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Treatment of Dye Waste Water by Bioreactor

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Abstract Waste water treatments are the major problem for the environment issue. Especially when it is related to toxic chemical this is used by industry. Different methods are adopted but there low efficiency is found for the secondary treatment of waste water. Now day advance technology membrane biotechnologies are introduced to the waste water. Highly polluting industrial wastewaters are preferably treated in an anaerobic reactor due to the high level of COD, potential for energy generation and low surplus sludge production. However in practical applications, anaerobic treatment suffers from the low growth rate of the microorganisms, a low settling rate, process instabilities and the need for post treatment of the noxious anaerobic effluent which often contains ammonium ion (NH4+) and hydrogen sulfide (HS−). In most applications, despite the efficiency of the anaerobic process is high, complete stabilization of the organic matter is impossible anaerobically due to the high organic strength of the wastewater. The final effluent produced by the anaerobic treatment contains solubilized organic matter. This is suitable for aerobic treatment, indicating the potential of using anaerobic–aerobic systems and subsequent post treatment using aerobic treatment is required to meet the effluent discharge standard. Phanerochaete chrysosporium immobilized on different support materials, such as polyurethane foam (PUF) and scouring web (SW), in shake cultures, was able to decolourize efficiently the textile industry effluent in a long-term repeated-batch operation.

Keywords: *dye wastewater, decolorization, fungi, microfiltration, membrane bioreactor*

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1. Introduction

The dye wastewater is rated as the most polluting among all industrial sectors considering both volume and composition of effluent [1]. It is a complex and highly variable mixture of many polluting substances ranging from inorganic compounds and elements to polymers and organic products [2]. It induces persistent color coupled with organic load leading to disruption of the total ecological/symbiotic balance of the receiving water stream. Dyes with striking visibility in recipients may significantly affect photosynthetic activity in aquatic environment due to reduced light penetration and may also be toxic to some aquatic lives due to metals, chlorides, etc., associated with dyes or the dyeing process. It is difficult to remove dyes from effluents since dyes are stable to light, heat and oxidizing agents and are non-biodegradable [3]. Several physico-chemical decolorization techniques have been reported (e.g. adsorption, chemical transformation, and incineration, and photocatalysis, ozonation or membrane separation), few, however, have been accepted by the textile industries. Their lack of implementation has been largely due to high cost, low efficiency and inapplicability to a wide variety of dyes. Biodegradation is an environmental friendly and cost competitive alternative but the conventional aerobic treatments are ineffective for textile wastewater [2]. Some anaerobic microorganisms can biodegrade dyestuffs by azoreductase activity, but highly biotoxic aromatic amines can be formed by reductive fission under anaerobic conditions. Besides, upon exposure of the anaerobic degradation product to oxygen, reverse colorization may take place. However, wood rotting 'white-rot' fungi are able to degrade aerobically a wide variety of recalcitrant organic pollutants, including various types of dyes through extracellular secretion of non-specific oxidative enzymes as a secondary metabolic activity in C or N-limited medium [4].

The application of white-rot fungi in large-scale waste treatment has been impeded by the lack of bioreactor systems that can sustain steady production of high levels of ligninolytic enzymes for a long period together with a controlled growth of fungi. The widely used systems were stirred tank reactor and air-lift and bubble column, fixed bed bioreactor, rotating disk reactor and silicone-membrane reactor. There are few reports specifically on dye decolorization in continuous bioreactors. Up to 80% decolorization of a disperse dye (Red-553) in a continuous (10-20 days) fixed-film bioreactor [5]. Some researcher also investigated continuous decolorization of an azo dye, Orange II, in a packed-bed reactor, achieving high decolorization efficiency 97% [6]. However, a number of operational problems such as formation of mycelia aggregates, electrode fouling and clogging emerged after a

