Analysis of 4-bromophenols degradation mechanism, kinetics and isotherms by Pichia kluyveri FM012: Experimental and modeling philosophy

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Analysis of 4-bromophenols degradation mechanism, kinetics and isotherms by *Pichia kluyveri* FM012: Experimental and modeling philosophy

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Highlights

- The *Pichia kluyveri* FM012 is effective biosorbent for 4-bromophenols.
- Microorganisms on the cell surface contribute to increased biodegradation.
- At low pH, protonation of H^+ combined with the fungal surface, increasing biosorption.
- The addition of glucose to *Pichia kluyveri* FM012 rised the removal of 4-bromophenol.
- Culture showed the highest degradation (96%) with yeast for 15 days of incubation.

Abstract

The use of toxic and persistent pesticides in agriculture results in serious and lasting environmental impacts. Although traditional methods such as physical and chemical reclamation give the best results, treating these contaminants requires high cost and expertise. Therefore, this study focuses on bioremediation recovery, which is more efficient, economical, and safer to remove. In this case, the newly isolated potential of *Pichia kluyveri* FM012 in degrading 4-bromophenol was investigated. The selected strain was isolated from the tropical rainforest in Selangor, Malaysia. The impact of optimized parameters such as agitation, pH, nitrogen and carbon source were also

studied. After extensive testing, the best optimal degradation was at pH 5 with 150 rpm stirrer speed. Glucose and yeast performed best compared to other carbon and nitrogen sources. The maximum biosorption capacity (q_m = 38.46 mg/g biomass) was predicted by the Langmuir model, but the Freundlich model adsorption gave a better value of R^2 = 0.999. The pseudo-second-order kinetic model fits the study of biosorption kinetics. The FTIR spectrum revealed the presence of asymmetric and symmetric vibration of the aromatic ring and was assigned C=C or C=O, and fungi showed biosorption ability in broad functional groups. These results provided interesting information about the ability of *Pichia kluyveri* FM012 and potential applications to remediate the resistant pesticide.

Keywords: *Pichia kluyveri*; biosorption; 4-bromophenol; biodegradation

1. Introduction

Bromophenols (BRPs) are a group of brominated phenolic compounds that can be made from both natural and synthetic processes. It has been extensively used in chemical industry, flame retardants, polymer materials, resorcinol precursors, pharmaceuticals and pesticides for agricultural field as well [1, 2]. Combustion of leaded petrol also rise the producing of bromophenol [2, 3]. In addition, several marine organisms have been reported in nature to produce brominated compounds, including sea sponges, algae, and bacteria [4-6]. However, due to their physicochemical properties such as toxicity, persistence and bioaccumulation [7-10], bromophenols are prohibited by the U.S. EPA listed as a Priority Pollutant due to harmful effects on the environment and organisms [11]. Bromophenol levels of up to 187, 1140 and 3690 mg L^{-1} have been reported in photographic industrial wastewater [12], river water [13] and estuarine sediments [14], respectively. All of these issues encourage researchers to develop an efficient and effective method for removing pollutants containing 4-bromophenols before they are released into the environment.

Several chemical and physical techniques such as photodegradation, photocatalysis, volatilization and advanced oxidation have been proposed and implemented to treat pollutants containing phenolic compounds. Chemical degradation of bromophenols by using advanced oxidation methods such as photocatalysts [15-22], metal oxides [23, 24], biomimetic catalytic systems using iron(III)-porphyrins and an oxygen [25-29] and

permanganate [15, 30] the positive result. In addition, physical processing treatment can also be used to degrade halogenated aromatic compounds, including UV/Fenton degradation, direct UV irradiation, UVvis/BiOBr, and related reactions [15, 17, 21, 31]. Although conventional methods such as physical and chemical treatments gave the appropriate results, the debromination efficiency for brominated aromatics still needs to be further improved due to the complexity of the aromatic reaction mechanism [32] and toxic by-products may be generated during the process. In addition, they required high costs and expertise in the treatment process. Therefore, a biological approach seems to be a good strategy to degrade brominated phenolic compounds.

