Desalination and Water Treatment
www.deswater.com
doi: 10.1080/19443994.2015.1060173

57 (2016) 13534–13539
June

Taylor & Francis
Taylor & Francis Group

Common filamentous *Trichoderma asperellum* for effective removal of triphenylmethane dyes

Sze Ying Chew, Adeline Su Yien Ting*

School of Science, Monash University Malaysia, Jalan Lagoon Selatan, Bandar Sunway, Petaling Jaya 46150, Selangor, Malaysia, email: syche54@student.monash.edu (S.Y. Chew), Tel. +603 5514 6105; Fax: +603 5514 6184; emails: adeline.ting@monash.edu, adelsuyien@yahoo.com (A.S.Y. Ting)

Received 12 February 2015; Accepted 2 June 2015

ABSTRACT

The dye-removal potential of a common environmental isolate *Trichoderma asperellum* on four triphenylmethane dyes was studied via biosorption experiments using free-cells and alginate-immobilized forms. *T. asperellum* demonstrated potential to remove TPM dyes, with higher efficiency when alginate-immobilized forms were used compared to free-cells. Amount of crystal violet (CV), methyl violet (MV), cotton blue (CB), and malachite green (MG) adsorbed by immobilized forms were 60.64, 50.29, 49.91, and 16.61 mg g^{-1} compared to 12.97, 12.54, 14.34, and 11.44 mg g^{-1} by free-cells, respectively. Enhanced dye removals by alginate-immobilized *T. asperellum* were attributed to the carboxyl and hydroxyl functional groups present on the surface of alginate. For free-cells, their surface comprises amine and alkane groups which were less effective in binding dyes. This study is the first to report the dye-removal potential of *T. asperellum* for triphenylmethane dyes, and we recommend immobilized forms as suitable for application.

Keywords: Alginate immobilization; Biosorption; Dye removal; *Trichoderma asperellum*; Triphenylmethane dyes

1. Introduction

Triphenylmethane (TPM) dyes are one of the most important groups of synthetic dyes used extensively in the textile, leather, food, pharmaceutical, cosmetic, and paper industries [1]. These dyes escape into the environment via untreated wastewater effluents, gradually accumulating to a hazardous level affecting living organisms and the environment. TPM dye molecules are typically made-up of benzidine and other aromatic structures, which renders them highly toxic and recalcitrant to natural degradation [2–4]. In the early days, physico-chemical approaches were implemented as measures to remove dye residues from the environment, which include chemical oxidation, membrane filtration, ion exchange, and electro-kinetic coagulation [5]. These approaches were gradually of concern as they result in the generation of toxic waste (sludge), as well as incurring high operational costs [3,5]. As an alternative, the biological approach using biosorbents is explored to manage TPM dyes in wastewaters.

Among the many types of biosorbents available, microbial biosorbents (bacteria, algae, yeast, and filamentous fungi) are highly preferred compared to plant residues or inert materials [6]. Microbial

*Corresponding author.

biosorbents have low production cost, are easy to handle, and amenable for upscale production [7,8]. Filamentous fungi as microbial biosorbent have many advantages. The surface of fungal biomass is typically a mosaic of functional groups [amide ($–NH_2$), carboxyl (–COOH), hydroxyl (–OH), phosphate (PO_4^{3-}), and thiol (–SH) groups] [9]. This surface heterogeneity presents a negatively charged surface which allows for efficient biosorption of dyes to the cell surface. Another advantage of using fungal biomass as biosorbents is that it is economically feasible, as either freshly prepared biomass or spent biomass (derived from food and pharmaceutical industries) can be used [10–12].