short time and made necessary the periodical removal of fungal biomass from the reactors. Recently introduced a pulsed flow bioreactor packed with immobilized fungi, which treated dye loads of 0.2 g dye/m^3 day at over 90% efficiency for several months [7]. In vitro dye decolorization by manganese peroxidase in an enzymatic membrane reactor in continuous operation has been studied the system allowed a very fast decoloration with over 90% efficiency under high dye loading rate of 2.4 g dye/m^3.d [8]. Similarly 70% decolorization of heat-treatment liquor of waste sludge by a bioreactor using polyurethane foam-immobilized white-rot fungus equipped with a side stream ultra filtration membrane was achieved [9]. In this regard an effort has been made to evaluate the decolorization efficiency of the collected white rot fungi strains through agar plate and liquid batch studies and, subsequently assess the feasibility of a submerged microfiltration membrane bioreactor implementing the fungi culture for treatment of textile dye wastewater. According to the authors' knowledge, no attempt has been made until now to use a submerged membrane bioreactor with white-rot fungi culture for decolorization of dye wastewater.

2. Methods and Materials

2.1. Microorganisms

The white-rot fungi strains used for this study were *P. chrysosporium* (ATCC 24725) obtained from the NITE Biological Resource Center (NBRC), Japan. The stock culture was grown, as prescribed by ATCC, on Potato Sucrose Agar (PSA) medium at 26.5°C (growth temperature range = 24-28°C). The culture was maintained at 4°C and refreshed every 30–40 days. ATCC was used in the bioreactor experiment because of its superior performance in the batch test.

2.2. Dyes and Chemicals

Poly R-478 (polyvinyl amine suffocated backbone with anthrapyridone chromophore, violet color) and Poly S-119 (polyvininylamine backbone with azo chromophore, orange color) were purchased from Sigma Chemical Co. The peak absorbance in the visible range corresponds to the wavelengths 520 nm and 472 nm for Poly R-478 and Poly S-119, respectively. Since textile effluent contains a range of dyes, successful decoloration of a single dye does not adequately indicate the suitability of an organism for a decoloration process. However, these two polymeric dyes represent the majority of the synthetic dyes [10]. All other chemicals used were of reagent grade.

2.3. Culture Medium

Unless otherwise indicated, the basal decolourization medium consisted of sugar refinery effluent (adjusted to pH 4.5 with HCl) supplemented with (final composition per litre): 5 g glucose; 2.0 g KH_2PO_4; 1.06 g $MgSO_4 \cdot 7H_2O$; 0.032 g NH_4Cl; 10 ml mineral solution and 10 ml thiamine solution (100 mg/l) and was sterilized by filtration (0.45 μm). The mineral solution composition is described elsewhere [11]. The growth medium used in the repeated-batch experiments for carrier selection was identical to the basal decolourization medium but contained 10 g/l glucose and 0.128 g/l NH_4Cl. Growth medium devoid of effluent was used in the experiments for optimization of decolourization medium composition and in the continuous treatment with RBC.

2.4. Repeated-Batch-Decolourization-Experiments

The immobilization of *P. chrysosporium* on the support particles was carried out in 500 ml serum bottles containing 18 ml support and 24 ml growth medium inoculated with 10% (v/v) inoculum, under orbital agitation at 100 rpm and 38°C, until the supports were completely colonized. After immobilization, the medium was withdrawn and replaced with decolourization medium. The medium was changed at specific time intervals under the same conditions for repeated-batch decolourization tests. The serum bottles were aerated everyday with pure oxygen (2 l/min) for 3 min. Samples were routinely taken for analysis. The support materials tested were polyurethane foam and scouring web (Scotch-Brite, 3 M Company, Spain) cut into approximately cubic pieces *(*5 mm × 5 mm × 7 mm*)*.

2.5. RBC Reactor Studies

The closed RBC was constructed from a polymethylmethacrylate cylinder, 25 cm in length and 18 cm in diameter. The main chamber of the reactor was divided into three identical stages, each composed of three disks of 14 cm diameter. A layer of polyurethane foam (PUF) of 2.5 mm thickness was attached on both sides of each plastic disk. The disks were mounted on a horizontal steel shaft and rotated at 4 rpm. During operation the disks were 40% submerged.

The reactor had a working volume of 1.5 l. The atmosphere was enriched by introducing a continuous flow (40 ml/min) of pure oxygen. The temperature was maintained at 38°C by means of a heating jacket.

