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Thermophilic treatment of paper machine white water in laboratory-scale membrane bioreactors

Cláudio Arcanjo de Sousa[a], Cláudio Mudado Silva[a,*], Nívea Moreira Vieira[b], Ann Honor Mounteer[c], Mateus Salomé Amaral[a], Marcos Rogério Tótola[b], Willian Gomes Nunes[a]

[a]*Department of Forest Engineering, Federal University of Viçosa, 36570-000, Viçosa, MG, Brazil*
Tel. +31-3899-2717; email: mudado@ufv.br
[b]*Department of Microbiology, Federal University of Viçosa, 36570-000, Viçosa, MG, Brazil*
[c]*Department of Civil Engineering, Federal University of Viçosa, 36570-000, Viçosa, MG, Brazil*

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ABSTRACT

Paper mills consume large quantities of water and consequently produce large volume of effluent. Direct water reuse is not always possible because of poor effluent quality. Membrane biological reactor (MBR) treatment of paper machine white water is a technology that could allow for water reuse. This study examined the technical viability of thermophilic treatment of paper machine effluents (white water) in a MBR. The research was divided into two experiments. The objective of Experiment I was to compare performance of MBR treatment under mesophilic (35°C), thermotolerant (45°C) and thermophilic (55°C) conditions. The results showed that the increase in temperature led to a reduction in COD removal efficiency. No filamentous bacteria were found at 55°C and flocculation was deficient. The objective of Experiment II was to evaluate sludge microbial diversity in aerobic MBRs operating under mesophilic and thermophilic conditions. Microbial community composition and structure was analyzed by polymerase chain reaction–denaturing gel gradient electrophoresis (PCR–DGGE) and FAME–MIDI analyses, respectively. It was found that increased temperature reduced reactor sludge microbial diversity and richness.

Keywords: Microbial diversity; Denaturing gradient gel electrophoresis; Fatty acid methyl ester; Membrane biological reactor; Paper mill effluent; Water reuse

1. Introduction

Paper mills consume large amounts of water and consequently generate large volume of effluent, or white water, whose quality varies according to the paper grade produced, raw material and equipment used, as well as mill environmental management practices. In general, mills consume from 5 to 20 m^3 of fresh water per ton of paper produced, although there are mills where the average fresh water consumption may reach 100 m^3 ton^{-1} of paper produced.

In recent years, much effort has been paid to water system closure in paper mills. This has led to reduced water demands and thus lower effluent discharges and several zero-discharge recycled paper mills operate currently [1,2]. Increasing system closure in paper mills leads to higher process water temperatures and thus the applicability of thermophilic treatment systems becomes increasingly important [3]. Normally paper mills treat their white water in biological processes at mesophilic temperatures (20–40°C) and cooling of the wastewater prior to treatment might be necessary.

It is therefore not surprising that thermophilic biological processes have gained considerable interest in

*Corresponding author.

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recent years for the treatment of high-strength, high-temperature industrial wastewaters [4]. Thermophilic treatment facilitates industrial water system closure through reuse of individual treated flows. Other benefits include high organic load biodegradation rates and reduced sludge production [5]. However, thermophilic activated sludge systems have found restricted application since the more deficient biological floc formation and poor sludge settleability that occurs at higher temperature can result in poorer effluent quality [3,6–9]. In particular, high amounts of dispersed particles, such as free bacteria and colloids, increase COD values in thermophilic effluents [3,8,10]. The membrane biological reactor (MBR) appears particularly well suited for retention of sludge biomass under these conditions [11]. MBR combines a biological treatment system and a membrane separation unit. MBR technology has been widely used to treat industrial wastewaters [12,13] at ambient temperatures. However, few studies have explored the potential of a thermophilic MBR treatment [7,14–16].

There is still a gap in the literature with regard to the application of MBR for paper mill white water treatment under thermophilic conditions and the potential for treated white water reuse. The present work was thus undertaken to study the application of thermophilic biological treatment of paper machine white water using lab-scale membrane bioreactors. The effects of increasing organic loading and temperature on treatment efficiency were investigated. The effects of increased operating temperature on reactor sludge composition and structure were also evaluated.

