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# A feasible method for growing fungal pellets in a column reactor inoculated with mycelium fragments and their application for dye bioaccumulation from aqueous solution

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ABSTRACT

In the present paper, a feasible method was developed to grow fungal pellets in an air lift column reactor inoculated with mycelium fragments for improving separation effect of biomass from solution and reducing clogging effect of biomass; bioaccumulation of dye by the growing fungal pellets in the case of mycelium fragments inoculation was investigated. The results showed that inoculation with the mycelium fragments without any pre-treatment did not witness the formation of pellets; only pre-treated fragments using maize as both nucleus and carbon source for 72 h incubation guaranteed the formation of pellets in the air lift column reactor. Nearly 100% of dye removal was obtained by bioaccumulation of the growing pellets in successive three batches of dye wastewater treatment. The formation of pellets not only resulted in low clogging effect to promote mass transfer and dye bioaccumulation but also caused quick separation of dye-loaded biomass from treated wastewater.



## 1. Introduction

Among industrial wastewater, dye wastewater from textile and dye production is one of the most difficult treatment (Fu and Viraraghavan, 2001). Adsorption has been proved to be the most promising option for the removal of dyes from aqueous streams (Aksu, 2005). Biosorption of dyes from aqueous solution by microbial biomass is a promising potential alternative to conventional adsorption processes (Crini, 2006) because of fastness, low cost, easy availability, easy operating conditions and high efficiency in detoxifying very dilute or concentrated effluents (Aksu, 2005). A wide variety of microorganisms including bacteria, fungi and yeasts are used for the biosorption of a broad range of dyes (Aksu, 2005). It has long been recognized that the use of nonviable biomass, i.e. the resting and dead ones, is more advantageous for dye biosorption as the nonviable biomass is not affected by toxic wastes and doesn't require a continuous supply of nutrients (Crini, 2006). However, in recent years the growing biomass has been drawing more attention for dye bioaccumulation as it allows simultaneous realization of biomass growth and dye removal in the same step, leading to simpler system control and lower operational cost (Aksu, 2003; Renganathan et al., 2006; Wang and Hu, 2008).

Up to now, there are a great number of reports on the biosorption/bioaccumulation of dyes by a wide variety of microorganisms, but this process has only been tested for limited practical applications on dye removal from effluents (Crini, 2006). Most of the past works focused on condition optimization, kinetic, thermodynamic and reaction mechanisms involving biosorption/bioaccumulation of dyes in lab scale as basic theory researches (Fu and Viraraghavan, 2001; Aksu, 2005). Few reports were available to settle the two major problems regarding practical industrial application, i.e. the solid–liquid separation and the clogging effect (Aksu, 2005; Crini, 2006; Gupta and Suhas, 2009). In many cases, the powdered or dispersed form of microbial biomass was utilized for dye biosorption in shaking flasks and the centrifugation was used routinely for the separation of biomass after biosorption, which were not generally practical in the industrial processes (Aksu, 2005). In order to solve these problems, the microbial biomass was immobilized in a supporting material, which could improve biomass performance, increase mechanical strength and facilitate separation of biomass from pollutant containing solution (Aksu, 2005; Crini, 2006). However, the immobilization not only increased the cost of biomass pre-treatment but also affected the mass transfer kinetics of organics uptake (Aksu, 2005; Crini, 2006). Recently, the self-immobilized microbial aggregates, namely, both aerobic granular sludge and anaerobic granular sludge, were attempted for biosorption of dyes due to their excellent settle ability, dense and porosity microbial structure (Gao et al., 2010; Sun et al., 2011). However, as a mixture of various microbial species, the granular sludge generally had low adsorption capacity (Gao et al., 2010) and a high cost

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modification was required for improving the biomass performance (Sun et al., 2011).

The filamentous fungus such as *Penicillium* sp., *Aspergillus* sp., *Trichoderma* sp. and so on could grow by way of self-immobilization to form fungal pellets with excellent settling ability, facilitating the separation of biomass from effluents and eliminating the clogging effect without any cost (Sumathi and Manju, 2000; Renganathan et al., 2006). In recent years, there were quite a few reports on bioaccumulation of dyes by a variety of growing fungal pellets (Sumathi and Manju, 2000; Renganathan et al., 2006; Ranjusha et al., 2010; Tastan et al., 2010; Taskin and Erdal, 2010). However, the dye bioaccumulation by growing fungal pellets was carried out in shaking flasks with spores inoculation which was not generally practical in industrial processes. Because sorption column permits simultaneous realization of biosorption and separation of the immobilized biomass beads or the granular biosorbents from treated wastewater as well as the low shear force of the sorption column is favorable to maintain integrity of the biosorbents, the sorption column is thought to be the most effective device for continuous operations (Aksu, 2005). So far, there were few reports on dye bioaccumulation performance by growing fungal pellets in column reactor which was necessary for industrial application of the process (Aksu, 2005). Lately, the bioaccumulation of Cu-complex reactive dye by the growing fungal pellets of *Penicillium oxalicum* in an air lift column reactor was realized successfully, fully demonstrating the advantages of fungal pellets in improving solid–liquid separation efficiency and decreasing clogging effect (Xin et al., 2010). Although the previous works moved the first step towards the practical application of this process, the spores inoculation to form fungal pellets still posed a great obstacle for its industrial popularization and wide application (Xin et al., 2010). The spores are generally obtained by solid fermentation method, which is complicated, costly and low efficient, especially when expensive agar is used for preparing the solid slant as a matrix in lab. Therefore, the spores inoculation cannot meet the need of practical application in industrial scale. On the contrary, the mycelium fragments derived from dye-loaded pellets as excess sludge after dye bioaccumulation can be obtained in large quantities without any cost. As a result, formation of fungal pellets in a column reactor inoculated with mycelium fragments, namely, formation of cycle of pellets–mycelium fragments–pellets, is very necessary for practical application of this process. However, there was no report available in literature regarding formation of fungal pellets inoculated with mycelium fragments in a column reactor.

