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Kinetics and Isotherms of a Genetically Engineered Saccharomyces cerevisiae EBY100 Strain Expressing Palladium Binding Peptides for the Biosorption of Pd (II) in a Batch Reactor

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Palladium is a rare platinum group metal concentrated in the Earth's core and mantle; because of its scarcity, its waste recovery and reuse options are economically appealing.Traditional strategies for extracting PGMs from effluents have a variety of flaws, prompting researchers to search for novel methods. One such method is biosorption.A novel biosorbent in the form of a genetically engineered strain of *Saccharomyces cerevisiae* EBY100 has been developed for the biosorption of palladium in aqueous solution.The genetically modified *Saccharomyces cerevisiae* EBY100 strain was created to display palladium-binding peptides on its surface. The purpose of this study was to characterize the adsorption of Pd(II) by a genetically modified strain of *Saccharomyces cerevisiae* EBY100 in a batch reactor using isotherm and kinetic studies.The maximum adsorption capacity of transformed S.cerevisiae EBY100 cells for Pd(II) ions was found to be 125 mg/g; adsorption was found to be best described by the Freundlich isotherm and pseudo-second order models.

1. Introduction

Palladium is one of the platinum group metals (PGMs);PGMs are extremely desirable for a range of applications, including manufacturing processes, automotive catalysts, and medical implants due to their unique physical properties (Harding *et al*., 2014).It is comprehensible that there is a rising and ongoing demand for palladium due to the crucial role it plays in various industries; as a result, it is important to manage its reserves. One of the ways in which this can be achieved is by means of its recovery from waste solutions. Solvent extraction, reduction of the metal precipitate by reagents and ion exchange resin adsorption are some of the most commonly used industrial recycling methods for recovering precious metals from wastewaters; however, these methods have the following disadvantages: they are expensive, labour and time consuming, and generate large amounts of secondary waste (Won et al., 2010).Due to the aforementioned disadvantages of these methods, new technologies are being sought after that are less expensive, environmentally friendly and produce less secondary waste when recovering precious metals from waste (Won et al., 2010) . One of these innovations is biosorption.Although the use of biosorption and its significance for the recovery of PGMs are well known, there is still a need for additional research and the development of biosorption systems that are more advanced.
Mashangoane and Chirwa (2022) effectively genetically engineered *Saccharomyces cerevisiae* EBY100 to synthesize the palladium binding peptide, 4R-PtPB, on the surface of the cell utilizing a yeast display vector, pYD5. Transformation settings were adjusted in their study to discover the best transformation conditions for *Saccharomyces cerevisiae* EBY100 cells bearing the pYD5/4R-PdBP as well as the optimal conditions for the expression of 4R-PdBP on the cell surface.The genetically engineered *Saccharomyces cerevisiae* EBY100 cells which exhibited the highest expression levels of the palladium metal binding peptide (4R-PtPB) after being induced in galactose were utilized to conduct biosorption studies; these were annotated P41C.The P41C cells reached a maximum biosorption capacity of 125 mg/g for Pd (II) from an aqueous solution.

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These results were comparable to those of wild type *Saccharomyces cerevisiae*, which reached adsorption capacity values of 23.0 mg/g - 23.3 mg/g (Saitoh et al., 2020) and 0.042 mg/g (Godlewska-Zykiewicz et al., 2019). These findings point to the potential application of 4R-PdBP surface exhibiting *Saccharomyces cerevisiae* EBY100 cells for the recovery of palladium from industrial waste. The objective of this study was investigate the adsorption isotherm and kinetic properties of Pd (II) removal by P41C in a batch reactor. Adsorption isotherm studies are a necessity for proper comprehension and interpretation of adsorption mechanism pathways; this is essential for the general design and improvement of an adsorption system (Rangabhashiyam et al.,2014). The Freundlich and the Langmuir Isotherms were utilized in this study to access the two different isotherms and their ability to correlate the experimental data of the biosorption of Pd (II) onto P41C. On the other hand, adsorption kinetic studies yields information on the adsorption rate, adsorbent performance and mass transfer mechanisms; understanding adsorption kinetics is also critical for designing adsorption systems (Wang and Gou, 2020). The Pseudo-First Order and Pseudo-Second Order models were utilized to analyse the experimental results for purposes of characterizing the adsorption kinetics Pd (II) onto P41C in this study.

