

Article- Environmental Sciences

Evaluation of a Combined System Based on an Upflow Anaerobic Sludge Blanket Reactor (UASB) and Shallow Polishing Pond (SPP) for Textile Effluent Treatment

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HIGHLIGHTS

- Shallow polishing pond step improved the discoloration performance during the operation time.
- Residual pretreated yeast cells showed to be effective in supplying nutrient source for biological activity for color removal in the UASB reactor.

Abstract: Color removal from textile effluents was evaluated using a laboratory-combined process based on an upflow anaerobic sludge blanket (UASB) reactor followed by a shallow polishing pond (SPP). The anaerobic reactor was fed with a real textile effluent, diluted 10-times in a 350 mg/L solution of pre-treated residual yeast extract from a brewery industry as nutrient source. The parameters color, COD, N-NH₃ and toxicity were monitored throughout 45 days of operation. According to the results, decolorization and COD removal were highest in the anaerobic step, whereas the effluent was polished in the SPP unit. The overall efficiency of the complete UASB-SPP system for COD and color were 88 and 62%, respectively. Moreover, the N-NH₃ generated by the residual yeast extract ammonification was below 5 mg/L for the final effluent. Finally, no toxicity was detected after the treatment steps, as shown by the *Vibrio fischeri* microscale assay.

Keywords: anaerobic-aerobic treatment; residual yeast; textile effluent toxicity; *Vibrio fischeri*.

INTRODUCTION

Textile industries consume large volumes of water and chemicals during the wet processing of fabrics. It is estimated that approximately 700,000 tons of dyes are used worldwide each year in the textile industry [1]. Among the totality, about 15% of dyes are discharged to the environment due to their incomplete fixation during the step of fiber dyeing. Moreover, some dyes and other degradation by-products might be carcinogenic and have mutagenic properties, especially those containing the azo-aromatic function as a chromophore [2-4]. Therefore, the textile effluent treatment is crucial for environment and health protection.

Several techniques have been studied to provide an efficient treatment of textile wastewater, in terms of color, organic matter and toxicity removal. Among the main techniques used, combined physical and chemical methods, such as coagulation followed by flotation and/or sedimentation, showed satisfactory results for particulate matter removal, although these methods are ineffective for discoloration and often involve additional operational costs [5].

Some researchers highlight that biological processes represent the most economically viable routes for the removal of these contaminants [2,4,6,7]. Biological aerobic and anaerobic processes are based on the activity of heterotrophic and/or autotrophic microorganisms. Anaerobic decolorization processes are generally simpler and promote a more efficient color removal, when compared to other physical/chemical and aerobic biological methods [2,6]. The biological treatment in anaerobic reactors is extremely advantageous in the textile context, since the dyes are the final electrons acceptors, increasing the efficiency of color removal [6]. To improve color removal in the anaerobic reactor, application of redox mediators have been investigated due to their ability to enhance the kinetics of azo dye reduction. Some components, such as vitamin B2 (Riboflavin) and quinone, are excellent redox mediators, since they are reversible organic molecules able to undergo oxidation and reduction reactions [6]. For instance, the application of immobilized redox mediators in microbial fuel cells was tested for electricity generation with simultaneous azo dye removal due to facilitated electrons transfer from bacteria to anodes and azo dyes [8]. In this context, the use of low cost redox mediators have been suggested, such as commercial yeast extract (source of riboflavin) [2,9,10] or residual yeast from the brewing industry [11,12]. Dyes are deficient in carbon and nitrogen sources, and the biodegradation of dyes without any supplement of these sources may be hampered. In this manner, the addition of yeast extract provides macro and micronutrients easing the biological activity. Despite improving the anaerobic degradation of azo dyes, recent studies demonstrated that yeast extract addition to UASB reactor, at a concentration of 350mg/L, did not promote changes in the microbial structure [10].