Experiments were started in batch mode to immobilize fungal biomass onto the disks. 1.5 l of growth medium was sterilized and placed in the RBC reactor, inoculated with 10% (v/v) inoculum and then operated for 4 days. After immobilization, the growth medium was removed and the decolourization medium was continuously fed into the reactor at a flow rate of 0.35 ml/min (hydraulic retention time of 3 days).

2.6. Analytical Procedures

Colour was measured at 420 nm after pH adjustment to pH 9.0 with 0.012 M borate buffer. Total phenols were determined using the Folin and Ciocalteau reagent, based on the method described by Singleton and Rossi [12]. The chemical oxygen demand (COD) was determined according to the closed reflux colorimetric method [13]. An HACH COD digestion system and a spectrophotometer HACH-2000 were employed. The proportion of the total COD derived from sugar refinery effluent was calculated by subtracting the COD of added glucose from the total COD. Glucose concentration was measured by using an enzymatic Boehringer-Mannheim/716251 Kit.

The system was first inoculated with fungi grown for two weeks in 1 liter Erlenmeyer flasks each containing

500 ml of the culture media and the reactor was operated in batch mode for a week after which the continuous operation was started with a MLSS concentration less than 2000 mg/l. Specific amount of sludge was wasted from the reactor and membranes were cleaned (off site manual cleaning with water) when membrane fouling was so severe that the transmembrane pressure exceeded 60 kpa or so.

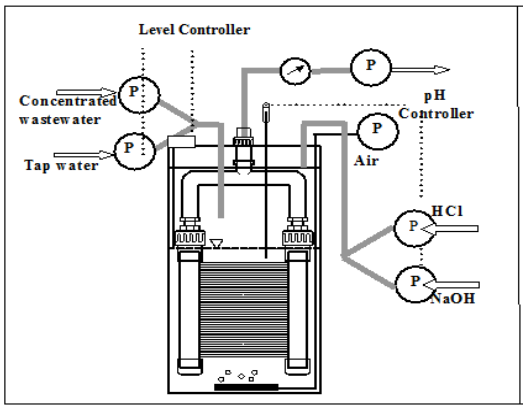

Figure 1. Setup of reactor for reducing dye waste water [14]

2.7. Analytical Methods

Total organic carbon was measured with a Total organic carbon analyzer (TOC-V, Shimadzu). Prior to the measurement, the samples for TOC analysis in batch tests were homogenized (Branson sonifier 450) for 5 minutes (30% duty cycle, output control of 3). The samples were not filtered because starch, being poorly soluble in water, would be retained by a filtering unit of 0.45 µm. Samples from the membrane bioreactor were free from suspended solid and hence did not require any treatment before TOC measurement. Color measurements were carried out spectrophoto metrically using a spectrophotometer (U-2010, Hitachi) to measure the absorbance of the sample at the peak wavelength of the dye used. The concentration of dyestuff was calculated from a calibration curve of 'absorbance versus concentration' and concentration values were used for calculations of decolorization efficiency. The sample from batch test for absorbance measurement was filtered through a Dismic -25 hydrophilic filtering unit (0.45 µm, mixed cellulose ester). The absorbance measurement on the reactor supernatant and final effluent was made after centrifuging the sample (H-3R centrifuger, Kokusan) for 10 mins. at 3000 rpm. MLSS concentration was measured according to the Standard methods (20th edition, 1998).

3. Results and Discussion

3.1. Batch Decolourization Tests

The longevity of the decolourization activity of *P. chrysosporium* immobilized on two different carriers previously selected [15], polyurethane foam (PUF) and scouring web (SW), was measured in repeated-batch tests.

Cultures immobilized on PUF or SW behaved similarly, as can be seen in Figure 2. The time needed to obtain significant decolourization was reduced from 8 days (first batch) to 3 days (third batch). It should be stressed that fungal growth on the supports was observed during batch operation, and especially on the first one. On the third batch it is likely that the fungus had reached optimum growth, thus being more adapted to the effluent environment. Because of this adaptation and growth, the immobilized mycelia exhibited rapid decolourization, thereby reducing the processing time.