Biodegradation is one of the most important natural processes that has been extensively researched. Due to its many benefits, including lower treatment costs, a lack of secondary contamination, and an environmentally benign treatment process, it has showed potential over these techniques [33]. Depending on the structure of the pollutant and the type of microorganisms, the degradation of the brominated phenolic compounds can take place under aerobic and anaerobic conditions. Algae [34], actinomycetes [35], bacteria [36, 37], yeast [38, 39], and fungi [40, 41] have all been used in studies on the biodegradation of aromatic compounds by microorganisms. Recently, bioremediation research has focused more on bacteria and fungi. Bacteria have been shown the ability to degrade the aromatic compound due to their strong adaptability with high activity and wide distribution [42]. However, the bacterial activity in the degradation of aromatic amines is limited and inhibited [43]. Furthermore, it has been demonstrated that fungi degrade a complex organic material through extracellular ligninolytic enzymes [44] when an efficient system such as the solid-liquid separation method is applied [45].

However, the presence of a halogenated group in the aromatic ring increases resistance to microbial attack [46]. Therefore, the degradation of halogenated aromatic compounds by microorganisms has attracted the attention of researchers, and some bacteria and fungi have been shown to enhance the degradation process [47, 48]. Some microorganisms such as *Achromobacter piechaudii* strain TBPZ [49], *Ochrobactrum* sp. strain TB01 [50] is said to degrade bromophenol. In addition, *Sphingopyxis chilensis* S37 and Sphingopyxis-like strain S32 have been reported to degrade tribromophenol [51]. Arthrobacter chlorophenolicus has been shown to complete the degradation of 4-chlorophenol within 24 hours by using chlorophenol as a carbon and energy source [52].

Therefore, the present study aims to investigate the potential of Pichia kluyveri FM012 for dehalogenation of brominated aromatics in liquid media batches. In particular, we focused on: (1) the efficiency of bromophenol removal, (2) the impact of the typical factors, and (3) studies on debromination kinetics and isotherms. It is very useful to determine the highest degradation of 4-bromophenol over a short period of time using wild isolated fungi.

2. Materials and methods

2.1 Materials

The chemicals used in the study were obtained from 4-bromophenol Fluka (Switzerland), while D(+)-galactose, chloramphenicol and Remazol Brilliant Blue R were obtained from Acros Organick (Belgium). D(-)-fructose, Malt extract and D(+)-glucose monohydrate obtained from Merk (Germany). Macherey-Nagel (Germany) was used to get silica gel. Bacteriological peptone was obtained from Oxiod (England). Dichloromethane, ammonium nitrate, hydrochloric acid, chloroform, N,N-dimethylformamide and ethyl acetate were purchased from QreC (New Zealand). Tween 80, ammonium tartrate and sodium hydroxide pellets were obtained from Sigma Aldrich (USA) and toluene was purchased from Deajung (Korea).

2.2 Microorganism

Pichia kluyveri was collected from tropical rainforest by Forest Research Institute Malayisia (FRIM) Selangor, Malaysia. The isolated strain was chosen based on its ability to remove Remazol Brilliant Blue R (RBBR) in a solid medium containing 20 mL of malt extract agar (2% (w/v), 2% (w/v) glucose, 0.2% (w/v) yeast, 0.03% (w/v) chloramphenicol and 50 ppm RBBR in a Petri disk, followed by incubation at room temperature for 7 to 15 days transferred to fresh agar obtaining a pure strain. Potential strain changed the hue of the RBBR dye from blue to yellow.

2.3 Liquid culture condition

The selected fungi were screened in several liquid media to find the optimal medium for degrading 4-bromophenol. The liquid medium contains 1% (w/v) glucose, 1% (w/v) yeast and 0.2% chloramphenicol. The experiments were carried out in a 100 mL Erlenmeyer flask containing 20 mL liquid medium and 40 ppm 4-bromophenol dissolved in N,N-dimethylformamide, Tween 80 and distilled water. The liquid medium

was sterilized at a temperature of 121 °C for 15 min. Three mycelial plugs of the fungus were punched out with a cork borer from the outer rim of an actively growing culture on an inoculum plate before inoculation into the Erlenmeyer flask. The duration of the pre-incubation was varied between 7 and 15 days to obtain a similar radial. Each liquid medium was supplemented with 40 ppm 4-bromophenol and then incubated for 7-day intervals in the dark at a temperature of 25 °C. Incubation of the liquid medium with 4-bromophenol without inoculum was used as a comparison to determine loss of initiation. During the incubation process, the concentration of 4-bromophenol was also carried out. This investigation was carried out three times.