The use of fungal biosorbents is however, limited by their small particle size, low mechanical strength, and poor density, which led to clogging of water systems [9,13]. To mitigate this, cell immobilization is introduced to immobilize or entrapped fungal cells within or on an inert matrix [14]. Polyvinyl alcohol, polysulfone, polyurethanes, agar, gelatine, cellulose-derived polymers, and alginate are the examples of commonly used polymer matrices [15]. Of the many matrices, alginate polymers are highly favoured as they are relatively cheaper, easily prepared [13], have minimal clogging problems, and are amenable to solid-liquid separations [4]. Alginate-immobilized biosorbents often demonstrate enhanced biosorption activities, attributed to improved cross-linking of functional groups and having more binding sites for the dye molecules.

In this study, the isolate *Trichoderma asperellum* was evaluated for potential in removing TPM dyes. The four TPM dyes selected were common pollutants in the environment, nevertheless are the less studied compared to other dyes such as azo and anthraquinone dyes. Both free-cells and alginate-immobilized cells of *T. asperellum* were developed and tested for efficient removal of the TPM dyes. The isolate *T. asperellum* has to date, demonstrated metal-tolerant characteristics [16] and excellent potential in removing Cu(II) in alginate-immobilized forms [17]. Therefore, by establishing potential to remove TPM dyes further enhances the role of *T. asperellum* as fungal biosorbent for both dye and metal removal.

2. Material and methods

2.1. Preparation of dye solutions

The four TPM dyes used were crystal violet (CV) (Merck), methyl violet (MV) (Merck), cotton blue (CB) (Sigma-Aldrich), and malachite green (MG) (Friedemann-schmidt). Stock solutions (1,000 mg L^{-1}) of each dye were prepared using milliQ (Sartorius) water and diluted to 100 mg L^{-1} for subsequent experiments. The initial pH for all dye solutions was adjusted to pH 5 ± 0.2 with 0.5 M HCl and NaOH.

2.2. Preparation of biosorbents

The fungal isolate *T. asperellum* was isolated by [16] from the Penchala River (Kuala Lumpur, Malaysia). Culture was initiated by inoculating five mycelial plugs of *T. asperellum* into 250 mL of potato dextrose broth (Difco) and incubated for 5 days (150 rpm, 30 ± 1˚C). The fungal biomass was recovered by filtration (Whatman No. 1) and divided into two parts; one for preparation of free-cell biosorbents, the other for alginate immobilization. The biomass meant for free-cell biosorbent was autoclaved (121˚C, 20 min), followed by oven-drying (50 ± 1˚C, overnight), grinding, and sieving (particle sizes ≤ 250 μm). The fungal biomass (free-cells) was stored at room temperature (25 ± 3˚C) until use. For alginate immobilization, the filtered biomass (2.5 g) was suspended in 50 mL of milliQ (Sartorius) water, homogenized (LabGEN 125 homogenizer), and gradually mixed with 50 mL of sodium alginate (Sigma) (4%, w/v) [17]. The mixture (2.5% fungal biomass and 2.0% Na-alginate) obtained was autoclaved (121˚C, 20 min) and after cooling was extruded into 200 mL of 3% w/v $CaCl_2$ solution in a drop-wise manner (using a 35-mL syringe). The $CaCl_2$ solution was stirred constantly to prevent aggregation of alginate beads. The beads were cured in the $CaCl_2$ solution for 1 h at room temperature (25 ± 3˚C). The procedure was repeated by substituting fungal biomass with sterile-distilled water to produce plain alginate beads (2% w/v) (control). All beads were kept in 1.0% w/v $CaCl_2$ solution at 4 ± 0.5˚C until use.

2.3. Biosorption studies

Free-cells of *T. asperellum* (0.25 g) were added into 50 mL of CV solution (100 mg L^{-1}) (subsequently repeated with 100 mg L^{-1} of MV, MG, and CB) and incubated (30 ± 2˚C, 200 rpm). At 15, 30, 60, 120, 240, 360, and 480 min, 0.5 mL of dye solution was sampled, centrifuged (microcentrifuge Thermo Scientific, 20 s) and the supernatant collected for spectrophotometric analysis. The absorbance was read at 590 nm (MV at 584 nm, MG at 617 nm, CB at 599 nm) using a microplate reader (TECAN). Absorbance values obtained were compared against standards constructed using 2–10 mg L^{-1} of respective dyes. The amount of dye

