Abstract—A rotating basket contactor reactor immobilized with \textit{Phanerochaete chrysosporium} on polyurethane foam cubes (1.0 × 1.0 cm$^3$) was developed to treat a synthetic wastewater containing 1,000 µg/L of carbamazepine. The reactor was continuously operated for 160 days under non-sterile condition. The removal efficiency for carbamazepine exceeded 90% after one month of fungal adaptation through the addition of external carbon source in the influent. Nutrients and oxygen were effectively transferred to fungi, which contributed to the excellent treatment performances of the bioreactor in the long-term continuous operation. This study would provide an effective and feasible way to use the white-rot fungus bioreactor for the removal of carbamazepine under non-sterile conditions.

Keywords—Carbamazepine, Intermittent operation mode, Rotating Basket Contactor Reactor, White-rot fungus.

I. INTRODUCTION

CARBAMAZEPINE is an antiepileptic drug known by its recalcitrant nature [1-3] and consequently the lower removal efficiency (usually <10%) through the conventional activated sludge process [4]. It has been detected in various water bodies worldwide [5, 6]. The existing processes capable of more effectively removing this compound mainly include ozonation, advanced oxidation processes, membrane separation, and activated carbon sorption [7-9]. However, the disadvantages of these technologies include the high operational costs and the formation of toxic intermediates or by-products. The biological process able to degrade the contaminant at a low cost has gained an increasing attention as a potent technology to remove the recalcitrant pharmaceutically active compounds.

White-rot fungus has been reported the only microorganism to effectively degrade carbamazepine [10, 11]. However, most studies have been conducted under sterile conditions [11, 12]. Moreover, the removal efficiency for this compound varied, from around 20% to higher than 90% [12, 13]. It should also be noted that even though a satisfactory performance has been achieved under non-sterile conditions, it was still difficult to maintain the performance for a long-term due to the bacterial contamination [14]. Rodarte-Morales et al. [12] obtained a removal efficiency as high as 93% for carbamazepine through a stirred tank reactor operated with free pellets of \textit{P. chrysosporium} under the continuous feed mode. However, the stable removal performance was maintained for 55 days and then decreased subsequently.

It is important to keep the activity and dominance of white-rot fungi over the bacterial overgrowth in order to maintain a long-term stable performance in the bioreactor. The important role of the external carbon source in the \textit{P. chrysosporium} growth was confirmed by Kirk et al. [15]. They reported \textit{P. chrysosporium} would neither grow nor degrade the lignin if no co-substrate (glucose or cellulose) added due to the low energy produced from the lignin biodegradation. As an aerobic microorganism, oxygen is crucial to the \textit{P. chrysosporium} growth and development [16]. Leisola et al. [17] indicated that the partial oxygen limitation was the main reason for the slow and incomplete degradation of lignin by \textit{P. chrysosporium} in non-agitated cultures.

The immobilization of white-rot fungi shows many advantages. The immobilized cultures tend to have a higher level of activity and are more resilient to the environmental perturbations such as pH or the exposure to toxic chemicals concentrations than the suspended cultures. The cell immobilization would reduce the apparent broth viscosity and make the rheological features more favorable for oxygen supply and mass transfer. Another advantage of cell immobilization is the reduction in the protease activity and the contamination risk [18].

The objective of this study is the establishment of a proper bioreactor configuration for the white-rot fungus growth under non-sterile conditions to further improve the removal of carbamazepine from a synthetic wastewater by enhancing the enzyme production.

II. MATERIALS AND METHODS

A. Microorganism

The white-rot fungus strain \textit{P. chrysosporium} BKM-F-1767 was purchased from Guangdong Institute of Microbiology (China). The strain was transferred and subcultured onto a fresh PDA medium plate which consisted of 20 g glucose, 3 g KH$_2$PO$_4$, 1.5 g MgSO$_4$, 0.1 g CaCl$_2$, 8 mg thiamine, and 20 g agar in 1 L of 20% potato extract. The spores formed on the plate were scraped and prepared into a spore suspension with the concentration of 15 × 10$^6$/mL through the dilution. The spore suspension was stored in a refrigerator until use.

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B. Chemicals
Carbamazepine was purchased from Sigma-Aldrich (USA). The stock solution (1.0 g/L) was prepared in methanol and kept at 4°C. All other chemicals used were of analytical grades.

C. Carrier
The polyurethane foam cubes (density, 74 kg/m³) were used as the support for the P. chrysosporium immobilization. Polyurethane foam is considered an excellent support for the microbial immobilization due to its stability, mechanical strength, and high porosity for the immobilized cells [19].