2. Materials and Methods

2.1 Preparation of P41C biomass

The expression of Pd (II) binding peptides (4R-PdBP) in P41C cells was achieved by incubating the P41C cells in a galactose-containing media as described in Mashangoane and Chirwa (2022). The induced cells were centrifuged at 3000 x g for 10 minutes at 4°C. The supernatants were discarded and the pellets were resuspended in ultrapure water. The cells were centrifuged again at 3000 x g for 10 minutes at 4°C. The cells were autoclaved at 121°C for 5 minutes to obtain dead biomass and were immediately frozen at -70ºC overnight. The cells were transferred to a freeze dryer (model) for 48hrs; fine powdered biomass obtained. The freeze dried cells were then stored at freezer at -70ºC.

2.2 Preparation of stock solution

The stock solution of Pd (II) (1000ppm) were prepared by dissolving measured quantities of PdCl2 (Merk) respectively in ultrapure water. The stock solution was diluted with ultrapure water to obtain the desired initial Pd (II) concentrations of 10mg/L, 20 mg/L, 30 mg/L and 50 mg/L. The pH level of the solutions was adjusted to 3 using dilute HCl or NaOH.

2.3. Biosorption Equilibrium Isotherms

Equilibrium isotherm studies were conducted at the following varying initial metal ion concentrations; 10mg/L, 20 mg/L, 30 mg/L and 50 mg/L for Pd (II). Experiments were conducted under optimum conditions; i.e. pH of 3, adsorbent (P41C) dosage of 0.1 g at room temperature. The experiments were carried out in duplicates whereby 1ml of samples were taken at pre-determined time intervals over 6 h from 500 ml of metal solutions in 1000 mL Erlenmeyer flasks. The collected samples were analysed for metal concentration using Inductively Coupled Plasma mass spectrometry (ICP, Spectro Arcos FHS12, Boschstroisse, Germany). The Langmuir and Freundlich isotherm models were used to analyse equilibrium isotherm data for purposes of describing biosorption data at equilibrium and showing the correlation between the mass of solute adsorbed per unit mass of sorbent at equilibrium. The amount of metal ions adsorbed per unit biomass was obtained using equation (1):

$$q_e = \frac{(C_0 - C_e)\, x\, V}{m} \quad (1)$$

where q_e is the is the amount of adsorbed metal (mg/g) at equilibrium (mg/g), C_0 and C_e are the initial and final equilibrium metal concentrations (mg/L), V is the volume (L) and m is the mass of the biosorbent (g).

The Langmuir model was represented in the following form:

$$\frac{1}{q_e} = \left(\frac{1}{q_{max}}\right) + \left(\frac{1}{q_{max}.K_L}\right) \quad (2)$$

where q_e is the amount of adsorbed metal (mg/g) at equilibrium (mg/g), q_{max} and K_L are Langmuir isotherm constants which are related to the maximum monolayer sorption capacity (mg/g) and energy or net enthalpy of adsorption (L/mg), respectively.The Freundlich model was represented in the following form:

$$q_e = K_f C_e^{1/n} \quad (3)$$

where q_e is the amount of adsorbed metal (mg/g) at equilibrium, C_e is the equilibrium concentration of the metal in solution (mg/L), K_f and n are Freundlich isotherm constants which are related to biosorption capacity (mg/g) and biosorption intensity respectively.

2.5. Adsorption kinetics

Biosorption kinetics studies are paramount for providing valuable insights into reaction pathways and mechanisms in which adsorption reactions occur. In this study, the kinetics of Pd (II) onto P41C was studied using the pseudo-first and pseudo-second order adsorption kinetic models. The pseudo-first-order kinetic model is expressed as follows:

$$\frac{dq_t}{dt} = k_1(q_e - q_t) \quad (4)$$

where q_t is the concentration of metal ion species on the biosorbent at time t (mg/g), q_e is the concentration of the metal at equilibrium (mg/g) and k_1 is the equilibrium biosorption constant of pseudo-first-order (1/min).
The pseudo-second-order kinetic model is expressed as follows:

$$\frac{dq}{dt} = k_2(q_e - q_t)2 \quad (5)$$

where q_t is the concentration of metal ion species on the biosorbent at time t (mg/g), q_e is the concentration of the metal at equilibrium (mg/g) and k_2 is the equilibrium biosorption constant of pseudo-second-order (g/mg/min).