adsorbed by the biosorbents was calculated using the following equation:

$$q_t = [C_0 - C_t]\, V_t/M$$

where q_t: adsorption of respective dye per gram of biosorbent (mg g^{-1}) at time t; C_0: initial dye concentration (mg L^{-1}); C_t: dye concentration at time t (mg L^{-1}); V_t: volume of dye solution; M: dry weight of biosorbent (g). The experiment was repeated using 1.0 g of alginate-immobilized *T. asperellum* and 1.0 g of plain alginate beads to substitute the free-cells.

2.4. FTIR analysis

The FTIR analysis was performed to characterize the functional groups present on the surface of free-cells, alginate-immobilized *T. asperellum*, and plain alginate beads. The biosorbents were first oven-dried (50°C, overnight) and ground to powder form. Single-reflection attenuated total reflection spectra (within 400–4,000 cm^{-1}) were acquired using the FTIR spectrophotometer (Thermo Scientific Nicolet 1810, diamond crystal) conducted under ambient temperature. For each biosorbent tested, a total of 16 scans were collected, averaged and the resolution determined at 4 cm^{-1}. Data were collected within the range of 4,000–700 cm^{-1} to eliminate interference.

2.5. Statistical analysis

The experiments were conducted in triplicates, and the results presented as mean values. Data collected were analyzed with one-way ANOVA and means compared using Tukey's Test ($HSD_{(0.05)}$). Analysis was performed using the software Statistical Package for the Social Sciences (SPSS) (version 20.0) (IBM).

3. Results and discussion

3.1. Biosorption studies

This study revealed the potential of *T. asperellum* in removing TPM dyes, an accomplishment for a common environmental isolate that is lesser studied for dye removal. Immobilized *T. asperellum* has higher removal efficiency for CV (60.64 mg g^{-1}), MV (50.29 mg g^{-1}), CB (49.91 mg g^{-1}), and MG (16.61 mg g^{-1}) compared to free-cells (12.97, 12.54, 14.34, and 11.44 mg g^{-1}, respectively) (Fig. 1). Free-cells demonstrated rapid dye uptake in the first 15–30 min with the maximum biosorption attained by 60 min. However, there was no increase in dye uptake thereafter (Fig. 2). It is postulated that the amount of dye adsorbed by free-cells of *T. asperellum* may be limited by the available binding sites on the cell surface. In the absence of alginate polymers, the available binding sites on the cell surface would have been exhausted by 60 min. A similar observation indicating limited functional groups on biomass were associated with the gradual stationary trend in dye uptake was reported by [18]. On the contrary, alginate-immobilized *T. asperellum* required a longer time to achieve maximum dye uptake (240–360 min) and recorded higher dye-removal rate by the end of 480 min compared to free-cells (Fig. 2). Higher biosorption efficiency observed in immobilized biosorbents was attributed to the presence of the polymer matrix (alginate), which allowed for more complex cross-linking of functional groups [18]. The benefits of alginate immobilization were evident in the removal of CV, MV, and CB by alginate-immobilized *T. asperellum* and plain alginate beads (40–70 mg g^{-1}) compared to free-cell forms (15–20 mg g^{-1}). The recalcitrant dye MG was also efficiently removed by immobilized *T. asperellum* and plain alginate beads, due to the prominent presence of alginate. Evidently, the presence of alginate with its complex cross-linking binding sites interacts well with the different steric conformation and charge distribution of the dye molecules, leading to efficient dye removal [3,18]. Immobilization is, therefore, pertinent to efforts in improving dye biosorption.