2. Material and methods

2.1. White waters (raw effluents)

Raw effluents were collected from the paper machines at two Brazilian paper mills: i) **White water 1** – Companhia Suzano S.A., Brazil—an integrated bleached eucalyptus fibre kraft pulp and paper mill that produces writing and printing paper and ii) **White water 2** – Klabin, Ponte Nova Unit, MG, Brazil–a recycled fibre mill that produces outer linerboard and inner corrugating sheets for production of paperboard boxes. The white waters were collected in 50 l polypropylene containers and stored in a cold chamber at 5°C until used. Fibres were removed from the effluents by filtering through 120 mesh screens.

The white waters from the two mills were mixed in appropriate amounts to produce samples with three different COD concentrations. This permitted varying the organic loading rate in each experiment while maintaining a constant hydraulic loading rate. Filtered white water 1 had a COD of 698 mg l^{-1} and white water 2 a COD of 5540 mg l^{-1}. Filtered white waters 1 and 2 were mixed proportionally 13,5:1, 2,8:1 and 0,6:1 to produce the organic loading rates used in Phases I, II and III, respectively. Before starting treatment, white water pH was adjusted to 7.0 ± 0.2 and nitrogen (N) and phosphorous (P) were added to obtain a COD:N:P ratio of 100:5:1.

2.2. MBR treatment studies

Three lab-scale (2.0 l) MBRs were operated at 35, 45 and 55°C, with temperatures controlled using TIC 17 Model Full Gauge microprocessors. The MBRs were inoculated with sludge from an industrial activated sludge treatment plant. White water was fed continuously to the MBRs at a flow rate of 200 ml h^{-1}, corresponding to a hydraulic retention time (HRT) of 10 h. Sludge age (Θc) was fixed at 10 d. The HRT and Θc values are typical of conventional activated sludge systems. An attempt was made to keep the dissolved oxygen (DO) concentration above 2.0 mg l^{-1} using porous stone aerators. White water was fed to the MBRs using a HV 07523-50 Model Masterflex peristaltic pump mounted with three 7518-00 Model Masterflex heads. Sludge ultrafiltration was carried out using a pump with a set of heads identical to the feeding system. The membrane modules were constructed from *Zenon*® polymeric membranes with a nominal cut off point of 0.02 μm. The nominal surface area of each membrane module used for ultrafiltration was 0.0158 m^2. The specific average flow of mixed sludge for each membrane was 12.5 l m^{-2} h^{-1}. Fig. 1 illustrates the experimental set up used during the study.

No back washing was applied. Membrane module was substituted by a clean module every 10 d Membrane cleaning was performed submerging the module for 24 h in a 0.5% NaOCl solution.

Sludge was acclimatized at 35°C until COD removal remained constant in the three reactors. Temperature

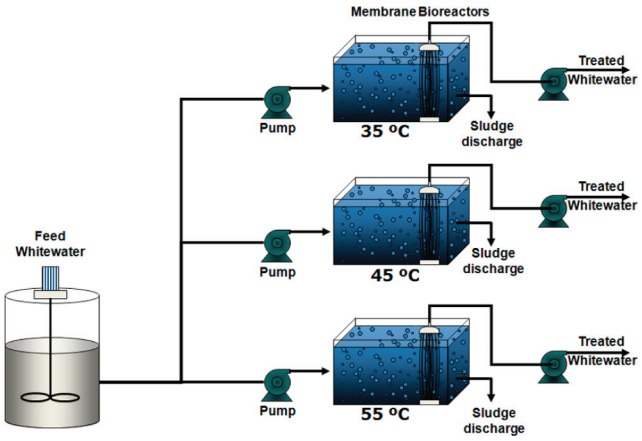

Fig. 1. Diagram of the lab-scale bioreactors used during the study.

was increased 1°C every day until Reactor 2 reached 45°C and Reactor 3 reached a temperature of 55°C.

The MBR operating conditions used are presented in Table 1. The study was carried out in three phases, at different COD loading rates. The COD volumetric load in Phase I was 2.57 kg·m^{-3} d^{-1}, and was established based on the typical MBR organic loading rates of 1.2 to 3.2 kg COD m^{-3} d^{-1}, as reported in Ref. [17]. In Phase II, the organic load was increased to 4.75 kg·m^{-3} d^{-1} and in Phase III to 9.43 kg·m^{-3} d^{-1}.