In this paper, *Trichoderma* sp. and Acid Brilliant Red B were used respectively as tested biosorbent and model dye. The objectives of the present studies were (i) to develop a feasible method for growing pellets inoculated with mycelium fragments in an air lift column reactor; (ii) to realize dye bioaccumulation by the growing pellets in the case of fragments inoculation in the column reactor; (iii) to assess the performance of the growing pellets in improving settle ability and reducing clogging effect.

## 2. Methods

### 2.1. Chemicals and strain

The model dye Acid Brilliant Red B with maximum absorbance wavelength ($\lambda_{max}$) at 524 nm was presented by Taixing Jinji Dyestuff Co. Ltd. whose purity was more than 99% and the chemical structure was shown in Fig. S1 in Supplementary material. All the other chemicals were analytical grade and purchased from Beijing Chemical Industry. *Trichoderma* sp. (AS 3.4263) as tested biosorbent was purchased from China General Microbiological Culture Collection Center.

### 2.2. Media and culture of the fungus strain

The media composition and detailed procedure for growing the spores in slants and the pellets in shaking flasks were described in the previous paper (Xin et al., 2010).

### 2.3. Preparation of the mycelium fragments and their pre-treatment

#### 2.3.1. Preparation of the mycelium fragments

The fresh wet pellets with diameter of about 3.0–4.0 mm were taken from the shaking flasks, and then crushed with a mortar and a pestle for 3–5 min to obtain the length of mycelium fragments less than 0.2 mm using optical microscopes. The mycelium fragments suspensions were prepared by adding different volume of saline solution to required turbidities.

#### 2.3.2. Pre-treatment of the mycelium fragments

The mycelium fragments were pre-treated in a half-solid medium containing maize, millet or potato in powdered form as both solid carbon sources and nucleus. The half-solid medium contained: powdered solid carbon sources, 100 g; $NH_4Cl$, 1 g; $KH_2PO_4$, 1 g; $MgSO_4{\cdot}7H_2O$, 0.5 g; deionized water, 1000 ml; pH 5.5. The half-solid medium of 500 ml was inoculated with the newly prepared mycelium fragments (10%, w/v) and incubated for a maximum period of 72 h in a self-made beaker fermentor with rod-heater at 37 °C, loop ventilation of air bubble by air pump at 1.0 l/min at the bottom of the beaker and mechanical agitation at 2 cm below the liquid level at a speed of 60 rpm. The 60 °C-drying maize, millet or potato were ground and sieved to obtain a mesh size of less than 0.15 mm as the powdered solid carbon sources.

### 2.4. Assessment of pellets formation and clogging effect in column reactor inoculated with the mycelium fragments with pre-treatment or not

The detailed structure and operation parameters of the air lift column reactor were described previously (Xin et al., 2010). Five hundred milliliter of liquid media was prepared and placed into the air lift column reactor without aseptic technique. Four successive experiments were conducted to develop a feasible way for formation of fungal pellets inoculated with mycelium fragments in the column reactor. (i) Mycelium fragments suspension at a turbidity of 400 FTU (Formazin Turbidity Unit) was inoculated at a rate of 5% (v/v) into the air lift column reactor; (ii) pre-treated mycelium fragments using different solid carbon sources as nucleus after 72 h of incubation were inoculated at a rate of 3% (v/v) into the column reactor; (iii) pre-treated mycelium fragments from different incubation periods with powdered maize as nucleus were inoculated at a rate of 3% (v/v) into the reactor; (iv) varied concentrations of pre-treated mycelium fragments with powdered maize as nucleus after 72 h of incubation were inoculated into the reactor. During the experiments, the samples were taken from the reactor, the mycelium was photographed to judge whether the fungal pellets were formed or not; the turbidity of solutions after pellets sank was measured with turbidity meter (HI93703-11, HANNA, Italy) to reflect roughly the mycelium biomass in dispersed form and its clogging effect. All the fungus biomass both in the dispersed form and in the pellet form was collected, washed and dried to determine the dry weight according to the previous paper (Xin et al., 2010). In the case of inoculation with mycelium fragments suspension, the controls were run without any inoculation; in the case of inoculation with pre-treated mycelium fragments, the controls were run with inoculation of mycelium fragments. All the experiments, including the controls, were carried out in triplicate.