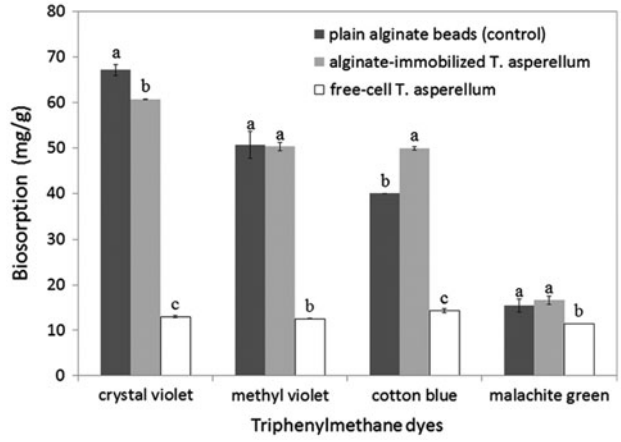

Fig. 1. Dye removal via biosorption by free-cell *T. asperellum*, alginate-immobilized *T. asperellum*, and plain alginate beads (control) on crystal violet (CV), methyl violet (MV), cotton blue (CB), and malachite green (MG). Means with the same letters for the same dye indicates no significant difference of biosorption by biosorbents according to Tukey comparisons ($HSD_{(0.05)}$).

