



Long-term performance and operational strategies of a poultry slaughterhouse waste stabilization pond system in a tropical climate

V. Del Nery^{a,*}, M.H.Z. Damianovic^b, E. Pozzi^b, I.R. de Nardi^c, V.E.A. Caldas^d, E.C. Pires^b

^a Rua Francisco Zavaglia, 405, 13569-590, São Carlos, SP, Brazil

^b Departamento de Hidráulica e Saneamento-EESC-Universidade de São Paulo, Av. Trabalhador São-carlense, 400, 13566-590, São Carlos, SP, Brazil

^c Centro Universitário Central Paulista, Rua Miguel Petroni, 5111, 13563-470, São Carlos, SP, Brazil

^d Instituto de Física de São Carlos-Universidade de São Paulo, Av. Trabalhador São-carlense, 400, 13566-590, São Carlos, SP, Brazil

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ABSTRACT

This paper discusses the performance of a poultry slaughterhouse wastewater treatment plant (WWTP) in a tropical country and operating strategies that were applied over 84 months to this WWTP. The WWTP consisted of a dissolved air flotation (DAF) system, anaerobic ponds (APs) and a facultative pond (FP). The organic loading rates applied to the APs ranged from 0.26 to 1.05 kg BOD/m³ day, and the hydraulic retention time ranged from 2.2 to 3.8 days. The resultant COD and BOD removal efficiencies were $42 \pm 11\%$ and $38 \pm 15\%$, respectively. The FP surface loading was up to 983 kg BOD/ha day. The main observed events in the WWTP processes over this period were organic overload, low chemical oxygen demand (COD) and biochemical oxygen demand (BOD) removal efficiencies and color change from green to reddish at the FP. Molecular biology techniques were used to identify microbial groups in the FP in the period of low organic matter removal efficiency and when the liquid became reddish. Sunny conditions favored anoxygenic photosynthesis, the growth of purple nonsulfur bacteria (such as *Rhodocyclales*-like organisms), and green nonsulfur bacteria (such as *Chloroflexi*-like organisms). Mechanical aeration at the inlet of the FP restored the water to its original color and increased the COD and BOD removal efficiencies. Based on the BOD removal efficiencies of 89%, after the installation of aerators, the operating strategies illustrate the WWTP system's capacity and versatility to adapt to load variations.

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1. Introduction

Waste stabilization ponds (WSPs) are one of the most common wastewater treatment systems used in different climate zones. Environmental conditions such as high temperatures and solar irradiation throughout the year are the main reasons for the extensive WSP use in tropical countries.

First proposed for the treatment of sewage, an anaerobic pond (AP) followed by a facultative pond (FP) is an effective arrangement for removing organic matter in a WSP system (Catunda and van Haandel, 1996). In addition, this arrangement meets the biological treatment requirements for various industrial effluents. These systems are inexpensive, have a simple design, low operating costs, simple operational strategies and are robust under hydraulic or organic shock loads (Mara, 1996; Mayo, 1996). Temperatures above 20 °C make the biological processes in these units highly effective (Yu et al., 1997; Hanqing et al., 1998).

As a primary treatment, APs are some of the cheapest BOD removal devices. Thus, APs should be integrated into WSP systems when possible (Saqqa and Pescod, 1995). However, due to design constraints, the organic loads applied to APs are lower than those in modern high-rate anaerobic reactors.

FP systems are characterized by natural processes of organic matter stabilization. Within these processes, the biochemical and hydrodynamic processes are strongly linked to meteorological factors, such as sun irradiation, wind, temperature, rainfall and evaporation (Racault et al., 1995; Kayombo et al., 2003; Tadesse et al., 2004).

Despite the simple technology, the ecological FP system consists of complex communities of algae, viruses, protozoa, insects, crustaceans and fungi (Kehl et al., 2009). Moreover, the wastewater strength influences the symbiotic activities of bacteria and algae (Kayombo et al., 2002).

Wastewater from agro-industrial processes (slaughterhouses, food canneries, dairies) is easily treated in stabilization pond systems (Mara, 1996; Pescod, 1996) because it contains significant concentrations of biodegradable organic matter.

The poultry slaughter industry (PSI) is one industrial segment that often uses ponds to treat their wastewater in Brazil

* Corresponding author. Tel.: +55 16 33074774.

E-mail address: vdelnery@terra.com.br (V. Del Nery).

(CETESB, 1999). In 2011, Brazilian PSI chicken meat production reached 13.06 million tons, and the quantity exported amounted to 3.94 million tons, ranking Brazil as the world's third largest producer and the world's largest exporter of PSI chicken meat. Poultry slaughterhouse wastewater is characterized by high concentrations of biodegradable organic matter, which primarily consists of lipids, proteins, suspended solids, oil, grease, nitrogen and phosphorus. These components vary from plant to plant, depending on the industrial process and water consumption (Del Nery et al., 2007; Valadão et al., 2011).

Despite the importance of WSPs for treating industrial wastewater in tropical countries, documentation of the specific design parameters and operating performance for these wastewater systems is lacking. This lack of information makes it difficult to evaluate the technologies used. Thus, the focus of this work is to report the performance of a poultry slaughterhouse wastewater treatment plant (WWTP) along with the operating strategies used to maintain its efficiency in removing organic matter under an organic overload. Molecular biology techniques were used to identify microbial groups in the facultative pond in the period of low organic matter removal efficiency when the liquid was a reddish color. In addition, plant upgrades, operational and design strategies (to enhance the plant performance to support the yearly load increase) and a slaughterhouse expansion are presented here.