3. Results and discussion

3.1 Adsorption Isotherms

Adsorption isotherms are derived from analysis of adsorption equilibrium data. The equilibrium data indicated that the biosorption capacity i.e. q_e (mg/g) of P41C for Pd (II) increased with the increase in metal concentration as previously mentioned in Mashangoane and Chirwa (2022). The highest adsorption capacities obtained by P41C for the biosorption of Pd (II) at initial metal concentrations of 10mg/L, 20mg/L, 30mg/L and 50 mg/L were and 40 mg/g, 60 mg/m, 80 mg/g and 125mg/g respectively. The results obtained indicated that the initial metal concentration does have an influence on the biosorption of Pd (II) by P41C cells. According to Sudha *et al*. (2007), at lower metal concentration the ratio of the initial number of metal ions available to the adsorption sites is low; thus the low biosorption capacity of the adsorbent; whereas at higher metal concentration, the available adsorption sites become fewer; thus the dependence of biosorption capacity of P41C on the initial concentrations of Pd (II). The Freundlich and the Langmuir isotherms were utilized in this study to access the isotherm model which best describes the adsorption data obtained. The Langmuir isotherm model is based on an adsorbate monolayer and is only applicable to homogenous surfaces (Syafiqah and Yussof, 2018). The Langmuir isotherm constants q_{max} and K_L are related to the maximum monolayer sorption capacity (mg/g) and energy or net enthalpy of adsorption (L/mg), respectively. Neither homogenous site energies nor restricted adsorption levels are presupposed by the Freundlich model (Syafiqah and Yussof, 2018); this means that the Freundlich model can describe experimental data for adsorption isotherms whether they occur on homogeneous or heterogeneous sites and it is unaffected by the formation of a monolayer (AL-Aoh *et al*., 2012). K_f and n are Freundlich isotherm constants which are related to biosorption capacity (mg/g) and biosorption intensity respectively. The results obtained by the application of the two-parameter models, Langmuir and Freundlich equations are presented in Table 1.

Table 1: Characteristics of isotherm parameters obtained for the biosorption of Pd (II) by P41C

Biosorbent	Isotherm model	Isotherm Parameter	Isotherm Parameter Values
P41C	Langmuir	R^2	0,826
		q_{max} (mg/g)	148,498
		K_L (L/mg)	0,135
	Freundlich	R^2	0,823
		n	3,496
		K_F ($L^{1/n}$/g.$mg^{1/(n-1)}$)	40,909

The biosorption of Pd (II) by P41C which was best described by the Langmuir isotherm; this was deduced from the correlation coefficient R^2 values of the linearized Langmuir and Freundlich isotherms. The correlation coefficient R^2 for linearized Langmuir isotherm, 0.826, is slightly higher than that for linearized Freundlich isotherm, 0.823 as indicated in figures 2A and 2B respectively.

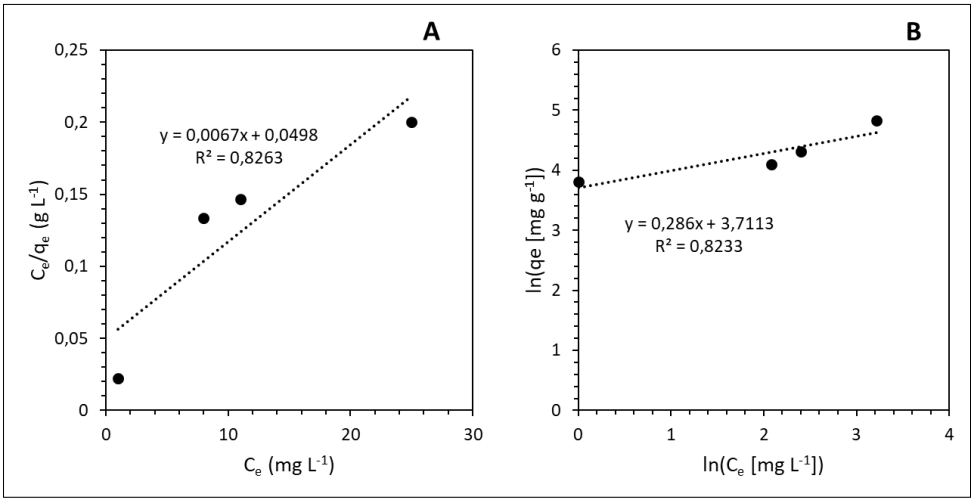

Figure 2: (A) Langmuir, (B) Freundlich adsorption isotherm of Pd (II) onto P41C.