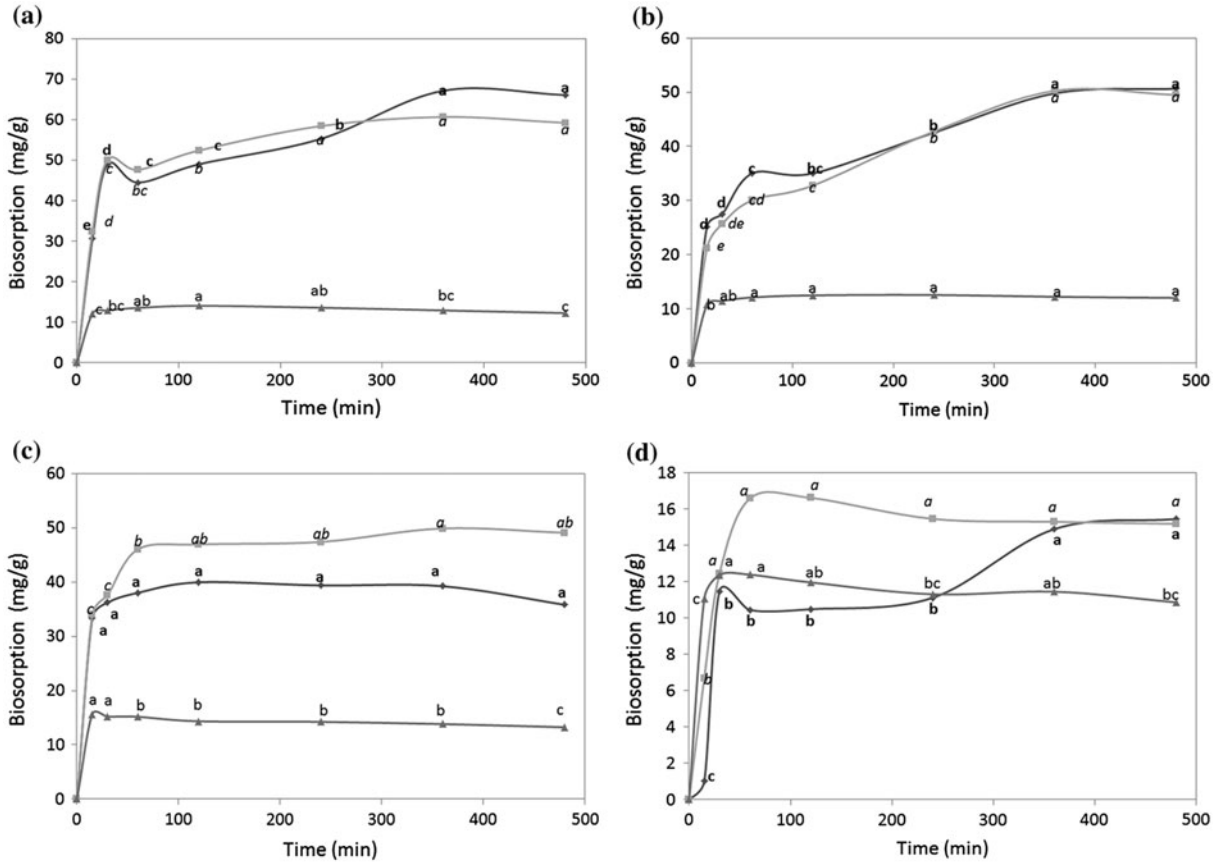

Fig. 2. Biosorption of (a) crystal violet (CV), (b) methyl violet (MV), (c) cotton blue (CB), and (d) malachite green (MG) by free-cells of *T. asperellum* (▲), alginate-immobilized *T. asperellum* (■), and plain alginate beads (control) (♦) at various time intervals. Means with same letters and caption for the biosorbent indicates no significant difference of biosorption at time intervals according to Tukey comparisons ($HSD_{(0.05)}$). Biosorption conditions include 100 mg/L dye concentration, pH 5.0 ± 0.2, 30 ± 2°C, and 200 rpm.

The amount of CV, MV, and MG removed by immobilized *T. asperellum* was similar to plain alginate beads, with the exception of CB (49.91 compared to 39.97 mg g^{-1}, respectively) (Fig. 1). The rate of dye removal for alginate-immobilized *T. asperellum* and plain alginate beads was similar; demonstrating rapid biosorption within the first 60 min, and maximum biosorption by 60 min (for CB and MG) and 360 min (for CV and MV) (Fig. 2). The lack of synergism between biomass and alginate polymers in removing TPM dyes such as MV, MG, and CV were contradictory to metal removal studies which reported that the presence of biomass contributes to more efficient metal removal compared to plain beads [17,19,20]. Nevertheless, this study is the first to reveal the removal of TPM dyes by *T. asperellum*. This is particularly interesting as it proposes the alternative use of non-white rot fungi as opposed to the well-known lignolytic fungi [21] for removal of dyes.

3.2. FTIR analysis

Free-cells have more functional groups present on their surfaces compared to alginate-immobilized *T. asperellum* and plain alginate beads (Fig. 3). Functional groups on free-cells comprise a variety of hydroxyl (–OH) (#1), amine (–NH_2) (#2), alkane (–CH_2– and CH_3–) (#3), and ester lipid (C=O) groups (Fig. 3), but alginate-immobilized *T. asperellum* and plain alginate beads have mostly hydroxyl (–OH) (i), carboxyl (–COOH), and other minor entities (ii–ix) (Fig. 3). These functional groups, particularly hydroxyl (–OH) and carboxyl (–COOH) groups, originate from the alginate matrix and were consistently detected in alginate-immobilized *T. asperellum* and plain alginate beads (Fig. 3). The complexity of functional groups on free-cells and immobilized *T. asperellum* agrees with the heterogenous nature of functional groups on organic biomass proposed by [22]. These groups are

