Biosorption of nonylphenol on dead biomass of *Rhizopus arrhizus* encapsulated in chitosan beads

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**Abstract**

The nonylphenol (NP) biosorption and desorption potential for fungal biomass used under batch conditions was investigated using kinetics and isotherm models. Fungal biomass of *Rhizopus arrhizus* TISTR 3810 exhibited preferential uptake of NP, an endocrine disrupting chemical. Sporangiospores, asexual spores, were immobilised in chitosan beads. The biosorption data of NP on the moist heat inactivated *R. arrhizus*–chitosan beads were analyzed using four popular adsorption isotherms and, by using non-linear least-regression with the solver add-in in Microsoft Excel, correlated in order with the Fritz–Schluender > Redlich–Peterson > Freundlich > Langmuir isotherms. The pseudo first-order kinetics was found to have the best fit with the experimental data. The diffusivity of NP in the *R. arrhizus*–chitosan beads was calculated using the shrinking core model, and the diffusivity values were in the ranges of $2.3736 \times 10^{-4} - 1.8950 \times 10^{-5} \text{ cm}^2 \text{s}^{-1}$. Desorption to recover the adsorbed NP from the beads was performed in methanol and was best described using a pseudo second-order kinetic model.

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**1. Introduction**

Nonylphenol (NP) is one of the organic pollutants found in aquatic environments as a consequence of the biodegradation of nonylphenol polyethoxylate (NPnEO), a non-ionic surfactant contained in industrial cleaning products and in household detergents (Ahel et al., 1993). Although the parent surfactant itself is less toxic, NPnEO released into the environment rapidly decomposes to form NP which is the most recalcitrant intermediate of NPnEO decomposition. Principally NP persists in sewage treatment plants and outflows (Nakada et al., 2006), and tends to accumulate in the fatty tissue of aquatic organisms when released into aquatic environments.

NP is known to be an endocrine disruptor compound, that is, it mimics endogenous hormones and disrupts important life processes. Substantial evidence exists to demonstrate that NP causes various disorders of the male reproductive system, including reduced testicular size and lower sperm production in rainbow trout (Nakada et al., 2006), and outflows (Nakada et al., 2006), and tends to accumulate in the fatty tissue of aquatic organisms when released into aquatic environments.

The nonylphenol (NP) biosorption and desorption potential for fungal biomass used under batch conditions was investigated using kinetics and isotherm models. Fungal biomass of *Rhizopus arrhizus* TISTR 3810 exhibited preferential uptake of NP, an endocrine disrupting chemical. Sporangiospores, asexual spores, were immobilised in chitosan beads. The biosorption data of NP on the moist heat inactivated *R. arrhizus*–chitosan beads were analyzed using four popular adsorption isotherms and, by using non-linear least-regression with the solver add-in in Microsoft Excel, correlated in order with the Fritz–Schluender > Redlich–Peterson > Freundlich > Langmuir isotherms. The pseudo first-order kinetics was found to have the best fit with the experimental data. The diffusivity of NP in the *R. arrhizus*–chitosan beads was calculated using the shrinking core model, and the diffusivity values were in the ranges of $2.3736 \times 10^{-4} - 1.8950 \times 10^{-5} \text{ cm}^2 \text{s}^{-1}$. Desorption to recover the adsorbed NP from the beads was performed in methanol and was best described using a pseudo second-order kinetic model.

Although the techniques of adsorption on activated carbon, photo-oxidation and ozone treatment for NP removal have been studied and found to be effective, cost-effectiveness is the main limitation against widespread practical use (Nevskaia and Guerre-ro-Ruiz, 2001; Kawasaki et al., 2001). The search for a low cost and easily available adsorbent has led the authors to investigate materials of agricultural and biological origin, and some industrial by-products as adsorbents.

