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# Comparative isocline analysis upon microbial decolorization in immobilized cell bioreactor using biocarriers

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## HIGHLIGHTS

- Unveil novel screening criteria for packing materials of cell immobilization.
- Present novel isocline-analysis upon performance of wastewater treatment.
- Disclose hydrophobicity and bed porosity as dominated factors to select matrices.

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## ABSTRACT

This study used various biocarriers (e.g., porites corals, Biolite™, porous ceramic filter media (PCFM)) to immobilize cells in fixed bed bioreactor (FBB) for wastewater decolorization. As prior studies proposed, an innovative graphical method of constant-slope isoclines to determine maximal allowable treatment capacity (MATC) was used as screening criteria for feasibility of packing matrices of immobilized cell systems (ICSs). Moreover, detailed inspection upon physical and chemical characteristics of packing matrices was also carried out to confirm the consistency of MTAC. The result of isocline analysis was in parallel with physical characteristics of biocarriers (i.e., porites coral > Biolite™ > PCFM). This first-attempt study successfully provided perspective in general terms to assess how the selected supporting materials were suitable to be packing matrices of ICSs for industrial applications (e.g., wastewater treatment).



## 1. Introduction

Due to the development of textile dyeing and paper-printing and food industry, residual dyes present in discharged wastewater from dyestuff or textile industry were a significant threat to the environment. Since physico-chemical processes apparently required to provide sufficient energy and supplemented chemicals and generated secondary effluent problems (e.g., formation of toxic by-product(s)) for treatment, their economic feasibility seemed to be not so promising. Therefore, bioremediation methods to treat dye-related problems provide several attractive advantages: promising environmental friendliness, appealing economic feasibility, less sludge production, nearly complete mineralization (Banat et al., 1996; Rai et al., 2005). That was why biological treatment was popularly being considered for wastewater treatment (e.g., dye-decolorization). For example, Novotný et al. (2012) investigated the degradation potential of myriads of dyes by fungal strain- *Dichomitus squalens* in biofilms and rotating biological contact reactors. Khouni et al. (2012) also showed the biological decolorization performance of textile dyes (e.g., blue bezaktiv S-GLD 150) using a sequencing batch reactor inoculated with acclimatized microbial consortia. Moreover, Singh et al. (2012) investigated ligninolytic enzyme activities of fungi (e.g., *Agaricus bisporus*, *Pleurotus sajorcaju* and *Volvariella volvacea*) attached onto different supported materials for textile dye-bearing wastewater treatment.

Among all emerging methods of biotreatment, immobilized cell systems (ICSs) could greatly improve the efficiency of bioreactor operation without risk of cell washout; for example, increasing process stability and tolerance to shock ladings, allowing higher treatment capacity per unit biomass and generating relatively less biological sludge. Thus, using reusable support materials for packing of ICSs is technically viable and environmentally sound to cradle-to-cradle wastewater decolorization. Of course, once candidate microbes with promising capability of dye decolorization were chosen, selecting most appropriate packing matrices for ICSs to maximize decolorization efficiency is evidently crucial to system

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optimization for biodecolorization. As known, ICSs could be popularly used for "high-cell density" biotreatment in several aspects (e.g., wastewater decolorization, whole-cell bioproduction), simple and plausible kinetic modeling to describe and predict transient dynamics of ICSs was still remained open to be explored for possible uses in industrial applications. According to Chen et al. (2009b) and Chen (2007), an innovative graphical method of constant-slope isoclines to determine maximal allowable treatment capacity (MATC) as shown herein was used as screening criteria for treatment feasibility of packing matrix of ICSs. Moreover, detailed inspection upon physical and chemical characteristics of packing matrices was also carried out to confirm the consistency of MTAC. This first-attempt study tended to provide perspective in general terms (e.g., constant-slope isocline analysis) to assess how the selected materials were biologically suitable to be packing matrices of ICSs for industrial applications (e.g., wastewater treatment).

Regarding comparison of characteristics of support matrices for immobilized cells in ICSs (Ludwicka et al., 1984), Tarjányi-Szikora et al. (2012) compared abiotic and biotic properties of three different biocarriers (i.e., Biolite™, Perl™ and zeolite) used to treat municipal wastewater. Their findings showed that biofilm formed on Biolite™ and Perl™ filter owned higher dehydrogenase activities and greater total nitrogen removal capabilities than natural zeolite. Moreover, Nacheva et al. (2008) also contrasted seven different natural and artificial materials as packing materials of biological aerated filter for domestic wastewater treatment, revealing removal performance of COD, TN, $NH_4^+$-N in terms of physical and chemical properties of carriers. In addition, Tziotzios et al. (2007) used two packing materials in immobilized beds for phenol biodegradation, indicating the association of specific surface area of packing matrices and the mode of operation on the performance of biodegradation. However, to the best of our knowledge, there are no reports in literature quantitatively revealing the correlation of overall biotreatment with abiotic/biotic characteristics in ICSs for conclusive remarks. This first-attempt study tended to introduce constant-slope isocline analysis to disclose whether candidate packing matrices could be well performed for biotreatment or not. Moreover, comparison of these novel analyses with physico-chemical characteristics of packing matrices was carried out to confirm the promising technical feasibility as isoclines-evaluation predicted.