The findings demonstrate that the monolayer adsorption of Pd(II) to P41C occurred on a homogenous surface without any interaction between the adsorbed Pd (II) ions. In addition, the Langmuir isotherm also assumes that all surface sites are alike and can only accommodate one adsorbed molecule, that a molecule's ability to be adsorbed on a given site is independent of its neighboring site occupancy, the adsorbed molecule cannot migrate across the surface or interact with neighboring molecules and adsorption is reversible; (Govindarajan *et al*., 2011); consequently this also applies to adsorption of Pd(II) to P41C. The Langmuir isotherm equation was used to calculate the adsorption constants for the Langmuir isotherm model parameters, qmax and KL, and the results are shown in table 1 as 148.498 mg/g for adsorption capacity and 0.135 mg/mg for adsorption rate, respectively. It is worth noting that the value of q_{max} calculated from the Langmuir model (148.498mg/g), was slightly higher compared to the experimental one (125 mg/g); indicating that more metal ions were required to form the monolayer coverage (Savastru *et al*., 2022).

3.2 Kinetics

Adsorption kinetic studies yields information on the adsorption rate, adsorbent performance, and mass transfer mechanisms; understanding adsorption kinetics is critical for designing adsorption systems. (Wang and Gou, 2020). In this study, the experimental data was analysed to characterize the adsorption kinetics of Pd (II) onto P41C using the pseudo-first order and pseudo-second order models. The values of the obtained of the coefficient of correlation R^2, calibrated adsorption capacity $q_{e\ cal}$ and rate constants K_1 and K_2 obtained from kinetic studies are presented in Table 2.

Table 2: Calculated parameters of the Pseudo-First Order and Pseudo-Second Order kinetic models for the adsorption of Pd (II) by P41C

Kinetic Model	Parameter	Metal: Pd (II) (mg/L)			
		10	20	30	50
	$q_{e\ exp}$ (mg/g)	45.000	60.000	80.000	125.000
	R^2	0.950	0.543	0.759	0.970
Pseudo-First Order	$q_{e\ cal}$ (mg/g)	43.321	57.000	82.000	124.412
	k_1	1.076×10^{-1}	104.947	$4{,}302 \times 10^{-2}$	5.779×10^{-2}
	R^2	0.859	0.804	0.822	0.964
Pseudo-Second Order	$q_{e\ cal}$ (mg/g)	42.000	63.000	75.000	126.894
	k_2	7.343×10^{-5}	2.791×10^{-5}	3.463×10^{-5}	4.282×10^{-5}

Graphical representations of the non-linear pseudo-second-order model for the adsorption kinetics of Pd (II) onto P41C; at initial metal concentrations of 10mg/g, 20mg/g, 30mg/g and 50mg/g; biosorbent dosage of 0.1g, pH 3 and room temperature are indicated in Figure 3.