Biosorption, the passive uptake of pollutants by non-growing or non-living microbial biomass such as bacteria, fungi and algae, is considered to have good potential for the removal of non-degradable pollutants from aqueous solution (Kapoor and Viraraghavan, 1995). Reports of biosorption of toxic organic compounds with biomass showed that better removal was obtained with dead biomass than with live biomass (Aksu, 2005). Immobilisation may also allow higher biomass concentrations, and facilitate separation and non-destructive recovery of biomass from pollutant-bearing solution (Fu and Viraraghavan, 2001). Immobilised fungal biomass has been used for the removal of various metals, dyes and some organic pollutants (Kapoor and Viraraghavan, 1995; Aksu, 2005) but no study has been conducted on the use of immobilised biomass for the removal of NP or other endocrine disruptor compounds. Therefore, there is a need to study the performance of the immobilised fungus system with NP.

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Nomenclature

\[ V \] volume of aqueous phase (L)
\[ M \] mass of biomass (g)
\[ q_c \] NP uptake rate at equilibrium (mg/g)
\[ q_e \] Langmuir adsorption isotherm constant (mg/g) (dependent on the maximum adsorption capacity of biosorbent)
\[ q_{\text{exp}} \] experimental NP uptake (mg/g)
\[ K_D \] desorption rate constant (s^{-1})
\[ q_{\text{cal}} \] theoretical NP uptake (mg/g)
\[ q_{\text{cal}} \] RMS root mean square

Greek letters

\[ \alpha, \beta \] Redlich–Peterson isotherm constants (dimensionless)
\[ \alpha_{\text{R}} \] Redlich–Peterson isotherm constant (mg/g)
\[ \alpha_{\text{P}} \] Redlich–Peterson isotherm constant (L/g)
\[ \beta \] Redlich–Peterson isotherm constant (dependent on the heterogeneity of the binding surface)

Chitosan, (1→4)-2-amino-2-deoxy-\( \beta \)-D-glucan, produced on an industrial scale by the alkaline deacetylation of chitin, one of the most abundant biopolymers in nature, was used in this study as the supporting material for living biomass immobilisation. It should be noted that the use of chitosan for living cell immobilisation has a limitation due to its anti-microbial activity (Helander et al., 2001). However, the fungal resting spores used in this study are tolerant to chitosan gelling because of their extra thick cell wall.

This study presents the biosorption characteristics of NP on dead chitosan-immobilised fungal beads, referred to as "dead beads". Suitable isotherm and kinetic models fitted by non-linear regression using the ‘solver’ add-in with Microsoft Excel 2007 are proposed. Also, the reusability of the dead beads, that is the stabil- ity to repeated adsorption–desorption cycles, is discussed in reference to their feasibility for industrial use.

1.1. Adsorption equilibrium

The capacity of an adsorbent can be described by the equilibrium isotherm, which is characterised by certain constants whose values express the surface properties and affinity of the adsorbent.

1.1.1. The Langmuir sorption isotherm

This model assumes an ideal, totally homogeneous adsorption surface with a finite number of binding sites and a few interactions between adsorbed molecules (Aksu and Balibek, 2007) is given by:

\[ q_c = \frac{q_a b C_e}{1 + b C_e} \]  

(1)

1.1.2. The Freundlich sorption isotherm

This model is suitable for a highly heterogeneous, energetic distribution of active sites, with interactions between adsorbed molecules (Aksu, 2001; Aksu et al., 2007), and is given by:

\[ q = K_F C_e^n \]  

(2)

1.1.3. The Redlich–Peterson sorption isotherm

This model is a combination of the Langmuir and Freundlich models and is given by Eq. (3). \( \beta \) lies between 0 and 1. For \( \beta = 1 \) the Redlich–Peterson model converts to the Langmuir model (Aksu, 2001)

\[ q = \frac{K_e C_e}{1 + a_{\text{P}} C_e^\beta} \]  

(3)

1.1.4. The Fritz–Schluender sorption isotherm

This isotherm for single-component systems more flexible to fit the experimental data since it contains more parameters than any other equilibrium isotherm (Fritz and Schluender, 1974; Yang and Al-Duri, 2005). It is usually written as:

\[ q = \frac{K_{\text{SC}} b_{\text{e}}}{1 + a_{\text{SC}} C_e^\beta} \]  

(4)

1.2. Adsorption kinetics

1.2.1. Kinetic models

Two simplified kinetic equations, from the pseudo first-order and pseudo second-order kinetic models initially proposed by Lagergren (1898) and Ho and McKay (2000), respectively, were used for a wide range of solute-sorbent systems.