The production of recalcitrant and toxic by-products from the anaerobic reduction of azo dyes requires an aerobic post-treatment unit, not only to degrade aromatic amines and recalcitrant COD, but also to eliminate toxicity from previous system [3]. Therefore, a treatment process with two steps may be necessary to make a complete treatment of azo-dye rich textile effluents. Results have shown the ability of an aerobic granule sludge reactor to perform color and nutrient removal under microaerophilic conditions showing aerobic and anaerobic metabolism in the same reactor, but the system still needs to be tested for real textile effluents [13]. Among the aerobic alternatives, polishing ponds, which involves the metabolic activity of bacteria and microalgae, seem to be the most promising approach. Microalgae species produce oxygen via photosynthesis, while aerobic bacteria oxidize the residual organic material alongside with oxygen respiration. In addition, there might be mixotrophic growth of certain microalgae, like *Chlorella* sp, which are able to use organic matter and even dyes as energy source [14]. There are few studies which investigated the ability of microalgae to perform color removal, most of them using dye solution with one or a mixture of different compounds [15-18]. However, so far there is no data concerning the use of microalgae to treat textile effluent.

Moreover, when textile effluent is not properly treated and disposed in the environment, it may interfere with aquatic ecosystems affecting an aquatic biota and public health [19]. In this manner, it is crucial to test the environmental toxicity of the treated effluent. A low-cost method consists in the use of a bioluminescent marine bacteria, i.e. *Vibrio fischeri*, which has shown to be efficient for predicting toxic substances [20]. For optimizing the test and for ensuring that many samples can be processed simultaneously, a microplate miniaturization may be carried out. A previous study has demonstrated the applicability of *Vibrio fischeri* microscale assay for tannery wastewater but so far, the test was not applied for textile effluents [21].

In the present study, residual yeast pre-treated by osmotic lysis was evaluated as a source of redox mediators and nutrients for the combined anaerobic/aerobic treatment of real textile effluent. For this, continuous system performance was evaluated using a laboratory-scale UASB reactor followed by a post-

treatment in a shallow polishing pond (SPP). In addition, miniaturized toxicity test using *Vibrio fischeri* was used to assess the quality of the effluent before and after its biodegradation.

MATERIAL AND METHODS

Residual yeast

Residual yeast biomass belonging to *Saccharomyces cerevisiae* species was obtained from a small brewery pretreated through osmotic lysis [11,12]. For this, yeasts were taken directly from fermentation vats and submitted to a clarification process, which consisted in a sequence of centrifugations and addition of ethanol solution 96% (10% M/M). The solid fraction was then submitted to osmotic lysis by the addition of NaCl (45% per yeast dry weight) aiming the release of riboflavin and other nutrients to the solution. The resulting suspension was then dried and macerated as described in previous studies conducted in our research group [12]. A solution with 350 mg/L of pretreated yeast cells was prepared and used as feeding solution. The amount of riboflavin released in the yeast osmotic lysis process ranged from 20.7 to 42.9 µg/g [11].

Continuous Laboratory-Scale Reactors

The laboratory-scale combined systems evaluated in this study were a UASB reactor followed by a post-treatment in a SPP Figure 1. The combined system was operated in continuous mode during 45 days. The UASB reactor had a 2.2 L working volume and operated at a 24 hours hydraulic retention time (HRT) at room temperature (~16-29 °C). The SPP reactor had a working volume of 6 L and operated at a 66 hours HRT. In this case, the UASB effluent followed to the SPP tank by gravity, and was exposed to ambient conditions, i.e. directly to sunlight, according to the natural climatic conditions (temperature variation of 15-32 °C). The water level of the SPP was monitored daily and maintained by tap water addition.

The UASB reactor was inoculated with anaerobic sludge from a pilot-scale UASB reactor for sewage treatment, whereas the polishing pond was inoculated with microorganisms from a small pond located the Federal University of Ouro Preto (Brazil). The sludge concentration in the mixed liquor had an organic matter concentration of 10g VSS/L in the UASB reactor and 6g VSS/L for SPP. The textile effluent was collected in the equalization tank of the industry, a stage that precedes its biological treatment. The crude effluent was diluted 10-fold in a pretreated yeast cells solution (350 mg/L) before being pumped into the proposed treatment system. This dilution aimed at reducing color, COD and the possible toxic effluent load, so that at first it did not impact the microorganisms in the reactors. A sample was collected for each analysis made in this work.