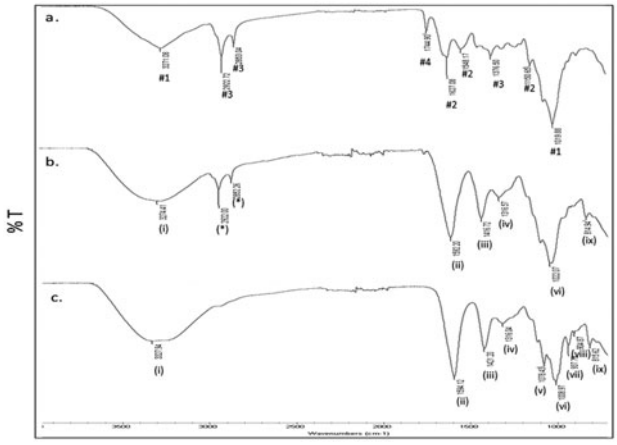

Fig. 3. FTIR spectra for (a) free-cell *T. asperellum*, (b) alginate-immobilized *T. asperellum*, and (c) plain alginate beads. For free-cell *T. asperellum*, #1 denotes hydroxyl, #2 for amine, #3 alkanes, and #4 for other groups. For alginate-immobilized *T. asperellum* and plain alginate beads, the presence of the following groups are highlighted (i) ν(OH), (ii) $\nu(COO)_{asym}$, (iii) $\nu(COO)_{sym}$, (iv) δ(CCH) + δ(OCH), (v) ν(OCO) ring (shoulder), (vi) C=O stretching vibrations, (vii) C–O stretching of uronic acid, (viii) C1–H deformation of mannuronic acid residues, (ix) mannuronic acid residues, and (*) symmetric and asymmetric CH_2– and CH_3– stretching vibration.

established dye-binding sites present in natural biomass and in polymers [23] and thus are evidently the key functional groups contributing to enhanced dye-removal efficacy compared to free-cell forms. The FTIR analysis did not quantify the number of functional groups present; nevertheless, it is assumed that the presence of alginate polymer may have enhanced the cross-linking of these functional groups with inert groups on biomass, leading to more available binding sites for greater removal of dye molecules. The various functional groups present on the surface of *T. asperellum* and alginate enhance dye-binding as they attract both the cationic (CV, MV, MG) and anionic (CB) TPM dyes. The positively charged functional group (=N^+H–) and anion (SO_3^-) in CB allows for the interaction of negatively charged and some positively charged functional groups on the biosorbents. Similarly, cationic dyes (CV, MV, MG) due to the presence of the amine groups (=N^+H– or $N^+(CH_3)_2$) bind to the negatively charged functional groups such as hydroxyl and carboxylic acid groups (COO^-) [24].

4. Conclusion

This study is the first to document the potential of a common filamentous fungus, *T. asperellum*, for the removal of TPM dyes from aqueous solutions. Alginate-immobilized *T. asperellum* has better biosorption of TPM dyes than free-cells, attributed primarily to the predominance of negatively charged carboxylic acid (COO^-) and hydroxyl (–OH) groups on the surface of the alginate polymer. The dye-removal potential of *T. asperellum*, established in this study, adds value to the use of *T. asperellum* as a biosorbent to remove both dyes and metals (determined in previous study).

Supplementary material

The supplementary material for this paper is available online at http://dx.doi.10.1080/19443994.2015.1060173.

Acknowledgments

This project supports a main project funded by the Ministry of Higher Education (MOHE) under the FRGS scheme (FRGS/2/2013/STWN01/MUSM/02/2). The authors are also grateful to Monash University Malaysia for the facilities to conduct the study.

References

[1] W. Azmi, R.K. Sani, U.C. Banerjee, Biodegradation of triphenylmethane dyes, Enzy. Microb. Technol. 22 (1998) 185–191.
[2] I.M. Banat, P. Nigam, D. Singh, R. Marchant, Microbial decolorization of textile-dyecontaining effluents: A review, Bioresour. Technol. 58 (1996) 217–227.
[3] V. Prigione, G.C. Varese, L. Casieri, V.F. Marchisio, Biosorption of simulated dyed effluents by inactivated fungal biomasses, Bioresour. Technol. 99 (2008) 3559–3567.
[4] K. Vijayaraghavan, Y.S. Yun, Bacterial biosorbents and biosorption, Biotechnol. Adv. 26 (2008) 266–291.
[5] T. Robinson, G. McMullan, R. Marchant, P. Nigam, Remediation of dyes in textile effluent: A critical review on current treatment technologies with a proposed alternative, Bioresour. Technol. 77 (2001) 247–255.
[6] M.T. Sulak, H.C. Yatmaz, Removal of textile dyes from aqueous solutions with eco-friendly biosorbent, Desalin. Water Treat. 37 (2012) 169–177.
[7] Z. Aksu, Application of biosorption for the removal of organic pollutants: A review, Process Biochem. 40 (2005) 997–1026.
[8] S. Chowdhury, P. Saha, Sea shell powder as a new adsorbent to remove Basic Green 4 (Malachite Green) from aqueous solutions: Equilibrium, kinetic and thermodynamic studies, Chem. Eng. J. 164 (2010) 168–177.
[9] M.Y. Arica, C. Arpa, B. Kaya, S. Bektaş, A. Denizli, Ö. Genc, Comparative biosorption of mercuric ions from aquatic systems by immobilized live and heat-inactivated *Trametes versicolor* and *Pleurotus sajur-caju*, Bioresour. Technol. 89 (2004) 145–154.