1.2.1.1. Pseudo first-order kinetic model (Lagergren’s equation). This model assumes that the biosorption rate is proportional to the number of unoccupied sites on the biosorption surface (Ozdemir et al., 2003). The kinetic model based on solid capacity gives:

\[ q = q_e(1 - e^{-k_1 t}) \]  

(5)

1.2.1.2. Pseudo second-order kinetic model (Ho’s equation). This model assumes the driving force for adsorption rate comes from the square of the difference between \( q \) and \( q_e \). It was transformed to non-linear form, giving as the equation:
\[
q = \frac{q_e^2 K_2 t}{1 + q_e K_2 t}
\]  

(6)

1.2.2. Diffusion models
Sorption kinetics is generally controlled by various factors including (i) bulk diffusion, (ii) film diffusion (external mass transfer), (iii) intraparticle diffusion, and (iv) solute adsorption at active sites by the mechanisms of ion exchange, precipitation, complexation or chelation. Different diffusion models propose different diffusion kinetic mechanisms and rate-controlling steps.

1.2.2.1. Intraparticle diffusion model. The model implies that the diffusion from the surface to intraparticle sites is the only rate-limiting step (Yang and Al-Duri, 2005). The model can be expressed as:

\[
q = k_p t^{0.5}
\]

(7)

The half adsorption time \(t^{0.5}\) is the time required for the adsorbent to take up half of the adsorbate it has at equilibrium. The rate constant, \(k_p\), obtained from the slope of Eq. (11), is related to the intraparticle diffusivity as:

\[
k_p = \frac{6D}{R}
\]

(8)

1.2.2.2. Shrinking core model. This model was applied to fluid-particle chemical reactions (Lavenspiel, 1972) and, more specifically, to solute adsorption by ion exchange, precipitation, complexation and/or chelation. These processes are controlled by liquid film diffusion, with the extent of the sorption being a function of time given by:

\[
X = \frac{3D}{4kC} \alpha
\]

(9)

If the film diffusion is the rate-determining process, a plot of \(X\) versus \(\alpha\) will give a straight line (Preetha and Viruthagiril, 2007). If the process is the particle diffusion control, the model is represented by:

\[
F(X) = 1 - 3(1 - X)^{2/3} + 2(1 - X) = \frac{6D}{R}C \alpha
\]

(10)

Consequently, in this case, a plot of the function \(F(X)\) versus \(\alpha\) will give a linear relationship, and the diffusivity can be obtained from the slope of such a plot using (Preetha and Viruthagiril, 2007):

\[
D = \frac{(slope)}{C0 R^2/6}
\]

(11)

2. Methods

2.1. Materials

2.1.1. Chemicals
NP was purchased from Fluka Chemical Industries, Ltd. It was a mixture of isomers and used without further purification. Chitosan was a gift from TC Union Company, Thailand. All other chemicals used were of reagent grade.

2.1.2. Microorganisms
Five fungal strains, i.e. Rhizopus arrhizus TISTR 3606, R. arrhizus TISTR 3610, Trichoderma harzianum, Aspergillus oryzae and Penicillium sp., available from a microbiological laboratory of Faculty of Liberal Arts and Science, Kasetsart University, were used in this work.

2.1.3. Instruments
Absorbance spectra were recorded on an Ultrospec 3000 UV–vis spectrometer. Freeze-dried samples were prepared using a Biofreezer and DW 3 Lyophlyzer (Scientific promotion Co., Ltd.).