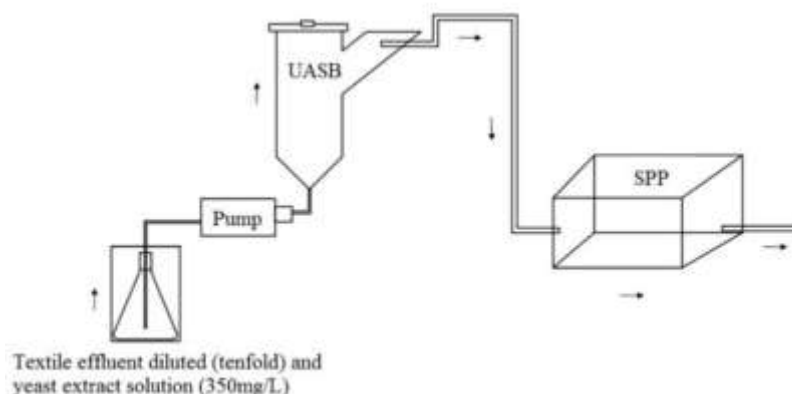


Figure 1. Diagram of the combined system employed for the treatment of textile effluent using an upflow anaerobic sludge blanket reactor (UASB) reactor followed by an aerobic post-treatment of shallow polishing pond (SPP) configuration

Analytical Methods

Continuous system performance was evaluated by means of pH, temperature, volatile fatty acids (VFA), N-NH₃, color and chemical oxygen demand (COD) analysis of both reactors influents and effluents. All samples were previously centrifuged at 3,600 rpm for 20 min (Fanem Excelsa II 206 BL) and the supernatant fraction was used for analysis. Temperature, color and pH of anaerobic and aerobic effluents were monitored periodically employing pH-meter (Metrohm, model 827). Color was analysed according to

the Standard Methods, using a 2120D Spectrophotometric Multi-Wavelength Method and a UV-Vis spectrophotometer (HP 8453) at 465 nm and mg/L PtCo scale. The COD and N-NH₃ (Kjeldahl method) were also performed according to Standard Methods [22]. Therefore, COD is analyzed three times a week and ammonia every fifteen days. VFA analyses were carried out by high performance liquid chromatography (HPLC) using an Aminex HPX-874W (BioRad) which was kept at 55°C under isocratic mode (0.6 mL/min of H₂SO₄ 0.01 mol/L). For this, 10 µL of the samples previously filtered in cellulose acetate membranes (0.45 µm) were injected in a Shimadzu equipment so that the separated VFA could be detected at 210 nm in a diode array detector (DAD). The main VFA analyzed were acetic, formic, propionic, butyric, isobutyric, valeric and isovaleric acids. VFA concentrations were used to estimate their contribution to the dissolved COD using stoichiometric coefficients as reported by Equation 1. [23].

$$\text{VFA(mgCOD/L)} = 0.35[\text{formate}] + 1.7[\text{acetate}] + 1.51[\text{propionate}] + 1.82[\text{butyrate} + \text{isobutyrate}] + 2.04[\text{valerate} + \text{isovalerate}] \quad (1)$$

Toxicity Assays

Toxicity assays were performed upon bioluminescent *Vibrio fischeri* in the system configuration composed by UASB/SPP, according to Standard Procedures [24]. In this test, light production is directly proportional to the metabolic activity of the bacterial population so that any inhibition of enzymatic activity causes a corresponding decrease in bioluminescence [25]. The test was carried out under miniaturized conditions (micro-scale) by applying a 10-fold reduction. Therefore 150 µL of bacterial suspension freshly prepared and 150 µL of test sample diluted in 2% saline solution were added to 96-wells microplates. Heptahydrate zinc sulfate was used as positive control in a concentration varying between 36 and 146 mg/L ($r^2 = 0.93$). The bioluminescence at 30 min from the bacterial suspension were analyzed using a microplate reader X3™ VICTOR Multilabel Plate Reader (Perkin Elmer). The initial and final measurement of luminescence were subjected to a series of calculations established by [24] to determine the luminescence inhibition values and effective concentrations for 20% population, EC₂₀.