2.4 Effect of physico-chemical parameters

The effects of pH on the bioremoval of 4-bromophenol were studied in the liquid medium containing mycelia and 30 ppm 4-bromophenol solution. In this study, pH values of 5 to 8 were adjusted. The effects of agitation speeds ranging from 0 to 150 rpm were evaluated. In addition, the effect of various carbon sources (glucose, galactose, lactose and starch) and nitrogen sources (yeast, peptone, ammonium nitrate and ammonium tartrate) were examined.

2.5 Analytical methods

Liquid medium was mixed with ethyl acetate to extract fungal enzyme. The medium was extracted separately with funnel separation three times (200 mL each). The liquid medium and mycelia were separated by filtration and then extracted with ethyl acetate. All extract solutions were evaporated using a rotary evaporator to remove the ethyl acetate solvent. The extracts were pooled for each culture and purified by column chromatography and loaded onto a silica gel column before eluting with 150 mL of dichloromethane. Then the extract solution was evaporated to about 2 mL using a rotary evaporator and then dissolved in 10 mL of toluene for the next analysis.

Gas chromatographic analysis of the extract solution was performed using an Agilent 7820A instrument fitted with a split/splitless injector and coupled to an ionization flame detector (FID). On an HP-5 capillary column (30 m, 0.32 mm i.d., 0.25 m film thickness), the compounds were separated. The oven's temperature was set at 80 °C for 1 min, followed by a linear ramp of 23 °C/min up to 280 °C and a 1-min hold at 310 °C. Temperatures at the injector and detector were 330 °C. The carrier gas used was nitrogen. The injection volume was 1 L, and the flow rate was 3 mL/min. By

171 contrasting the retention times between the sample and the control, the peak of the
172 sample was identified.
173
174 **2.6 Biosorption isotherm**
175 Two isotherm models, namely Langmuir and Freundlich, were used to describe the
176 biosorption equilibrium. The Langmuir model assumes that there is no interaction
177 between adsorbent and pollutant. In addition, the revisable adsorption or desorption
178 process takes place on a homogeneous surface upon formation of a saturated monolayer.
179 The Langmuir isotherm model can be expressed mathematically as: [53]

180 $$q_e = \frac{q_m . K_L . C_e}{1 + K_L . C_e} \quad (1)$$

181 where K_L is the Langmuir adsorption constant (L/mg) with respect to the adsorption
182 energy, q_m (mg/g) is the maximum adsorption capacity, q_e and C_e are the biosorption
183 capacity (mg/g) at equilibrium and equilibrium concentrations of pollutants (mg/ L).
184 A Freundlich model is proposed based on the heterogeneous surface adsorption. The
185 model is given as:

186 $$q_e = K_F . C_e^{\frac{1}{n}} \quad (2)$$

187 where K_F (L/mg) and n are Freundlich constants related to the capacity of the adsorbent-
188 biosorbent and the adsorption constant of the intensity of the biosorbent, respectively. q_e
189 is the equilibrium biosorption capacity (mg/g) and C_e is the equilibrium concentration of
190 the pollutant (mg/L).
191
192 **2.7 Biosorption kinetics**
193 In order to determine the kinetic behavior of 4-bromophenol biosorption, two pseudo-
194 first-order and pseudo-second-order kinetic models were used. The adsorbent was
195 mixed with concentrations (30 mg/L) of 4-bromophenol solution in 250 mL of an
196 Erlenmeyer flask. A total of 34 flasks were prepared to perform this experiment in
197 duplicate, maintained at constant stirring speed (150 rpm) and temperature (25 °C).
198 Control flasks without biosorbents were also prepared and these experiments were
199 performed. The sample was collected at different time points to determine the
200 concentrations of 4-bromophenol residues. The biosorption capacity (q) was determined
201 by following the following pseudo-first-order equation: [54]
202

$$\log(q_e - q_t) = \log(q_e - k_1)t \quad (3)$$

where q_t (mg/g) is the adsorption capacity at time t (h) and q_e (mg/g) is the equilibrium adsorption capacity and k_1 (h^{-1}) is the equilibrium pseudo first-order constant by plotting ln (q_e-q_t) vs. t.

The pseudo-second-order model is given by the following equation [55]:

$$\frac{t}{q_t} = \frac{1}{k_2 . q_e^2} + \frac{1}{q_e} \quad (4)$$

where q_t is the amount of pollutant adsorbed at time t ($mg^{-1}g^{-1}$), q_e is the steady-state biosorption amount ($mg^{-1}g^{-1}$), and k_2 is the pseudo-second-order rate constant (g/mgh).