2.2. Methods

2.2.1. Preparation of fungal biomass for NP biosorption
The five fungal strains were inoculated separately into a growth medium, Tryptone glucose yeast extract (TGY), composed of yeast extract (10 g/L), peptone (20 g/L) and dextrose (20 g/L) in distilled deionised water and cultivated in rotary shaking flasks for 4 days. The biomasses were autoclaved at 121.5 °C for 15 min and then harvested by filtering through a membrane filter. These were then washed thoroughly with deionised water and freeze-dried. Each sample of dried dead biomass was ground using a mortar and pestle. Particles passing through a 210 μm sieve were used as fungal powder for NP biosorption.

2.2.2. Pre-treatment of biomass
Five-day old living biomass samples, 5 g wet weight, were pretreated in six different ways: exposure to acid, basic or organic solution, in combination with and without heat treatment as followings: (i) washed and freeze-dried (untreated), (ii) washed and autoclaved for 15 min at 121 °C, (iii) immersed in a 1 M H2SO4 for 1 h washed and freeze-dried, (iv) immersed in a 2 N NaOH for 1 h, washed and autoclaved at 121 °C for 15 min, (v) immersed in a 2 N NaOH and autoclaved at 121 °C for 15 min, (vi) immersed in a 10% formaldehyde for 1 h, washed and freeze-dried and (vii) spread on a membrane filter and dried at 50 °C for 24 h. The treated fungal biomass was then dried and ground according to the above method 2.2.1. The NP biosorption ability of pre-treated samples was also compared with activated charcoal and commercial chitosan powder.

2.2.3. Biosorption study
Stock solution of NP (1.00 g/L) was prepared in methanol and stored under dark conditions. Fungal powder (0.02 g, duplicate samples) was added to 20 mL of the 100 mg/L of NP solution in 10% methanol in 125 mL conical flasks. The flasks were placed on a rotary shaker (100 rpm, 30 °C) for 1 h. Each mixture was filtered through a membrane filter (Whatman No.1), and then extracted with dichloromethane (20 mL) for NP determination. The concentration of NP in the organic layer was determined by comparing absorbance at a wavelength of 275 nm to a standard curve using dichloromethane as a solvent. The NP uptake was calculated by using:

\[
q = \frac{(C_0 - C) \times V}{M}
\]

(12)

In order to optimize the pH for maximum removal efficiency, experiments were conducted in the pH range from 2.0 to 10.0 using 0.02 g of R. arrhizus TISTR 3610 biomass with 20 mL of 100 mg NP/L solutions at room temperature. pH of the adsorbate solution was adjusted to set value with 0.01 M HCl and 0.01 M NaOH at the start of the experiment. It was confirmed through the preliminary experiments that the 6 h was sufficient to attain equilibrium between adsorbent and adsorbate. Each experiment was repeated twice and mean values were taken.

2.2.4. Preparation of the dead beads
The entrainment of R. arrhizus TISTR 3610 sporangiospores produced immobilised fungal bead biosorbents. Chitosan (2 g) was dissolved in acetic acid solution (3% v/v, 9.9 mL), asceptically heated at 100 °C for 5 min, then cooled and mixed with the R. arrhizus spore suspension (0.1 mL, about 3.7 × 10^9 spores/mL). The chito-
san suspension was added dropwise to sterilised sodium tri-poly-
phosphate (2% w/v, 100 mL) and let stand for 3 h to yield stable
2 mm diameter beads which were washed several times with
deionised distilled water. The beads with trapped spores were
transferred into TGY medium and incubated on a rotary shaker
(100 rpm) at 30 °C for 5 days. After this, the chitosan beads with
immobilised fungal mycelia were inactivated by autoclaving at
121 °C for 15 min, removed from the medium by filtration, washed
with distilled water and freeze-dried.

2.2.5. SEM studies
The cross-section and surface of the fungus immobilised beads
were observed by SEM using the preparation method of Pluemsab
et al. (2007).