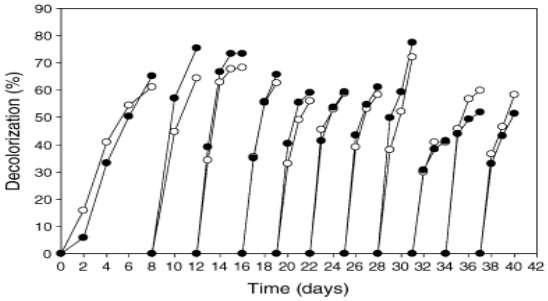

Figure 2. Batch decolourization tests using *P. chrysosporium* immobilized in different carriers materials

Therefore, further batches were conducted for 3 days each. During the 40 days of repeated-batch tests, average decolourization efficiencies of 62 and 60% were achieved with PUF and SW, respectively. The fungus maintained a relatively stable decolourization for a long period. The results demonstrate that *P. chrysosporium* immobilized on PUF or on SW is able to treat the effluent efficiently in a long-term operation. Polyurethane foam was selected for further studies because it is cheaper and mechanically more resistant than scouring web.

Observation from this experiment showed that the immobilized mycelia took a long time to grow in the presence of effluent before acclimating to the effluent environment.

This suggested that the fungus should be incubated in an appropriate growth medium without effluent before starting the decolourization phase. This would reduce the lag time in the decolourization process. Incubation of fungus on growth medium without effluent allows the immobilization on the support and growth to an optimum level before being used for decolourization. Therefore, in the remaining studies *P. chrysosporium* was previously grown in growth medium without effluent.

3.2. Continuous Effluent Treatment in RBC Reactor

A rotating biological contactor (RBC) containing *P. chrysosporium* immobilized on PUF disks was operated with optimized decolourization medium (basal medium without both thiamine and exogenous nitrogen) in continuous mode with a residence time of 3 days. The RBC reactor was monitored to determine the active life of the biocatalyst (Figure 3).

During the initial 17 days an average decolourization of 54% and an average total phenols reduction of 62% were observed. From the 17th day of continuous operation, a progressive decrease in colour removal was observed while the reduction of total phenols was reasonably stable. Minimum values of 27 and 56% were recorded on the 24th day, for colour and total phenols reduction, respectively. During the course of continuous decolourization it was observed that the biofilm thickness

in the first stage of the reactor increased more than in the remaining stages. The first stage was constantly exposed to the nutrient containing decolourization medium. Samples were taken from the three stages of the reactor, on days 9 and 19 of operation, for colour measurement. It was observed that, from days 9 to 19, the amount of colour removed, decreased by 58% in the first stage and by 23% in the second stage and no significant decrease occurred in the third stage. Therefore, the decrease in efficiency with the increase in the treatment period recorded was probably due to the loss of mycelial activity, primarily in the first stage, caused by diffusion limitations.

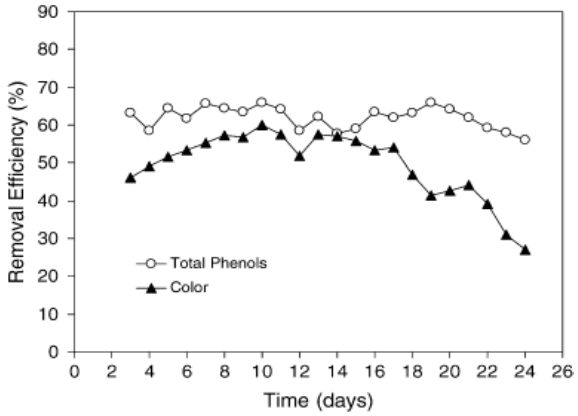

Figure 3. Colour and total phenols removal performance of continuous RBC reactor operated in one way feeding mode. Decolourization medium without addition of both thiamine and nitrogen was used in these experiments