RESULTS

Color Removal Performance

The UASB/SPP treatment system was operated during 45 days. During this period, the pH of the UASB varied naturally from 6.8 to 7.8, which is among the optimal range for anaerobic microorganisms. The SPP varied from 7.4 to 10.3, i.e. the system promoted an increase on the pH levels.

Color removal efficiency was evaluated from the soluble fraction considering color measurements in influent and effluent samples, while no color was visually observed from the solid fraction. Considering this, the UASB reactor removed around 50% of the textile effluent color at the beginning of the operation, as can be seen in Figure 2. In this case, color removal may have been achieved through the adsorption of dye into microbial granules, followed by biodegradation.

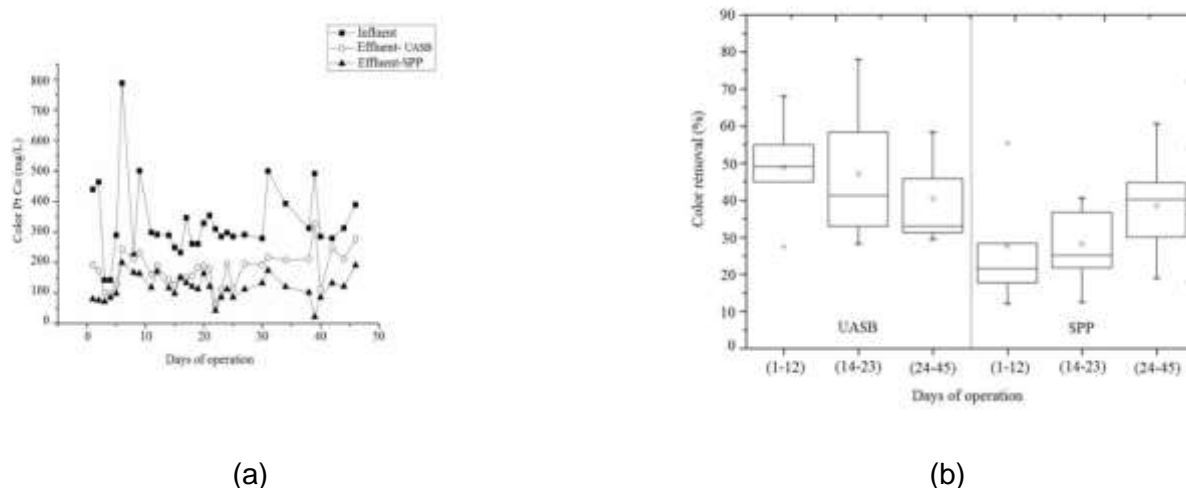


Figure 2. Variations in color concentration (A) and color removal (B) in UASB and shallow polishing pond (SPP) reactors fed with real textile effluent.

COD Removal

The COD removal efficiency of the anaerobic reactor remained high throughout almost the entire period, attaining in average 80% Figure 3. The values reached were higher than those compared to previous studies using the same HRT, although using lower organic loading rates (i.e. 50-79%) [2,3].