2.2.6. Batch kinetic experiments
Kinetic experiments were performed in continuously stirred
flasks (100 rpm at 30 °C) containing 100 mL NP solution at differ-
ent concentrations (50–300 mg/L) and 0.1 g of the dead beads.
Optimal solution pH was adjusted to 6.5–7.0, since NP was re-
moved more effectively by R. arrhizus TISTR 3610 biomass. Two
milliliters of samples were withdrawn at regular intervals and ex-
tracted with dichloromethane (2 mL). The organic layer was col-
lected by centrifuging at 3500 rpm at 4 °C. The residual NP
centration was determined by UV-vis spectroscopy at 275 nm
(Ultrospec 3000, Pharmacia Biotech Inc., Thailand). This was done
for duplicate samples of each treatment.

The validity of the isotherm and kinetic models was checked by
plotting Eqs. (1)–(11) with the observed data. The kinetic parame-
ters involved in the models were estimated using non-linear
regression analysis. By trial and error, and using the solver add-in
with Microsoft Excel spreadsheet application, the kinetic param-
ters were determined by minimising the difference between the
experimental data and fitted model.

2.2.7. Desorption studies
Desorption studies were conducted in methanol with equilib-
rium being reached within 90 min. Initially, a known weight of
dead beads was placed in 100 mg/L NP solution overnight, and
the NP adsorbed determined from the difference between the ini-
tial and final NP concentration. The beads were separated from
the solution by vacuum filtration.

The NP-beads were mixed with methanol and kept in sealed
flasks placed in a shaker at 30 °C overnight. The amount of NP des-
orbed was determined by UV-vis absorbance at 275 nm of wave-
lenght. Various desorption kinetic models were considered and
compared against the experimental data for fit. The desorption
process was repeated five times to check repeatability.

The desorption ratio was calculated from the amount of NP ad-
sorbed on the biomass and the final NP concentration in desorption
medium, that is:

\[
\text{Desorption ratio} = \frac{\text{Release nonylphenol (mg)}}{\text{Initially sorbed nonylphenol (mg)}} \times 100
\]  

2.2.8. Statistical analyses
The fit of kinetic expressions to the experimental data was
tested using the value of coefficient of determination, \( r^2 \) (Kumar,
2007), which is defined as:

\[
r^2 = \frac{\sum (q_{cal} - q_{exp})^2}{\sum (q_{cal} - \bar{q})^2 + \sum (q_{exp} - \bar{q})^2}
\]  

Also analyse used was the non-normalised root mean square
(RMS) that weights the actual error at all points (Yang and Al-Duri,
2005), and is defined as:

\[
\text{Non-normalised RMS} = \sqrt{\frac{\sum_{i=1}^{n} (q_{cal} - q_{exp})^2}{N}}
\]  

3. Results and discussion

3.1. Screening of fungal biomass for NP biosorption

Five fungal strains, obtained from the culture collection in a lab-
oratory of the Faculty of Liberal Arts and Science, Kasetsart Uni-
versity, were used for the NP biosorption study. Since NP solubility
in water is extremely low (5.43 mg/L at 20 °C) (Kim et al., 2005), it
was extracted from the water layer with dichloromethane before
analyses. From an initial concentration of 50 mg/L, the amount of
NP absorbed in each fungal mycelia was similar, about 69–76%, as
follows: 68.8 ± 2.1% for R. arrhizus TISTR 3606, 75.9 ± 0.6% for
R. arrhizus TISTR 3610, 70.6 ± 2.1% for T. harzianum, 70.5 ± 0.6% for
A. oryzae, and 69 ± 4% for Penicillium sp. The UV–vis spectra showed
decreases in all three main peaks of NP isomer (Fig. 1). The most
effective NP absorbent was the mycelia of R. arrhizus TISTR 3610,
therefore it was selected as a model of the fungal strains for NP bio-
sorption in this work. Also investigation was the use of non-viable
and viable R. arrhizus biomass to remove phenol and its derivatives
from aqueous solutions in batch reactors (Tsezos and Bell, 1989).