The intraparticle diffusion equation can be expressed as:

$$q_e = k_{diff} t^{0.5} + C \quad (5)$$

where C is the intercept of linear equation and k_{diff} is the intraparticle diffusion rate constant.

2.8 Characterization

The surface morphology of the sample was observed using scanning electron microscopy (SEM) (HITACHI S-3400N). In addition, the functional group of the sample was examined using Fourier Transform Infrared (FTIR) (Perkin Elmer, Spectrum One). The dried sample was mixed with KBr in a weight ratio of 100:1, followed by compression with a hydraulic piston to obtain a pellet shape with a diameter of 10 mm. The spectra were recorded with wave numbers between 400 and 4000 cm^{-1} with a spectral resolution of 4 cm^{-1}. All spectra were compared together between treated and untreated curves.

3. Results and Discussion

3.1 Effect of agitation

Fig. 1 shows the effect of agitation on the degradation of 4-bromophenol by *Pichia kluyveri* FM012. Agitation had two main effects on fungal incubation. First, a high agitation speed tends to ensure adequate oxygenation of the culture. On the other hand, it delivers high energy with high shear stress to the damaged culture cells and mycelium. The lowest degradation rate of 4-bromophenol was observed in the static

phase (88%) for 7 days. After increasing the agitation speed and time, the degradation rate was generally increased up to 96%. The best breakdown of 4-bromophenol was at a speed of 150 rpm. The success of decomposition is based on the correlation between the surface of the fungus and the oxygen supplied. This parallel association leads to an increased exchange of substances between the medium and the cells. In addition, the extracellular enzyme activities of fungi such as laccase and manganese also contribute to the degradation of 4-bromophenol [56]. The highest laccase enzyme productions are achieved in shake culture and promote degradation. In the stationary phase, degradation and biosorption limit oxygen transfer to the cells. This inhibits the oxidative enzymes and prevents optimal 4-bromophenol degradation. The lower rotation speed is probably related to the lower biosorption and resulted in insufficient contact between adsorbent particles in aqueous solution [57]. Therefore, the results obtained are consistent with [56] as they demonstrate that the degradation of pollutant compounds with enzymes increases over time.

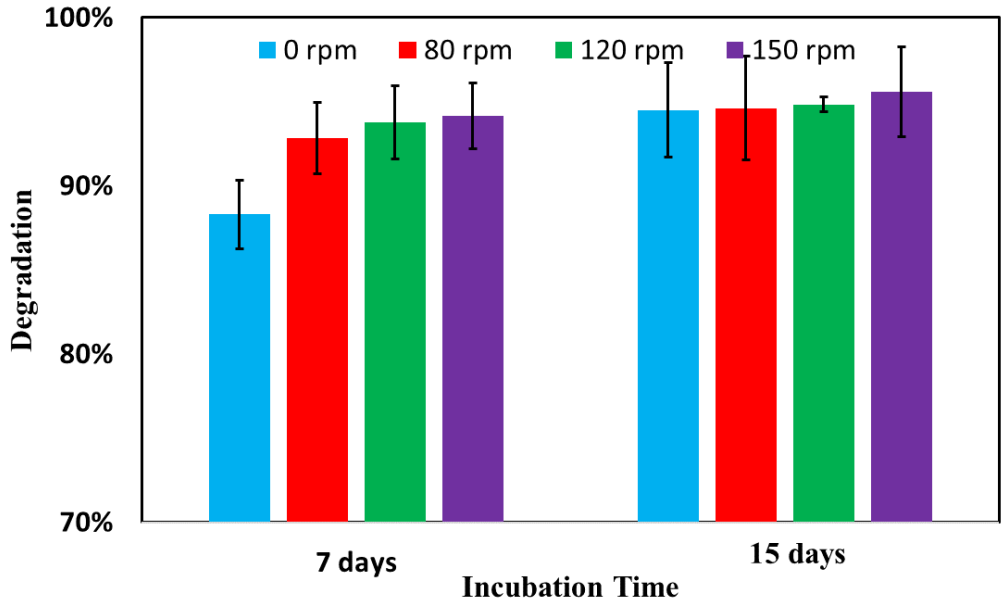

Fig. 1. Effect of agitation on degradation of 4-bromophenol by *Pichia Kluyveri sp.* FM012. Condition: glucose = 10 ppm, yeast = 10 ppm, pH = 5, temperature = 25 °C