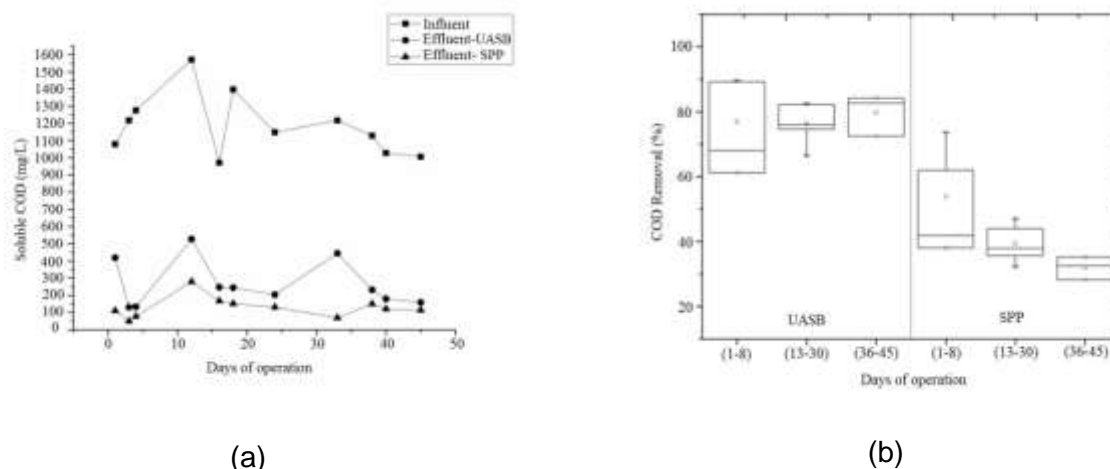


Figure 3. Variations in COD concentration (a) and COD removal (b) in UASB and Shallow polishing pond (SPP) reactor fed with real textile effluent

N-NH₃ and Toxicity

N-NH₃ concentration was monitored to evaluate the protein degradation from the pre-treated residual yeasts and the organic nitrogen present in the effluent, as well as to evaluate ammonia oxidation in the aerobic units. The results revealed that the N-NH₃ concentration was increased in the anaerobic stage due to ammonification reactions, reaching values up to 80 mg N-NH₃/L. However, this value dropped to very low values (below to 5mg/L) in the SPP.

Regarding the toxicity assays, no toxicity was shown in treated samples, in neither the UASB nor SPP effluents, which suggested that there was little toxic effects from the residual COD in both systems. Only the fed solution was toxic to *Vibrio fischeri* bacteria but at the lowest dilution factor (DF2). In this case, there was 20% of inhibition to the luminescence.

DISCUSSION

Higher pH values may be associated with the algal photosynthesis process, since the biomass consumes dissolved CO₂ from the pond, which in turn reduces the formation of carbonic acid and increases the pH. In accordance, a previous study demonstrated that the increase in the pH (up to 9) resulted in an increase in the decolorization rate for Malachite Green in a batch photobioreactor operated at a HRT of 12h [26]. Therefore, the natural increase in the pH at SPP may favour colour removal processes.

High color removal Figure 2 at the initial stages of the experiment seems to be due to high adsorption onto granules, since the adsorption capacity of the granules is higher at that time. However, overtime the efficiency dropped to around 35%. This can be explained by several factors, such as the saturation of microbial granules by dyes and the reduction of microbial mass due to the selection of microorganisms. Furthermore, by-products generated in this process may not be biodegraded anaerobically, but are readily biodegraded under aerobic conditions. It is worth mentioning that such discoloration rates were attained with a HRT of 24 hours and better efficiencies could be reached by increasing the HRT. In fact, previous authors achieved a discoloration percentage ranging from 60 to 90% in a UASB reactor treating real textile effluent at HRT of 48 hours [3]. Higher color removal efficiencies have also been reported for synthetic dye solutions and under batch conditions bioreactors [27,16].

Moreover, residual pretreated yeast cells showed to be effective in supplying nutrient source for biological activity for color removal in the UASB reactor. Previous studies also showed that the residual yeast could be used as the unique source of carbon and redox mediators in the anaerobic treatment of azo dyes. In those studies, the authors verified that the discoloration rates of dye solutions were practically the same (~72%) when using standard nutrient solution containing yeast extract and when using only residual yeast [11,12]. Moreover, color removal rates as high as 94% was observed for 10-fold diluted textile effluent supplied with commercial yeast extract in bench scale UASB [2]. In this case, the authors attribute

the good performance of the reactor to the presence of activated carbon inside the reactor, which may have enhanced dye reduction due to the presence of quinone groups on the adsorbent surface, as well as to the adsorption of inhibiting compounds. In addition, the researchers operated the treatment system for approximately 90 days, which ensured a greater time of selection of the microorganisms degrading the effluent. Nonetheless, in the present study using the same reactor, but only fed with residual yeast, showed lower discoloration rates. This was probably due to the short operation time, low temperatures and high complexity of textile effluents, which are normally composed of more than one class of dyes, as well as additives and auxiliaries used in the production process.