A similar continuous decrease in colour reduction of a bleach plant effluent after the 17th day of operation in RBC containing *P. chrysosporium* was observed by Yin et al. [16]. Kapdan and Kargi [17] also reported a decrease in the textile dye decolourization efficiency with time, in repeated-batch operation of RBC with *C. versicolor*. In both studies the decrease in activity was attributed to diffusion problems caused by excessive mycelial growth. A second attempt was made to avoid the excessive growth in the first stage of the reactor. The reactor was operated as follows: during the first 17 days the effluent was fed in the same way as previously. After day 17 the feed was reversed, i.e. the feed inlet was in the third stage, with the outlet being now in what was initially the first stage. In the initial 17 days the results were comparable to the ones obtained in the first run (Figure 4). After feed reversal, the activity was re-established after 3 days and an average decolourization of 53% and an average total phenols reduction of 62% were achieved in the following 16 days, i.e. until day 36 (Figure 4).

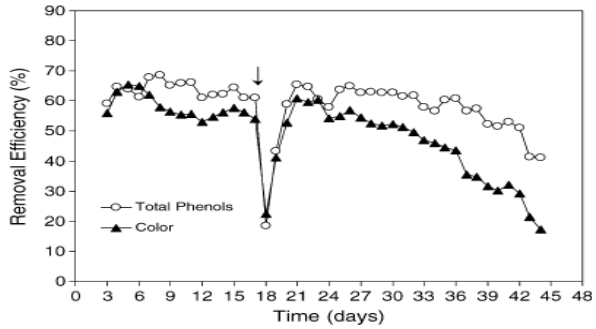

Figure 4. Colour and total phenols removal performance of continuous RBC reactor operated in alternated feeding mode. Arrow signs the inversion of reactor feeding. Decolourization medium without addition of both thiamine and nitrogen was used in these experiments

There was a significant decrease in the reactor efficiency after that period, probably due to an excessive biomass growth in all stages. The excess of mycelia could, in principle, be removed by scraping the surface of the disks and probably the decolourization ability could be recovered, thus increasing the operation period of the reactor. However, this was not performed because it was not possible to open the reactor during the operation.

The reactor with one way feeding (first run, Figure 3) was only able to operate for 17 days without clogging problems, whereas the reactor with alternated feeding (second run, Figure 4) was operated twice as long, maintaining at least 50% average colour removal.

Thus, it was possible to double the active fungal lifetime, with colour and total phenols being reduced, on average, by 55 and 63%, respectively. At the same time, 48% of COD in the initial wastewater was metabolized. Ohmomo et al. [18] reported the continuous decolourization of molasses wastewater in a bubbling column reactor with *C. versicolor* immobilized within Ca-alginate gel. With such a system an almost constant decolourization yield of 66% and a COD reduction of 46% were obtained during 16 days operation. In the continuous system described here, a slightly lower decolourization was obtained but the reduction of COD was similar, during 36 days operation. Although the alginate immobilized system was adequate for laboratory scale studies, it may not be attractive for large-scale applications. Drawbacks of the alginate system include (1) associated diffusion limitations that result in the localization of mycelia to a limited thickness near the outer surface of the alginate bead and (2) the susceptibility of calcium alginate to degradation [19].

The system described here seems to be more convenient for industrial application due to the special characteristics of RBC. Recently, Fujita et al. [20] studied the decolourization of melanoidin present in a synthetic effluent, using a bioreactor with *C. hirsutus* immobilized onto polyurethane foam cubes combined with an ultra membrane filtration unit. The contribution of the fungal bioreactor alone to the decolourization was only 45%, in a sequencing batch operation for 10 days. It may be concluded that higher decolourization efficiency was obtained in the continuous RBC here described, together with a longer operation time(36 days).

4. Conclusions

Results of the present investigation reveal that it is possible to treat the textile industry effluent continuously in a rotating biological contactor with *P. chrysosporium* immobilized on polyurethane foam disks and that the active fungal lifetime can be increased by simply reversing the feed inlet of the reactor. This system not only removed the colour of the effluent by 55% but also reduced total phenols and COD by 63 and 48%, respectively, suggesting its potential use in bioremediation of effluents.

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