Total suspended solids (TSS) and volatile suspended solids (VSS) in the MBRs were within the recommended ranges of 5 to 20 g l^{-1} for TSS and 4 to 16 g l^{-1} for VSS [17]. Food to microorganism (F/M) ratios were within the typical range of 0.1 to 0.4 kg COD kg TSS^{-1} d^{-1} in Phase I, but higher in Phases II and III [17].

2.3. White water and sludge analyses

The following analyses were carried out daily: flow across the membrane units, temperature, pH, DO, soluble COD, TSS, VSS, turbidity, hardness, electrical conductivity (EC), colour and microbiological observations. Physicochemical analyses were performed according to the Standard Methods for the Examination of Water and Wastewater [18]. Microbiological observations and filamentous bacteria identification were carried out using the methods described in Ref. [19].

2.4. Microbiological diversity

Microbiological diversity in the MBRs operated at different temperatures was evaluated using polymerase chain reaction-denaturing gradient gel electrophoresis (PCR-DGGE) and fatty acid methyl ester (FAME) analyses. The sludge samples used for these tests were collected during Phase II.

For this analysis, the samples were collected at temperatures of 35, 40, 45, 50 and 55ºC. The collection of samples at temperatures of 40 and 50°C were conducted during the increase in temperature of the reactors at 45 and 55°C, respectively. These collections were made when the COD removal efficiencies had stabilized at these temperatures.

2.4.1. PCR-DGGE

DNA from the sludge produced in each MBR was extracted using the PowerSoil DNA Isolation Kit (Mo Bio Laboratory Inc., USA). 16S ribosomal RNA (16S rRNA) gene fragments were amplified using the F984-GC and R1378 primer set [20]. A GC-rich sequence was attached to primer F984-GC [21]. The PCR mixture was as follows: 0.2 µm of each primer, 0.2 mm dNTP mixtures, 1x GoTaq® Green Reaction Buffer (Promega, Madison, USA), 1.5 mm $MgCl_2$, 5 µg/25 µl bovine serum albumin, 2% (v/v) formamide, and 1.5U/25 µl of GoTaq® DNA polymerase (Promega, Madison, USA). DNA amplification was performed by using 'touchdown' PCR [22,23] in order to reduce the formation of spurious by-products. For touchdown PCR the annealing temperature was initially set at 65°C, which was 10°C above the expected annealing temperature and was decreased by 2°C every second cycle until reaching 55°C, the annealing temperature used for the remaining 25 cycles. PCR conditions were: an initial denaturing step at 94°C for 5 min followed by 35 thermal cycles consisting of 1 min of denaturation at 94°C, 1 min for primer annealing at the appropriate temperature, and 2 min at 72°C for primer extension. Cycling was followed by a final extension step at 72°C for 10 min and cooling to 4°C.

The denaturing gradient in the polyacrylamide gel was adjusted from 40% to 60% (100% denaturing concentration corresponded to 7M urea and 40% (v/v) formamide). Electrophoresis was carried out at a constant voltage of 60 V and a temperature of 60°C for 16 h. After electrophoresis, DNA was stained with SYBR® Gold (Invitrogen) and bands were visualized in an Eagle Eye II Still Video System (Stratagene, USA). DGGE band profiles for the sludge samples withdrawn from the MBRs operated at different temperatures were compared. Each band produced corresponded to one operational taxonomic unit (OTU).

Table 1
MBR operating conditions during the three phases

Parameter	Phase I 2.57 kg COD m^{-3} d^{-1}			Phase II 4.75 kg COD m^{-3} d^{-1}			Phase III 9.43 kg COD m^{-3} d^{-1}		
Temperature, ºC	35	45	55	35	45	55	35	45	55
TSS, g l^{-1}	6.63	6.98	6.75	9.64	10.89	8.93	16.13	15.30	15.08
VSS, g l^{-1}	3.54	3.79	3.60	5.86	5.84	4.62	10.53	9.25	8.31
F/M, gCOD gVSS^{-1} d^{-1}	0.37	0.35	0.36	0.46	0.40	0.50	0.54	0.56	0.57
U, gCOD gVSS^{-1} d^{-1}	0.35	0.33	0.33	0.45	0.39	0.48	0.52	0.53	0.51
DO, mg l^{-1}	2.60	2.8	*	2.60	2.5	*	1.20	1.0	*

*DO could not be determined at 55°C.

