1997) and that is potentially a toxin producer (Kaebernick and Neilan, 2001). According to Post *et al.* (1985), the *Oscillatoria* genus is able to grow under dark

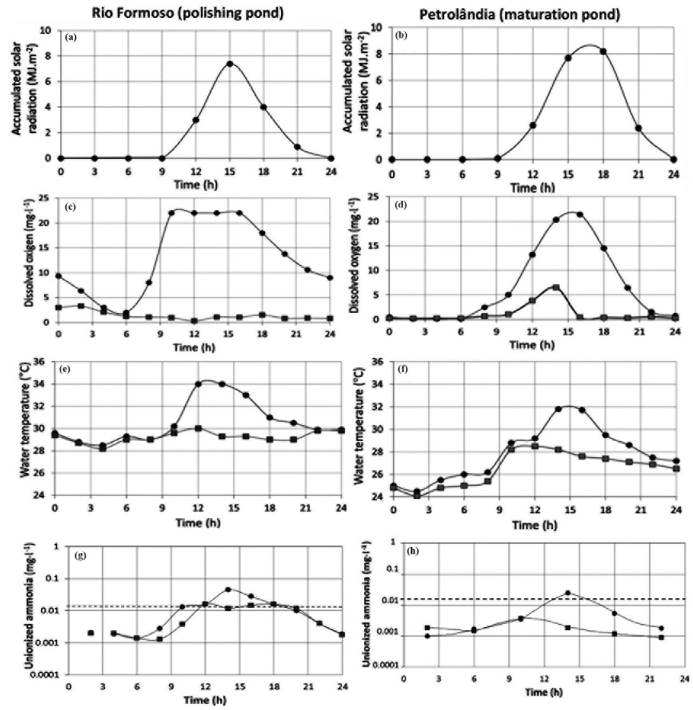

Figure 2. Variations in Rio Formoso and Petrolândia ponds during a 24h period. Accumulated solar radiation (a, b); dissolved oxygen (c, d); water temperature (e, f); total ammonia nitrogen (unionized ammonia) ($N\text{-}NH_3$) (g, h). —●— Surface. —■— Bottom. ······· Toxic limit.

TABLE II
TAXA OBSERVED IN THE PONDS

Division	Taxa	
	Rio Formoso (polishing pond)	Petrolândia (maturation pond)
Cyanophyta	*Oscillatoria* sp. *Microcystis aeruginosa* *Merismopedia tenuissima*	*Oscillatoria limosa* *Microcystis* sp. *Merismopedia tenuissima* *Chroococcus* sp. *Cylindrospermopsis raciborskii* *Limnothrix* sp. *Merismopedia punctata* *Pseudanabaena limnetica* *Raphidiopsis mediterranea* *Synechocystis aquatilis*
Chlorophyta	*Closteriopsis acicularis* *Scenedesmus acuminatu,* *Chlorococcum* sp. *Desmodesmus* sp. *Monoraphidium arcuatum*	*Closteriopsis acicularis* *Scenedesmus* sp. *Ankistrodesmus fusiformis* *Crucigenia fenestrata* *Crucigenia tetrapedia*
Euglenophyta		*Euglena acus* *Phacus longicauda* *Trachelomonas volvocina*

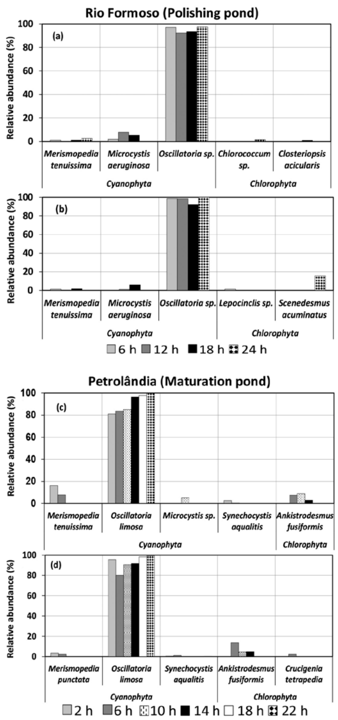

Figure 3. Relative abundance of the identified species in the studied ponds: (a) Rio Formoso at the surface and (b) at the bottom; and (c) Petrolândia at the surface and (d) at the bottom.

anoxic conditions at a rate similar to that under light conditions. This ability provides this genus with an advantageous adaptation, compared with other taxa. Another advantage is the fermentation capacity of certain species, e.g., *Oscillatoria limosa* (Heyer *et al.*,1989), which was identified in the Petrolândia pond. According to Stal and Moezelaar (1997), the species that do not ferment can be subject to cell lysis and will die after 2 to 3h when exposed to a dark anoxic environment, which might be the reason for the strong dominance of the genus *Oscillatoria* observed in the present study.

Conclusions

Variation of environmental conditions, such as water temperature and dissolved oxygen, in the studied ponds, favor an increase of the specific density of cyanobacteria. During the day, when the temperature is greater, the surface of the ponds warms, resulting in thermal stratification of the water column.

A high dissolved oxygen content due to oxygenic photosynthesis is directly related to the accumulated solar radiation. The diel variation in dissolved oxygen, from supersaturation during the day to anoxic conditions during the night, favors the predominance of the cyanobacteria *Oscillatoria* sp., which comprises >90% of the total phytoplankton at both surface and bottom of the water column. This species is able to ferment in the dark to obtain energy.

The high density of cyanobacteria that are potential toxin producers in the effluent of pond treatment systems is of great concern. Cyanobacteria can be a public health threat and an environmental problem, depending on the receiving water body.

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