D. Rotating Basket Contactor Reactor
Twelve baskets made of stainless steel mesh were uniformly distributed on a stainless disk (diameter, 14 cm). The size of each basket was 3.5 cm × 6.0 cm × 4.0 cm, filled with 1.0 × 1.0 × 1.0 cm³ polyurethane foam cubes immobilized with P. chrysosporium. An electric shaft rotated at 0.44 m/min drove the disk. The run and stop time of the disc could be controlled through the shaft connected to an intermittent switch (GB-60, Jinlong Electrical, China). The synthetic wastewater was pumped from an inlet tank into a reaction tank and the treated effluent was discharged into another storage tank.

There were two phases involved in the whole operational period, the comparison study during the first ten days and the continuous experiment during the remaining period. For the comparison study, both run time and stop time of the bioreactor were set at 5 min during the first five days, while the stop time was extended to 15 min during the next five days. The removal performances were compared between the two operational modes. Based on the optimized results, the continuous experiment in the second phase was carried out for around 160 days with the synthetic wastewater continuously fed into the bioreactor. The feeding glucose concentration was cut from 10 to 5 g/L on Day 74 and further cut from 2.5 to 1 g/L on Day 118. The hydraulic retention time (HRT) was three days. No biomass was replenished or discharged from the reactor during the continuous operation.

E. Analyses
The concentration of carbamazepine was measured using a Dionex UltiMate 3000 HPLC system (Thermo Scientific, USA) equipped with an Acclaim™ C30 reversed-phase HPLC column (5 μm, 4.6 × 150 mm) and a Dionex UltiMate 3000 diode array detector. The column temperature, detection wavelength, and injection volume were set at 30°C, 220 nm, and 100 μL, respectively. The mobile phase was composed of acetonitrile and deionized water buffered with 50 mM phosphate solution (pH 3.0) at 3:7 (v/v) and the eluent went through the column at the flow rate of 0.5 mL/min.

The manganese peroxide (MnP) activity was measured, according to the method described by Tien and Kirk [20] with modifications. Ten milliliters of the reaction system contained 0.4 mM guaiacol, 0.2 mM MnSO₄, and 0.1 mM H₂O₂ added to trigger the chemical reaction. The pH value in this system was adjusted to 4.5, buffered with 50 mM sodium succinate. After the reaction at 30°C for 5 min, the changes in optical density (OD) were recorded at 465 nm using a spectrophotometer (UV Mini-1240 Spectrophotometer, Shimadzu, Japan). One unit (U) of the MnP activity was defined as the amount of enzyme required to oxidize 1 μmol of Mn⁴⁺ to Mn³⁺ per minute.

Chemical oxygen demand (COD), total nitrogen (TN), and ammonia nitrogen were measured according to the standard HACH method. The pH values were measured using an S220 Seven Compact™ pH meter (Mettler-Toledo Group, Switzerland). The supernatant obtained from the centrifugation of mixed liquid was used for the bacterial plate counting and the spread plate technique followed the standard method [21].

III. RESULTS AND DISCUSSION
A. Optimization of Operational Conditions
The operational mode of run/stop cycle favors the treatment performance of the bioreactor. The polyurethane foam cubes filled the basket with high porosity (the average porosity in each basket was around 60%), good for the circulation of liquid and air flows. When the basket was rotating in the liquid medium, the liquid would flow into the basket while the air was squeezed out. On the other hand, when the basket was leaving the medium surface, the liquid would flow out the basket by gravity while the air immediately filling up. Therefore, the rotation would make P. chrysosporium immobilized on polyurethane foam cubes experiencing the nutrient and oxygen supply regularly. After the basket rotated out of the medium surface, the free water in the pores between the carriers aggregate would flow out until only a thin layer of liquid left between biofilm and air, thus the resistance of oxygen transfer into the biofilm could be reduced significantly.

The removal efficiency for carbamazepine achieved under 5 min run/15 min stop (mode II) was approximately 20% higher compared to under 5 min run/5 min stop (mode I), 93.00 ± 21.51% vs. 71.00 ± 11.20%. There were statistically significant differences in carbamazepine removal efficiency (0.0024) between modes I and II. A longer stop time means a longer contact time between white-rot fungus and oxygen in the air. It is well known that the lignin degradation is an oxidative process, requiring a high O₂ level [16]. Since a nitrogen-limited culture medium was used in this study, in case of the excessive carbon source, the mass transfer of oxygen would be counter checked due to the excessive extracellular amylase excreted by the white-rot fungus [22]. This problem can be solved through the application of a longer stop time since it can provide more contact between microorganism and oxygen. As a result, the enzymatic production of white-rot fungus could be improved [12] and a relatively high removal efficiency for carbamazepine was achieved.