According to the results, the SPP step improved the discoloration performance during the operation time. This is in accordance with previous studies that observed an increase in dye degradation by previous contact with microalgae species [16]. During our study, the color removal ranged from 20 to 40% during the experiment, suggesting an adsorption phenomena (increased with the increase of biomass) and/or adaptation of the microorganisms (microalgae and bacteria) on decolorization performance. However, even considering the maximum decolorization measured in this study the value attained was lower than those reported in the literature [28,26]. However, the referred studies were carried out with a synthetic wastewater; which is far less complex than the actual textile wastewater applied in this study. Additionally, the efficiency shown on color removal was enough to allow the treated effluent to be discharged considering national legislation [29].

VFA accumulation in the UASB reactors was low. Acetic acid was the highest VFA measured, ranging from 0.12 to 0.32 mg/L. Previous studies from our research group using the same reactor, type of effluent and similar influent COD concentration (900 mg/L) found higher values, around 82 mg/L, even at controlled temperature and pH [2]. It is known that in a stable reactor, operated under optimal conditions of microbial growth there will be no significant VFA accumulation, since microorganisms are in equilibrium [30]. For this reason, the results lead us to believe that the addition of pretrated residual yeast favoured under some aspect the microbial degradation. Considering that less than 1% of the residual COD belonged to VFA, the remaining residual COD in the anaerobic effluent (which was around 300mg/L) is probably due to aromatic amines and soluble microbial products produced during treatment, as well as residual dyes and recalcitrant compounds present in the textile effluent.

Regarding to the COD removal at the SPP unit, there was a decrease during the operation from around 80% to 30% at the final week, which is in a contrast with the color removal profile which was low at the beginning and high at the end of the period. The residual COD was probably composed by sub-products from partial degradation of dyes, such as aromatic amines in the case of azo dyes and even organic products generated during the microalgae metabolism along the biomass growth.

Nevertheless, a COD removal of 40% promotes a desirable final polishing to the system effluent. According to the current Brazilian legislation, textile effluents can be discharged at up to 250 mg/L of COD. As can be seen, soluble COD from the SPP effluent was always below this requirement Figure 3. In addition, the current federal legislation (CONAMA 357) states that for assessing the removal of organic load by stabilization ponds the samples from these systems should be filtered, as well as the state of Minas Gerais (COPAM/CERH-MG N.º 1, de 05 de Maio de 2008), which establishes the same parameters and conditions [31]. Therefore, the results presented here were highly promising and as far as we know this is the first time that a SPP has been used as a polishing step for treatment of textile effluents.

Since yeast extract composition is around 48% protein [32], the observed nitrification values are due to probably due to nitrification and/or algae assimilation, although no measurements of nitrate or algae were performed in this study. Such observation indicated that yeast extract might have played an important role as nutrient source during the anaerobic-aerobic treatment of textile effluent.

Concerning toxicity, considering that the raw effluent was diluted 10 times, the real toxicity is probably higher. The toxicity may be due to dyes or additives in the complex textile effluent. Previous authors evaluated the toxic potential of Peristal BFL, an auxiliary raw material used in the dyeing process, by means of toxicological tests using *Vibrio fischeri* [33]. According to the study, at concentrations of 9 and 45 mg/L, inhibition of 20 and 50%, respectively were observed after 30 min of exposition. For synthetic dye solutions higher levels of toxicity have been reported [34,35].

CONCLUSIONS

A combined anaerobic-aerobic system is often necessary for a complete treatment of textile effluents. The investigated system, composed by an UASB reactor followed by a shallow polishing pond, was able to remove 88% of COD and 62% of color. Also, no toxicity was detected after the treatment, as shown by the

Vibrio fischeri microscale assay. Addition of residual yeast extract was able to support biological activities during the degradation processes, by providing nutrients and redox mediator (riboflavin) for the UASB-SPP system to be burned to fuel the treatment. However, it is necessary to operate this system of reactors using the textile effluent closer to the real (less diluted) to know the real possibility of application on a full scale.

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