## 2. Methods

### 2.1. Microorganism and cultivation method

The model dye-decolorizing bacterium-indigenous *Aeromonas hydrophila* (Chen et al., 2009a,b; Hsueh et al., 2009) was predominantly isolated from a fountain spring in Lin-Mei of northeast Taiwan. *A. hydrophila* was first cultivated aerobically at 30 °C using Luria-Bertani (LB; Difco) broth medium. The LB broth (Difco) consisted of tryptone (10 g $L^{-1}$), yeast extract (5 g $L^{-1}$) and NaCl (10 g $L^{-1}$). A loopful of *A. hydrophila* seed taken from an isolated colony on a LB-streak plate was precultured in 50-mL LB broth for 12 h overnight at 30 °C, pH 7.0, 125 rpm using a water-bath shaker (SHINKWANG, SKW-12). Then, 1% (v/v) precultured broth was inoculated into fresh LB broth for 24-h culture in order to be used for cell immobilization. Note that the pH was not controlled for all cultures. As showed in previous studies (Chen et al., 2009c), 0.3 × LB was the most biologically favorable for optimal attachment of immobilized cells. Therefore, 0.3 × LB (i.e., 30% LB medium, but NaCl concentration still remained 10 g $L^{-1}$ for preventing osmotic pressure to cells) was used as culture medium for the work. The determination of cell and dye concentration was described elsewhere (Chen et al., 2009c).

### 2.2. Cell Immobilization and dye decolorization

The ICSs for biological filter used herein was made of acrylic plexiglass, with the inner diameter of 5.4 cm, outer diameter of 6 cm, and the internal packing height of 34.5 ± 0.2 cm (refer to Chen (2007) for system schematics). Three kinds of packing materials-porites corals (obtained from Pacific Ocean side of Hualien County in eastern Taiwan; specific gravity 2.60 and pH ~8.37), Biolite™ (Degremont), porous ceramic filter media (PCFM, We-Je Applied Material Co., Ltd.) were used in the biological-filter column as biological carriers for immobilized cells (packing weight of 790.84 g for porites corals, 686.60 g for Biolite™, and 293.43 g for PCFM). To inspect inhibitory effects of packing carriers upon *A. hydrophila* for cell immobilization, biotoxicity assessment upon biocarriers was also conducted via respirometric measurement using automated Columbus Micro-Oxymax Respirometer equipped with $CO_2$ sensors (Chen et al., 2012). However, the result revealed that toxicity potency of packing matrices was not significant (data not shown). That is, these packing materials were feasible carriers for ICS operation.

First, the packing matrix was washed with sterile tap water to remove solid particles and then successively washed for overnight (O/N) with deionized-and-distilled water. Regarding packing matrices, moist–heat sterilization was carried out to eradicate microbiota present within pores of packed materials. Three kinds of biocarrier materials were also dewatered in 121 °C oven until constant dry weight (±3%) was approximately achieved. The packing matrix was thus filled in randomly into column bioreactor. To ensure complete sterilization prior to cell immobilization, total recycle of 3% $H_2O_2$ (ca. 50 mL $min^{-1}$ in total volume 1.0 L) was conducted for two days.

#### 2.2.1. Cell-immobilization step

After complete sterilization through total recycle of 3% $H_2O_2$, 10 L sterile deionized-and-distilled water was continuously pumped into the ICS at 50 mL $min^{-1}$ for total washout of residual $H_2O_2$. In addition, sterile conditions were frequently confirmed via traditional plate-count techniques. Prior to experiments, precultured cells (ca. 600 mL) were then drawn into the operation system for cell immobilization via total downflow recycle at ca. 60 mL $min^{-1}$. For column operation, temperatures were controlled at 25 ± 1.0 °C. Immobilization step was continuously taking place for 2 weeks to ensure cells stably entrapped onto packing materials. Since cells in the logarithmic growth phase would attach faster onto carriers than the cells in the stationary growth phase (Prieto et al., 2002), approximately 300 mL cell broth was thus replaced by fresh, sterile LB medium every 3 days for two-week period of cell immobilization.

#### 2.2.2. Decolorization step

To provide better attachment onto packed matrix for decolorization in the ICS, 0.10 g $L^{-1}$ $MgSO_4$ and 10 mL $L^{-1}$ glycerol were also amended in the inlet dye-bearing stream. Note that supplementation of $MgSO_4$ and glycerol was the most biologically viable to have maximal capacities of immobilized cells on the packing matrix (data not shown) since such augmentations might trigger effective expression of some vital enzyme(s) for cell growth/immobilization and reductive decolorization (also refer to Chen (2007)). During decolorization, constant hydraulic residence time (ca. 10 h) was applied to upflow-packed bed reactors. To exclude effects of non-biotic decolorization (i.e., adsorption), Phase (I) (i.e., Fig. 2 in Chen et al. (2009b)) of operation in a designated manner of staircase was applied prior to the Phase (II) (Fig. 2 in Chen et al. (2009b)) for practical decolorization. To inspect the maximal capacity for biodecolorization, staircase ramp input of dye concentration was applied as indicated in Phase (II) (Fig. 2 in Chen et al.