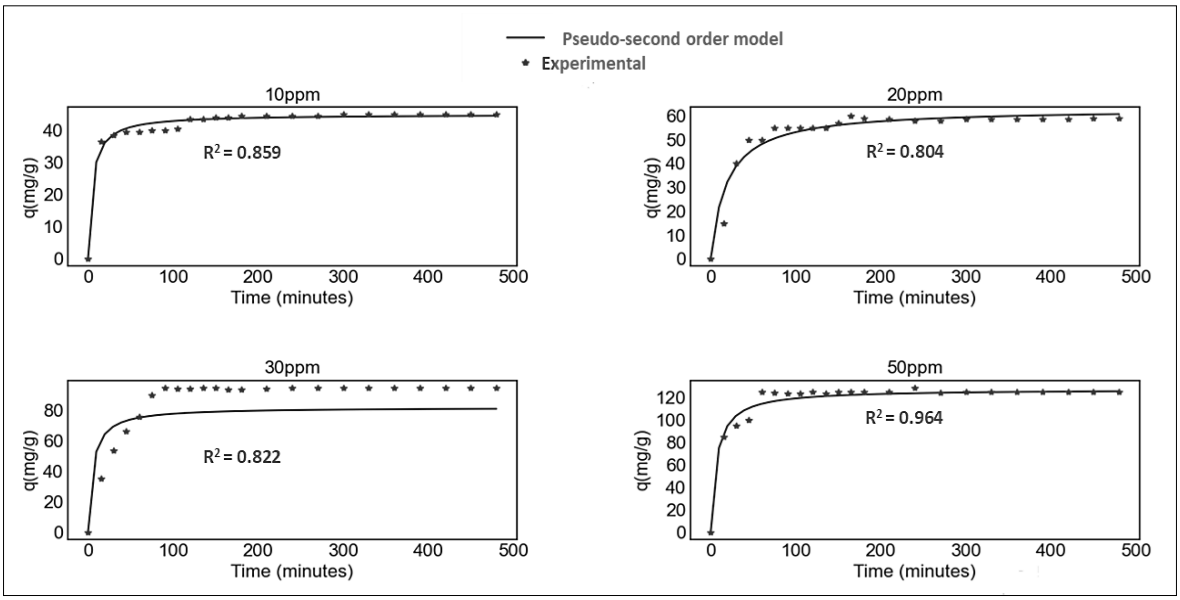

Figure 3: Non-linear representations of the Pseudo-second-order kinetics for the removal of Pd (II) by P41C at initial Pd (II) concentrations of 10 mg/L, 20mg/L, 30 mg/L and 50 mg/; biosorbent dosage of 0.1g, pH 3 and room temperature.

The pseudo-second-order model was found to explain the adsorption kinetics of Pd (II) onto P41C most effectively as indicated by the coefficient of correlation R^2 value in table 2. According to literature the rate-limiting step of the pseudo-second-order model is the surface adsorption that involves chemisorption, whereby the removal of compounds from a solution occurs as a result of physicochemical interactions between the two phases (Wang *et al*., 2007); therefore this applies to the biosorption of Pd (II) onto P41C. It is worth noting that the $q_{e\ cal}$ values were very close to the experimental values for Pd (II); the highest deviation was 6% across all the initial metal concentrations of 10mg/g, 20mg/g, 30mg/g and 50mg/g as indicated in table 2.

4. Conclusions

The adsorption isotherm and kinetic studies were effective in comprehending and interpreting the biosorption mechanism pathways for the adsorption of Pd (II) by P41C. The biosorption experimental results from this study indicated that Pd (II) was adsorbed by P41C on a homogeneous surface via monolayer adsorption, this was supported by the Langmuir isotherm model, which was found to be effective in explaining the experimental results. It is also worth noting that the value of q_{max} calculated from the Langmuir model (148.498mg/g), was

slightly higher compared to the experimental one (125 mg/g); indicating that more metal ions were required to form the monolayer coverage. The adsorption kinetics of Pd (II) onto P41C were found to be best explained by the pseudo-second-order model, demonstrating that surface adsorption involves chemisorption, whereby the removal of compounds from a solution occurs as a result of physicochemical interactions between the two phases.

Nomenclature

q_e - amount of adsorbed metal at equilibrium, mg/g

C_0 - initial metal concentrations (mg/L),

C_e - metal concentrations at equilibrium, mg/L

V - volume (L)

q_{max} - Langmuir isotherm constant related to maximum monolayer sorption capacity; mg/g

K_L - Langmuir isotherm constant related to energy or net enthalpy of adsorption L/mg

K_f - Freundlich isotherm constant related to biosorption capacity; mg/g

n - Freundlich isotherm constant related biosorption intensity

q_t - concentration of metal ion species on the biosorbent at time t , mg/g

k_1 - equilibrium biosorption constant of pseudo-first-order (1/min).

k_2 - equilibrium biosorption constant of pseudo-second-order (g/mg/min).

m - mass of the biosorbent (g).

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