B. Bioreactor Performance
To our best knowledge, there has been no study reporting about the PhACs removal using a white-rot fungus enhanced rotating biological contactor (RBC). Vasiliadou et al. [23] treated a synthetic wastewater spiked with four kinds of PhACs including carbamazepine by a two stages activated sludge-RBC, with the total removal efficiency for
carbamazepine at 49.4% under 4-day HRT. In the current study, after one month of adaptation period, the removal efficiency for carbamazepine reached >90%. However, the removal efficiency decreased from Day 74 when the glucose loading in the influent was cut from 10 to 5 g/L, and on Day 75-118, the average removal efficiency for carbamazepine was 86.29 ± 3.29%. Starting from Day 118, the feeding glucose concentration further decreased from 2.5 to 1.0 g/L and the carbamazepine removal efficiency also further declined to 75.36 ± 10.18% (Fig. 1). This result is in a good agreement with some other studies in terms of the effect of the feeding carbon source on the contaminant removal performance through the white-rot fungus reactor. Pakshirajan and Kheria [24] found the maximum decolourization efficiency (83%) was achieved when 10 g/L of glucose was used in the media through an RBC treating the textile wastewater. The system performed poorly in decolorizing the wastewater without any supplementation of carbon source. The decolourization efficiency was poor when the media contained glucose at concentration <5 g/L. Blanquez et al. [25] also found the decolourization efficiency dropped from around 70 to 60% when the feeding glucose concentration was cut from 3 to 2 g/L through a Trametes versicolor enhanced RBC for treating the textile wastewater.

The average residual TN concentration was 36.23, 280.50, and 86.51, 84.38, and 86.47%, respectively (Fig. 2). During the periods of Day 31-74, Day 75-118, and Day 119-160, the average COD removal efficiencies were 86.51, 84.38, and 86.47%, respectively (Fig. 2).

After one month of fungal adaptation, the COD removal efficiency reached 85% and remained at this level even when the concentration of the feeding carbon source was gradually reduced. During the periods of Day 31-74, Day 75-118, and Day 119-160, the average COD removal efficiencies were 86.51, 84.38, and 86.47%, respectively (Fig. 2).

On the other hand, there was a completely different performance occurred in case of the TN removal. During the first month, TN was almost completely removed (removal efficiencies >95%) and after that, it began to gradually decline. The average residual TN concentration was 36.23, 280.50, and 140.64 mg/L, respectively, during Day 31-74, Day 75-118, and Day 119-160. This phenomenon has also been observed in other studies. Cruz-Morato et al. [26] found the concentration of ammonium nitrogen increased under the nutrient scarcity condition in a batch fluidized bed reactor inoculated with T. versicolor. Zhang and Geißen [27] also observed the nitrogen accumulation in the degradation of carbamazepine and attributed to the lysis of fungal mycelia under starvation conditions.

Fig. 1. The residual concentrations of carbamazepine (-■-) and the corresponding removal efficiencies (-○-) using the rotating basket contactor reactor in a continuous operation.

Fig. 2. The variation of the residual concentrations of COD (-●-) and TN (-Δ-) in the reactor effluent.

There was a gradual decline in pH value of the effluent before Day 74, dropping from nearly 4.0 to around 2.5, and then increased slowly. The average pH values were 4.6 and 6.2 during the periods of Day 75-118 and Day 119-160, respectively. The activity of the extracellular enzyme remained at a relatively low level during the whole period. The detected activity of MnP was always <3.5 U/L. The low activity of enzymes was also observed by other studies in terms of operating the continuous white-rot fungus reactor under non-sterile conditions [24, 27, 28]. In addition, the bacterial counting also showed the viable bacterial number was continuously decreased to <2.0 × 10⁸ CFUs/mL during the reactor operation under non-sterile conditions.

IV. CONCLUSION

A rotating basket reactor immobilized with P. chrysosporium on the polyurethane foam cubes (1.0 × 1.0 × 1.0 cm³) was continuously operated for around 160 days under the non-sterile condition, to remove carbamazepine from a synthetic wastewater. More than 90% removal efficiency was achieved with the glucose addition (10 g/L) into the influent as the external carbon source, with the 5 min run/15 min stop operational mode. The removal efficiency dropped about 15% when the glucose loading was gradually reduced to 1 g/L.

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