2.4.2. FAME analyses

MBR biomass was harvested by centrifugation. Bacterial membrane FAME analyses were performed according to the MIDI protocol (Microbial Identification System, Microbial ID Inc., Newark, Delaware, USA) [24]. The FAME profiles obtained by gas chromatography were compared using the Sherlock software (MIDI Inc., Newark, Delaware; version 4.5).

2.5. Statistical analysis

Removal efficiencies at different temperatures for the various white water parameters evaluated were compared by ANOVA followed by the Tukey test, at 5% probability, using the *GENES software* [25].

3. Results and discussion

3.1. Physicochemical parameters

The results of the physicochemical characterization of raw and treated effluents, as well as the treatment efficiencies, are shown in Table 2. High COD removal efficiencies were found at all organic loading rates and temperatures, although COD removal was lower at 55°C than at the lower temperatures. In each phase the increase in temperature caused significant reductions in COD removal efficiency. COD removal only fell below 90% at the highest COD load and temperature. As observed in Fig. 2, effluent COD increased on day five of Phase I at all temperatures, indicating a possible toxic load present in the effluent. Reduced COD removal lasted approximately seven days, after which the treatments at 35°C and 45°C recovered their initial efficiencies, while the thermophilic treatment (55°C) did not, suggesting that the thermophilic microorganisms were not able to recover from the variations in effluent quality.

During Phases I and II treated effluent COD was consistently below 100 mg l^{-1}, regardless of temperature and organic loading rate. This low COD in the treated effluent permits considering reuse of the effluent in different paper mill processes, such as water cleaning devices, low-pressure showers, sealing waters and dilution water for paper additives. Average COD values of the effluents treated at 35°C were always lower than at the higher temperatures. When the input COD load increased to 9.43 kg·m^{-3} d^{-1} (Phase III), the treated effluent COD increased significantly over the values obtained in Phases I and II, with average values of 173 ± 42, 265 ± 70 and 405 ± 93 mg l^{-1}, at 35, 45 and 55°C, respectively.

The MBR system lost efficiency at an organic loading rate greater than 4.75 kg·m^{-3} d^{-1}. According to Metcalf

Table 2
Physicochemical characterization of raw and treated white water, and treatment removal efficiencies in MBR at varying temperatures and organic loading rates

Parameter	White water	Phase I 2.57 kg COD m^{-3} d^{-1}			Phase II 4.75 kg COD m^{-3} d^{-1}			Phase III 9.43 kg COD m^{-3} d^{-1}		
		35°C	45°C	55°C	35°C	45°C	55°C	35°C	45°C	55°C
COD, mg l^{-1}	Raw	1070	1070	1070	1980	1980	1980	3930	3930	3930
	Treated	48	62	87	51	87	99	173	263	405
	Removal %	95.5	94.2	91.9	97.4	95.6	95.0	95.6	93.3	89.7
TSS, g l^{-1}	Raw	0.19	0.19	0.19	0.50	0.50	0.50	0.54	0.54	0.54
	Treated	0	0	0	0	0	0	0	0	0
	Removal %	100	100	100	100	100	100	100	100	100
VSS, g l^{-1}	Raw	0.10	0.10	0.10	0.29	0.29	0.29	0.36	0.36	0.36
	Treated	0	0	0	0	0	0	0	0	0
	Removal %	100	100	100	100	100	100	100	100	100
Turbidity, NTU	Raw	247	247	247	296	296	296	479	479	479
	Treated	0.11	0.24	1.13	0.10	0.16	1.01	0.87	2.00	4.34
	Removal %	99.9	99.9	99.5	100	99.9	99.6	99.8	99.5	99.0
Conductivity, µS cm^{-1}	Raw	1727	1727	1727	1969	1969	1969	2957	2957	2957
	Treated	1795	1809	1834	1894	1906	2012	2802	2845	3135
	Removal %	–3.9	–4.4	–5.8	2.8	2.1	–3.4	5.0	3.3	–7.1
Hardness, mg l^{-1} as $CaCO_3$	Raw	280	280	280	466	466	466	671	671	671
	Treated	258	203	160	439	350	292	639	565	495
	Removal %	7.9	28.0	42.9	6.4	25.1	36.9	4.9	16.0	26.4
Colour, mg l^{-1}	Raw	316	316	316	112	112	112	218	218	218
	Treated	24	23	102	30	36	127	46	58	163
	Removal %	92.4	92.6	67.8	72.7	68.2	–14.0	79.1	73.6	25.0