2. Materials and methods

2.1. Climate description

This study was carried out at the WWTP of the Ideal Ltda poultry slaughterhouse in Pereiras, São Paulo, Brazil. The WWTP is located 500 m above sea level at a latitude of 23°03'45"S and a longitude of 47°56'15"W. The average annual air temperature ranges from 18.2 to 24.9 °C. The average rainfall is 1420 mm/year, and the average evaporation is 1157 mm/year. The average solar radiation at the site is 5 kWh/m² day.

2.2. WWTP description

The original WWTP consisted of rotary screens, a static screen, a diffused-air flotation system, two parallel APs and an FP. The original design was modified following industrial effluent characteristic changes after construction. These changes included the addition of an equalization tank, the replacement of the diffused-air flotation system by a dissolved-air flotation (DAF) system with full-influent pressurization and the installation of mechanical aeration in the first third of the FP (3250 m²). After aeration was introduced (from the 16th month on), the FP became an aerated facultative pond (AFP). Table 1 contains the design parameters for the equalization tank, DAF system, APs and FP. The WWTP simplified layout of the WWTP and photographs of the APs and AFP are shown in Figs. 1 and 2, respectively.

2.3. WWTP monitoring

The WWTP monitoring parameters were evaluated monthly over 84 months of operation. Samples were collected every 30 min during the sampling period (24 h) and composed based on the flow rate. The biochemical oxygen demand (BOD), chemical oxygen demand (COD), oil and grease (O&G), total Kjeldahl nitrogen (TKN) and total phosphate (TP) were analyzed at the Laboratory of Water and Wastewater Examination at the University of São Paulo, São Carlos. Sample preservation and examination were performed according to the *Standard Methods for the Examination of Water and Wastewater* (1998).

Table 1
WWTP units design parameters.

Equalization tank ^a			
Volume (m ³)	50		
Air saturator ^a			
Volume (m ³)	1.63	APs	
Saturation pressure (kPa)	200–400	Number of units	2
A/S ratio (mL air/mL SS)	0.013	Depth (m)	4
		Area (m ²)	275
		Volume (m ³)	1100
Flotation tank			
Depth (m)	4	AFP	
Surface area (m ²)	16	Number of units	1
Volume (m ³)	64	Depth (m)	1.5
Effluent recycling	None	Area (ha)	1.3
Chemical addition	None	Volume (m ³)	19,500
		Aerated area (m ²)	3250
		Aeration (hp) ^b	108

^a Installed in the 27th month of the operating period.

^b Installed from the 16th to the 21st and from the 57th to the 65th month of the operating period.

2.4. Statistical analyses

Statistical analyses were performed with the SigmaStat 3.5 software to compare before and after results as the plant design and operational procedure changed. In addition to descriptive statistics, the following tests were used: normality test (Kolmogorov–Smirnov), mean comparison (Mean–Whitney Rank Sum Test), *t*-test, ANOVA, and linear correlation and regression. The nonparametric tests were used whenever the normality tests failed. Not all results are shown in this work. Considering the measurement and sampling procedures, the data were considered paired. The set of results does not contain missing values.

2.5. Molecular analysis and microscopy

Microbial diversity was assessed using 16S rRNA clone libraries obtained in a specific operating period. In this way, microbial groups present in the FP during times of low organic matter removal efficiencies and that caused the liquid to take on a reddish color were identified. This was an attempt to identify mitigating approaches to improve the efficiency of the system.

The total bacterial DNA from the microbial biomass was extracted with the procedure described by Griffiths et al. (2000). The PCR amplifications were carried out using the universal bacterial primers 27f (5'-AGA GTT TGA TCC TGG CTC AG-3') and 907r (5'-CCG TCA ATT CCT TTG AGT TT-3'), as described by SO and Young (1999). The 16S rRNA fragments were cloned into a plasmid pCR2.1 TOPO-TA easy vector system (Invitrogen®) and transformed into *Escherichia coli* DH5α (as suggested by the manufacturer). Clones were randomly selected from 300 original colonies and were screened for positive inserts with M13 primers following the manufacturer's instructions. In total, 120 randomly chosen positive clones were sequenced in an ABI 377 DNA Sequencer (Perkin–Elmer, Waltham, MA, USA) using M13 primers (forward and reverse, separately). The sequences obtained were aligned and clustered using the Phred/Phrap package (Ewing et al., 1998; Ewing and Green, 1998) and the Cap3 package for the assembly of the “contigs” program (Huang and Madan, 1999). After alignment and detection of the presence of chimeras, the nucleotide sequences were compared with the electronic database of the Ribosomal Database Project (RDP) (Cole et al., 2007) to determine their phylogenetic identity. The phylogeny was estimated by maximum likelihood criterion using the “Satè” (simultaneous alignment and tree reconstruction) (Liu et al., 2009) program. The search was made in the best topology for each group of data. A biological research microscope (Olympus BHTZ, Japan) equipped with phase contrast was used to observe the microbial samples.

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