3.2. Pre-treatment of fungal biomass and solution pH for NP
biosorption

Research has shown that some physical or chemical pre-treat-
ment processes can facilitate more efficient uptake of sorbate as
compared to untreated biomass. Six pre-treatments of mycelial
biomass of R. arrhizus were prepared to observe various chemical
and physical effects on both cell walls and intracellular compart-
ments. Heat treatment by autoclave at 121 °C for 15 min was found
to be the most effective for biosorption with an uptake of
73.7 ± 1.0 mg NP/g (Table 1). Treatments with drying at 50
°C for 24 h or soaking in 10% (v/v) formaldehyde for 1 h were also
fairly effective, with NP uptakes larger than that of untreated
biomass at 54 ± 12 mg NP/g. However, the chemical treatments with strong
3.3. Properties of the dead beads

There are a number of immobilisation methods using dead grilled fungal biomass encapsulated in polymer matrix, including calcium alginate and poly(vinyl alcohol) (Wu and Yu, 2007). In the present study, asexual spores of *R. arrhizus* were entrapped into chitosan beads by a liquid curing method in the presence of TPP, and then cultivated for 5 days for complete spore germination. After this period, the hyphae would convert to a sporeulation cycle. The bead surface was found to have homogeneously distributed exuberant mycelia inside, and outward growing mycelia covering the whole particle surface. The dry weight of immobilised fungus in the support was $9.7 \times 10^{-4}$ g/bead. The dried chitosan-immobilised fungal beads had a diameter of approximately 4 mm with a spherical shape which was completely different from the 1–1.5 mm diameter dried plain beads. It also improves the affinity of the biosorbent for the solute by providing directly exposure without any barriers. The dead beads were also stronger and more stable than plain chitosan beads when immersed in acid solution (data not shown), even at pH 1.

3.4. Evaluation of adsorption isotherms

To model NP biosorption and gain a better understanding of the sorption process, the four adsorption isotherms, Eqs. (1)–(4) were compared to the experimental data for fit. The adsorption isotherm constants with the corresponding correlation coefficients, Eq. (14) and non-normalised RMS, Eq. (15) for each model are presented in Table 2. Although linear regression has been the most commonly used method to obtain the parameters and determine the fit of the kinetic expressions, non-linear regression provides a better measure of fit (Kumar, 2007). As compared to non-linear analysis, the correlation coefficients ($r^2$) of 0.8912 (Redlich–Peterson), 0.9320 (Freundlich) and 0.7561 (Langmuir), except that for the non-linearized Fritz–Schluender, were obtained from the fitting parameters by linear regression. From the results shown in Table 2, the four-parameter isotherm models of Fritz–Schluender gave the best fit with $r^2 = 0.9984$, followed by the three-parameter isotherm models of Redlich–Peterson ($r^2 = 0.9932$), Freundlich ($r^2 = 0.9287$) and Langmuir ($r^2 = 0.9271$). Yang and Al-Duri (2005) also observed the superiority of the Fritz–Schluender model over the other models for biosorption of three reactive dyes onto activated carbon. Based on the Freundlich isotherm plot, the adsorption capacity ($K_F$) was found to be 30.25 mg/g with an adsorption intensity ($n$) of 1.87. These were similar values to those for the biosorption of pentachlorophenol onto live *R. arrhizus* which has a $K_F$ of 32.13 mg/g and an $n$ of 1.79 (Tszeos and Bell, 1989). The high value of the maximum adsorption capacity, $Q_0$ of 312.17 mg/g and the low value of the Langmuir constant, $b$ of 0.05 L/mg were obtained with the Langmuir isotherm. In general, a small value of $b$ indicates that the adsorbate has a high binding affinity for the biosorbent (Ariza et al., 2003). A similar observation was reported for 2,4-dichlorophenol biosorption onto free and calcium alginate immobilised biomass of white-rot fungus *Phanerochaete chrysosporium* with $b$ values of 0.039 and 0.053 L/mg, respectively (Wu and Yu, 2007). The values of $K_F$ and $Q_0$ also indicate the great affinity for NP by the beads in this study.