3.2 Effect of pH

Fig. 2 shows the influence of adsorption and degradation of 4-bromophenol. The optimal pH in this reaction was pH 5.0 with values of 88 and 96% within 7 and 15 days

of incubation, respectively. The lowest percentage biosorption was observed at pH 8.0. Increasing the pH from 5.0 to 8.0 for 15 days has a significant effect on the degradation and biosorption of 4-bromophenol. At lower pH, protonation of H^+ can combine with the surface of the fungi to increase degradation and biosorption [7]. However, as pH continues to increase, the degradation and biosorption process decreases due to the low solubility of hydroxide precipitation. Lesser precipitates disrupt the biosorption process and make them unavailable for biosorption. These results have been confirmed by many reports and studies showing that the pH range between 4.0 and 6.0 is an optimal pH for degradation and adsorption [58-61].

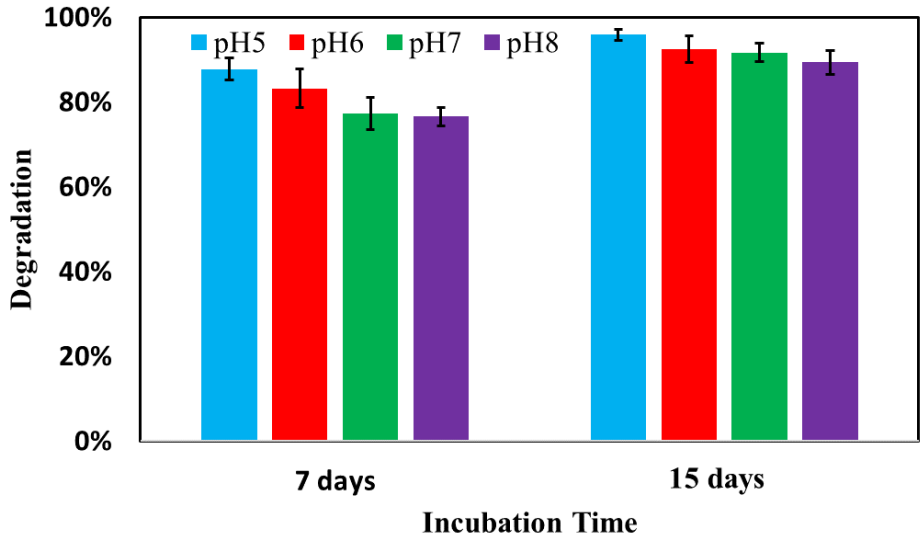

Fig. 2. Effect of pH on degradation of 4-bromophenol by *Pichia Kluyveri sp.* FM012. Condition: glucose = 10 ppm, yeast = 10 ppm, agitation speed = 150 rpm, temperature = 25 °C

3.3 Effect of Carbon Sources

Fig. 3 shows the effects of different carbon sources on the degradation of 4-bromophenol. Glucose, fructose, galactose and starch were used as carbon sources and added to liquid medium containing *Pichia kluyveri* FM012. Of all the carbon sources tested, the lowest degradation rate of 4-bromophenol was observed with strengths of 81 and 91% for 7 and 15 days of incubation, respectively. After 15 days, glucose showed the highest degradation rate of 4-bromophenol with a value of 96%. In the same period, the degradation rate of fructose and galactose reached 91% and 95%, respectively. 4-bromophenol readily degradable by *Pichia kluyveri* FM012 supplemented with glucose under aerobic conditions. This is because glucose is a compound with the simplest source of carbon and is easily consumed by fungi. The addition of an easily degradable

carbon source such as glucose can stimulate the biodegradation of pollutants and shorten the degradation lag time in liquid medium [62] showed that mechanisms of the stimulating effect of an easily accessible carbon source such as fructose and glucose. The pattern of the presence of carbon sources was influenced in the fungal degradation of 4-bromophenol. In general, fungi consume the carbon source as the main energy for growth by producing extracellular enzymes and secondary metabolites for biodegradation [63]. Due to the more easily degradable carbons, they discovered that the relationship between the microbial response and organic additions increased the breakdown of 4-bromophenol and hindered the mineralization of the pollutant.