[10] E. Luef, T. Prey, C.P. Kubicek, Metal ion resistance in fungi: Molecular mechanism and their regulated expression, J. Cell. Biochem. 45 (1991) 30–40.
[11] E. Fourest, J.C. Roux, Heavy metal biosorption by fungal mycelial by-products: Mechanisms and influence of pH, Appl. Microbiol. Biotechnol. 37 (1992) 399–403.
[12] L. Svecova, M. Spanelova, M. Kubal, E. Guibal, Cadmium, lead and mercury biosorption on waste fungal biomass issued from fermentation industry. I. Equilibrium studies, Sep. Purif. Technol. 52 (2012) 142–153.
[13] D. Park, Y.S. Yun, J.M. Park, The past, present, and future trends of biosorption, Biotechnol. Bioprocess Eng. 15 (2010) 86–102.
[14] S.K. Mehta, J.P. Gaur, Use of algae for removing heavy metal ions from wastewater: Progress and prospects, Crit. Rev. Biotechnol. 25 (2005) 113–152.
[15] T. Akar, Z. Kaynak, S. Ulusoy, D. Yuvaci, G. Ozsari, S.T. Akar, Enhanced biosorption of nickel(II) ions by silica-gel-immobilized waste biomass: Biosorption characteristics in batch and dynamic flow mode, J. Hazard. Mater. 163 (2009) 1134–1141.
[16] A.S.Y. Ting, C.C. Choong, Bioaccumulation and biosorption efficacy of *Trichoderma* isolate SP2F1 in removing copper (Cu(II)) from aqueous solutions, World J. Microbiol. Biotechnol. 25 (2009) 1431–1437.
[17] W.S. Tan, A.S.Y. Ting, Efficacy and reusability of alginate-immobilized live and heat-inactivated *Trichoderma asperellum* cells for Cu (II) removal from aqueous solution, Bioresour. Technol. 123 (2012) 290–295.
[18] S. Luo, X.J. Li, L. Chen, J.L. Chen, Y. Wan, C.B. Liu, Layer-by-layer strategy for adsorption capacity fattening of endophytic bacterial biomass for highly effective removal of heavy metals, Chem. Eng. J. 239 (2014) 312–321.
[19] Y. Yalçınkaya, L. Soysal, A. Denizli, M.Y. Arıca, S. Bektaş, Ö. Genç, Biosorption of cadmium from aquatic systems by carboxymethylcellulose and immobilized *Trametes versicolor*, Hydrometallurgy 63 (2002) 31–40.
[20] T. Tsuruta, Biosorption and recycling of gold using various microorganisms, J. Gen. Appl. Microbiol. 50(4) (2004) 221–228.
[21] D.S.L. Balan, R.T.R. Monteiro, Decolorization of textile indigo dye by ligninolytic fungi, J. Biotechnol. 89(2–3) (2001) 141–145.
[22] J.L. Wang, C. Chen, Biosorbents for heavy metals removal and their future, Biotechnol. Adv. 27 (2009) 195–226.
[23] A. Srinivasan, T. Viraraghavan, Decolorization of dye wastewaters by biosorbents: A review, J. Environ. Manag. 91 (2010) 1915–1929.
[24] J. Shi, N.M. Alves, J.F. Mano, Drug release of pH/temperature-responsive calcium alginate/poly(N-isopropylacrylamide) semi-IPN beads, Macromol. Biosci. 6 î(2006) 358–363.