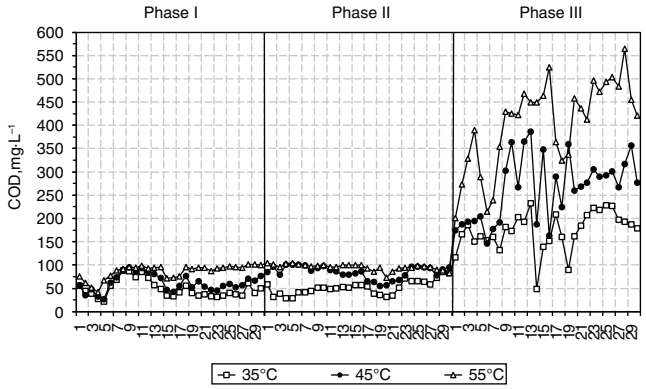

Fig. 2. Paper mill white water COD after MBR treatment at organic loading rates of 2.57 (Phase I), 4.74 (Phase II) and 9.43 (Phase III) kg COD m^{-3} d^{-1}, and temperatures of 35, 45 and 55°C.

and Eddy [17], the COD load used in MBR systems typically ranges from 1.2 to 3.2 kg·m^{-3} d^{-1}. The COD load of 9.43 kg·m^{-3} d^{-1} applied was approximately three times higher than the maximum load recommended by these authors, and resulted in a F/M ratio (0.54–0.57) approximately 1.4 × higher than the recommended range of 0.1 – 0.4 kg COD kg VSS d^{-1}.

MBR treatment completely removed TSS and VSS and presented very high turbidity removal efficiencies. However, as shown in Fig. 3, treated effluent turbidity was higher at higher treatment temperatures and increased significantly at the highest organic loading rate. In spite of the negative effect of increasing temperature on turbidity, the average turbidity values of 1.13, 1.01 and 4.34 NTU for Phases I, II and III, respectively, were relatively low. A possible explanation for the increase in turbidity at 55°C is that pore expansion occurred in the polymeric membranes, causing entrainment of small fractions of solids and colloids. Another possibility is that calcium compounds precipitated after ultrafiltration, resulting in an increase in turbidity.

MBR treatment had no effect on white water conductivity and was not very efficient in removing hardness (Table 2). Increasing temperature had a positive effect on hardness removal, which can be explained by the precipitation of calcium and magnesium bicarbonates, responsible for the so-called "temporary hardness", at higher temperatures, which were retained by the membranes.

Another parameter of industrial interest for writing and printing paper mills is white water colour. Colour removal decreased as the treatment temperature and organic loading rate increased (Table 2).

3.2. Microbial community composition and structure

At 35ºC there was a predominance of large, very compact and dense flocs, over 500 µm in diameter. A 021N type filamentous bacteria were observed in abundant concentrations, associated with the flocs [19]. These filamentous microorganisms were present in the sludge collected at the industrial treatment plant used to inoculate the MBR reactors and they continued to survive in the laboratory reactors operated at 35ºC. Morphological changes occurred in biological sludge with increasing temperature. The flocs observed at 45ºC were small-sized, below 150 µm in diameter, and presented a weak and open structure, essentially due to the presence of 0581 type filamentous bacteria. Medium-sized, compact flocs ranging from 150 to 500 µm, were observed at 55ºC. Although the flocs were compact, they contained no filamentous bacteria, and were therefore weak structures with high concentrations of floc forming bacteria.