### Table 1

<table>
<thead>
<tr>
<th>Biomass/biosorbent</th>
<th>NP uptake (mg/g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pre-treatment</td>
<td></td>
</tr>
<tr>
<td>Untreated</td>
<td>54.4 ± 11.9</td>
</tr>
<tr>
<td>Autoclaved (121 °C, 15 min)</td>
<td>73.7 ± 1.0</td>
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<tr>
<td>1 M H$_2$SO$_4$ for 1 h</td>
<td>49.8 ± 5.1</td>
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<tr>
<td>1 N NaOH for 1 h</td>
<td>37.1 ± 0.0</td>
</tr>
<tr>
<td>1 N NaOH at 121 °C for 15 min</td>
<td>45.3 ± 3.4</td>
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<tr>
<td>10% (v/v) formaldehyde for 1 h</td>
<td>68.3 ± 0.4</td>
</tr>
<tr>
<td>Dried at 50 °C for 24 h</td>
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### Table 2

| Parameters from fitting the adsorption equilibrium data to various isotherm models. |
|-----------------------------------|-----------------------------------|
| Langmuir constants                |                                    |
| $Q_0$ (mg/g)                      | 312.17                            |
| $b$ (L/mg)                        | 0.05                              |
| $r^2$                             | 0.9271                            |
| Non-normalised RMS                | 20.4090                           |
| Freundlich constants              |                                    |
| $K_F$                             | 30.25                             |
| $n$                               | 1.87                              |
| $r^2$                             | 0.9287                            |
| Non-normalised RMS                | 18.5402                           |
| Redlich–Peterson constants        |                                    |
| $K_P$                             | 180.94                           |
| $a_P$                             | 5.26                              |
| $r^2$                             | 0.9302                            |
| Non-normalised RMS                | 18.4784                           |
| Fritz–Schluender constants        |                                    |
| $K_S$                             | 46.87                            |
| $a_S$                             | 0.67                              |
| $b_S$                             | 0.74                              |
| $b_S/n_S$                         | 0.35                             |
| $K_S/a_S$                         | 70.47                            |
| $r^2$                             | 0.9984                            |
| Non-normalised RMS                | 18.4617                           |

*Note: Parameters determined using non-linear regression in Microsoft® Excel (Microsoft Corporation, USA).*

* Table 1

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* Note: Initial NP concentration of 100 mg/L, performed at 100 rpm, at 30 °C for 1 h.
3.5. Evaluation of adsorption kinetics

The rate constants for the pseudo first-order ($K_1$) and pseudo second-order ($K_2$) sorption reactions were obtained by plotting the non-linear curves fitting Eqs. (5) and (6). Kinetic rate constants for NP uptake by the dead beads are presented in Table 3. From the slope corresponding to a slower adsorption process. Thus, it can be implied that the rate-controlling step of NP adsorption was extremely low solute concentration remaining in the solution, and relates to adsorption of the solute into micropores. The slopes of the linear sections indicate the rates of adsorption, a shallower slope corresponding to a slower adsorption process. Thus, it can be implied that the rate-controlling step of NP adsorption was essentially intraparticle diffusion. In addition, relatively low to moderate levels of initial NP concentration (20.12–189.91 mg/L) induced adsorption into micropores as observed from the nearly parallel third section of the plots. The intraparticle diffusivity ($D$) and intraparticle diffusion rate constant ($k_p$) significantly increase with increased initial NP concentration (Table 4). On the other hand, using a shrinking core model and plotting $F$ against $a$ shows significant linear relationships (Fig. 4b), as compared to plotting $X$ against $a$ (Fig. 4a). The diffusivity values are in the range of $2.37 \times 10^{-4}–1.89 \times 10^{-4}$ cm$^2$ s$^{-1}$. Clearly, this implies that intraparticle diffusion is the rate-controlling step for the NP biosorption in this work. Although intraparticle diffusion and shrinking core models can describe the biosorption process for NP uptake by the dead beads reasonably well, an initial NP concentration was quite limited lower than approximately 200 mg/g (Fig. 4).