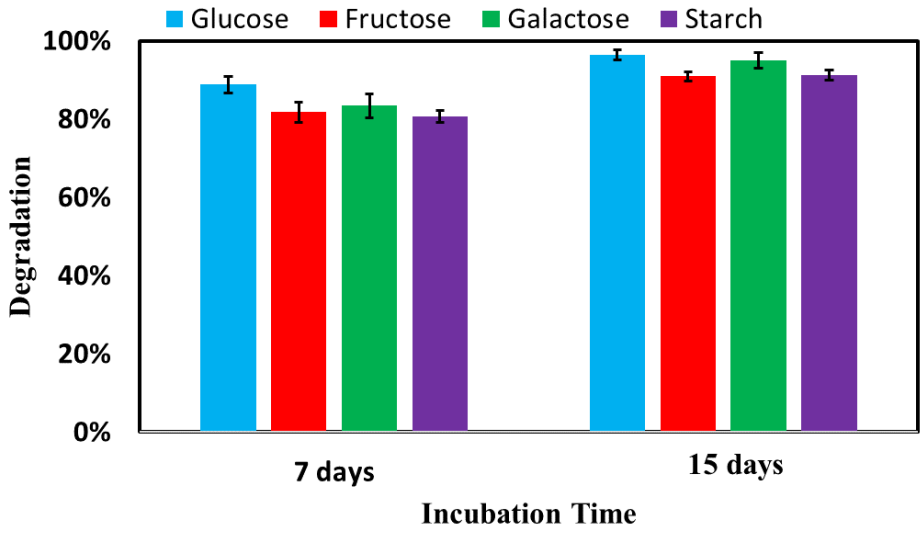

Fig. 3. Effect of carbon source on degradation of 4-bromophenol by *Pichia Kluyveri sp.* FM012. Condition: yeast = 10 ppm, agitation speed = 150 rpm, pH = 5, temperature = 25 °C

3.4 Effect of nitrogen sources

Various sources of nitrogen are shown in Fig. 4. Four nitrogen sources were added to the *Pichia kluyveri* FM012 cultures. Yeast, peptone, ammonium nitrate and ammonium tartrate were used as nitrogen sources in incubation for 7 and 15 days. The culture showed the highest degradation rate of 4-bromophenol in shake flask liquid cultures observed with yeast 88 and 96% for 7- and 15-days incubation, respectively. On the other hand, degradation cultures supplemented with peptone and ammonium tartrate reached 90 and 89% after 15 days of incubation. Due to the oxygen and nutrients that are present in aerobic conditions, bacteria can breakdown a variety of contaminants in groundwater and soil [3]. Adding a yeast as the simplest nutrient would increase degradation due to the ease of utilization of the more easily degradable nutrient. Meanwhile, the performance of ammonium nitrate in breaking down 4-bromophenol

was not very effective for 7 days, but increased rapidly after 15 days later. Therefore, yeast was selected as the best and most suitable nitrogen source for fungi to break down 4-bromophenol.

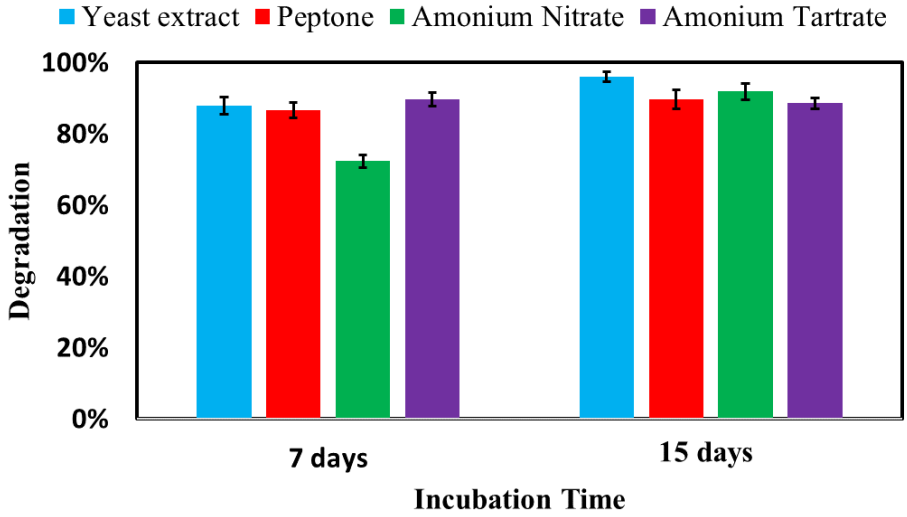

Fig. 4. Effect of nitrogen source on degradation of 4-bromophenol by *Pichia Kluyveri sp.* FM012. Condition: glucose = 10 ppm, agitation speed = 150 rpm, pH = 5, temperature = 25 °C