The analyses of genetic diversity using the DGGE technique demonstrated that there was a significant

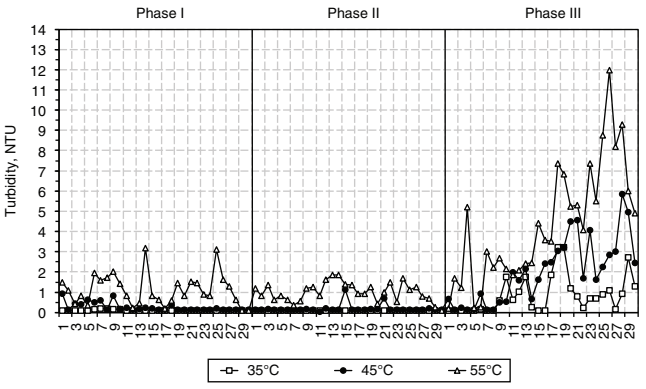

Fig. 3. Turbidity in paper mill white water after MBR treatment at organic loading rates of 2.57 (Phase I), 4.74 (Phase II) and 9.43 (Phase III) kg COD m^{-3} d^{-1}, and temperatures of 35, 45 and 55°C.

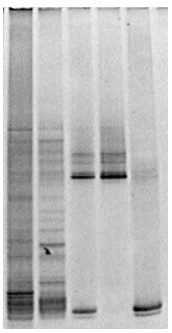

Fig. 4. DGGE band separation patterns of PCR-amplified segments of 16S rRNA genes isolated from biomass withdrawn from MBR reactor treating paper mill white water at 35ºC (lane 1), 40ºC (lane 2), 45ºC (lane 3), 50ºC (lane 4) and 55ºC (lane 5). 16S ribosomal RNA (16S rRNA) gene fragments were amplified using the F984-GC and R1378 primer set.

reduction in the number of OTU with the increase in temperature in the MBR treatment (Fig. 4). The sludge samples withdrawn from the MBR operated at 35 and 40ºC produced similar DGGE band patterns. Treatments at 45 and 50ºC also produced similar band patterns, but with a much lower number of denser bands than the lower temperature treatments. The smallest number of bands was observed at 55ºC. The reduction in OTUs indicates that the increase in temperature favoured natural selection for some microbial species and/or groups. The reduced microbial diversity had a negative effect on white water COD removal efficiency, and could be expected to occur in an industrial treatment plant operating at higher temperatures. These results are in agreement with other authors who observed fewer distinct phylotypes were present in thermophilic bioreactors as determined by band counting [26,27]. Our results illustrate how the use of molecular biology techniques such as DGGE can lead to increased understanding of the relationship between microbial community structure and function.

FAME analyses indicated similar microbial community structures in the reactors operated at 35 and 40ºC. An intermediate and different group was formed at 45ºC. A third group of microorganisms was formed above 45ºC. These results support the hypothesis that the different bacterial community structures were established during thermophilic MBR operation that negatively affected white water COD removal efficiency.

4. Conclusions

MBR treatment of paper machine white water afforded greater than 90% COD removal efficiencies when carried out at temperatures of 35 to 55°C and organic loads up to 4.75 kg COD m^{-3} d^{-1}. Treatment at 55°C resulted in lower COD removal efficiency than at 35 and 45°C. At an organic load of 9.43 kg COD m^{-3} d^{-1}, only the mesophilic system (35°C) achieved greater than 95% COD removal.

Turbidity in the MBR treated effluents was practically nil at 35 and 45°C, but residual turbidity (1–4 NTU) remained after treatment at 55°C. The highest treated effluent turbidity values were found at the highest organic loading rate (9.43 kg COD m^{-3} d^{-1}).

White water hardness removal efficiencies were low (<45%) with the highest efficiencies observed for the treatments at 55°C.

The thermophilic treatment (55°C) presented significantly lower colour removal efficiency than the lower temperature treatments.

Increasing the treatment temperature altered sludge morphology and microbial diversity. Sludge flocs were smaller and weaker at higher temperatures and filamentous bacteria were absent at 55°C. The number of OTUs was greatly reduced for treatment at 45°C or higher.

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