3.6. Desorption kinetics

Desorption studies elucidate the mechanism of adsorption and recovery of NP from water, its interaction with the adsorbent. The adsorbed NP remains mostly stable on the adsorbent and can be desorbed using methanol. It can be seen that the desorption process of NP can well described by a pseudo second-order model using a non-linear regression method (Fig. 5). This was evident from the high $r^2$ value of 0.9997 and low non-normalised RMS value of 5.2708 as shown in Table 3. NP desorption by methanol also occurred rapidly ($K_d = 4.98 \times 10^{-3}$ g/mg min). Although, it has been reported that distilled water (Wu and Yu, 2007), CaCl$_2$ solution (Benoit et al., 1998) and NaNO$_3$ solution (Daughney and Fein, 1998) can be used to desorb solutes from phenol-loaded and chlorophenol-loaded biomass, using methanol as the desorbing agent allows for easy regeneration as it can be separated from nonylphenol by vaporisation. The NP waste can then be further degraded in Fig. 2. Adsorption kinetics of nonylphenol by the dead beads described with the pseudo first-order model Eq. (5).

**Fig. 2.** Adsorption kinetics of nonylphenol by the dead beads described with the pseudo first-order model Eq. (5).

![Fig. 2. Adsorption kinetics of nonylphenol by the dead beads described with the pseudo first-order model Eq. (5).](image)

**Fig. 3.** Intraparticle diffusion kinetics for nonylphenol biosorption on the dead beads (multi-linearity) described with Eq. (7).

![Fig. 3. Intraparticle diffusion kinetics for nonylphenol biosorption on the dead beads (multi-linearity) described with Eq. (7).](image)
by chemical or physical treatments (Maniero et al., 2008). The regenerated dead beads are also reusable and, from this study, active in four batches (Fig. 6). The weight loss of dead beads was 43% by the end of the fifth batch use.

4. Conclusion

The use of chitosan-immobilised beads was effective for an innovative treatment of low soluble recalcitrant pollutant from wastewater. The fungal dead biomass could be prepared readily on a commercial scale as an alternative low-cost biosorbent for use in wastewater treatment plants. In this work, the dead beads were pre-treated with moist heat which substantially increased their capacity of NP biosorption. Equilibrated NP adsorption with the dead beads best fitted the Fritz–Schluender isotherm. A maximum monolayer capacity of the dead beads was over 312 mg/g. The rate of NP biosorption was rapid in the first 40 min of contact time. The kinetics for adsorption of NP onto the dead beads was well described by the pseudo first-order equation, while that for desorption was well described by the pseudo second-order one. Both intraparticle diffusion and shrinking core models were found suitably consistent with the adsorption experimental data, especially at the NP concentration less than 200 mg/L. The regeneration of used dead beads with methanol was effective for at least 4 batch cycles in this study. As mentioned, the production of the dead beads and their possible reuses provides an alternative biosorbent

<table>
<thead>
<tr>
<th>Initial concentration of NP (mg/L)</th>
<th>Intraparticle diffusion</th>
<th>Shrinkage model</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Slope</td>
<td>k (mg/l min$^{0.5}$)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>20.12</td>
<td>1.21</td>
<td>9.79 x 10$^1$</td>
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</tr>
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<td>189.91</td>
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</tr>
<tr>
<td>245.15</td>
<td>14.70</td>
<td>1.31 x 10$^4$</td>
</tr>
</tbody>
</table>

Bead dosage = 0.1 g, NP solution volume = 100 mL, bead amount = 18 beads.

Note: Parameters determined using non-linear regression in Microsoft® Excel (Microsoft Corporation, USA).

Fig. 4. Shrinkage core model for (a) external film diffusion control and (b) particle diffusion control.

Fig. 5. Comparison of two desorption kinetic models for NP removal by the dead beads. Curve fitting performed with SigmaPlot 11.0 (Systat Software Inc., USA).

Fig. 6. Desorption efficiency of the dead beads after repeated NP biosorption and NP desorption with methanol.
for NP removal, as well as for other hydrophobic aromatic recalcitrant pollutants in wastewater.

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References