3.5 Effect of contact time

The contact time has a parallel correlation with the biomass surface area in adsorption studies [64]. It is important to design batch adsorption studies by monitoring the percentage of 4-bromophenol degradation. The sample was observed for two days during a 17-day incubation to assess fungal growth. The biosorption of 4-bromophenol almost reached equilibrium within 17 days of contact time and remained constant with increasing time (Fig. 5). Thereafter, there was no significant change in biosorption and the maximum adsorption capacity reached after 17 days.

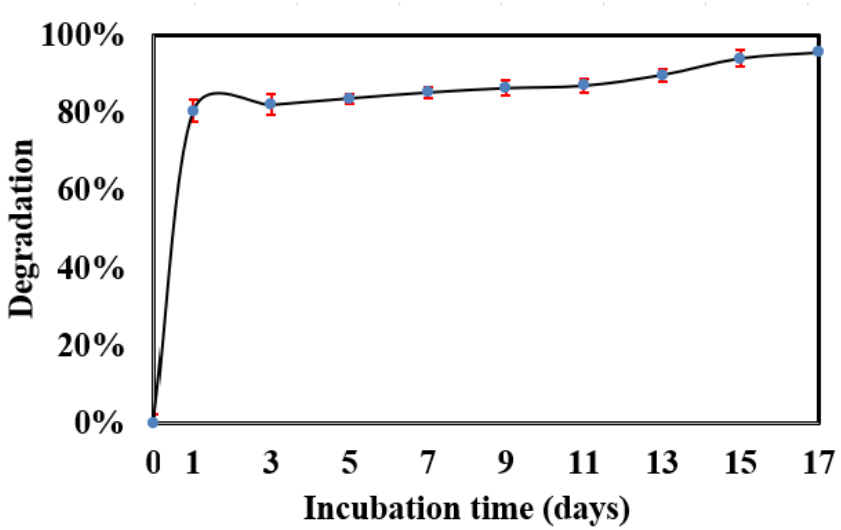

Fig. 5. Effect of contact time on degradation of 4-bromophenol by *Pichia Kluyveri sp.* FM012. Condition: glucose = 10 ppm, yeast = 10 ppm, agitation speed = 150 rpm, pH = 5, temperature = 25 °C

3.6 Isotherms Adsorption

An equilibrium sorption isotherm is important to develop an equation that describes the capacity of an affinity and surface biomass properties. Table 1 shows that two biosorption isotherm equilibrium data were fit into the linearized Langmuir and Freundlich models. To use the isotherm equation, it was assumed that this is a monolayer cover equilibrium model and adsorption sites are equally probable. For both tested isotherm models, the correlation coefficient (R^2) of both models was mostly close to 1, but the value of the Langmuir correlation coefficient ($R^2 < 0.97$) was slightly lower than that of the Freundlich isotherm ($R^2 > 0.99$), which shows that the Freundlich model better represents the equilibrium biosorption of 4-bromophenol by *Pichia kluyveri* FM012. These observational results imply heterogeneous surface conditions (Fig. 6).

A plot of C_e/q_e versus C_e of the Langmuir equation gives a straight-line plot with an intercept of b and a slope of $1/q_{max}$. Therefore, the Langmuir model shows that the maximum adsorption capacity (q_m) for 15 days is 38.46 (mg/g) and the K_L value (-1.733) is a Langmuir constant related to the adsorption/desorption energy [65, 66]. The Freundlich isotherm equation was used to analyze the adsorption intensity of the sorbent. The linearized Freundlich equation was plotted with log q_e versus log C_e to give a straight-line graph with intercept (log K_F) and slope ($1/n$). Values of n (3.802) and K_F (1.396) are Freundlich constants. A comparison between Langmuir and Freundlich R^2 values shows that the biosorption of 4-bromophenol on *Pichia kluyveri* FM012 fits the Freundlich isotherm model better than the Langmuir isotherm model and

shows the correlation between the equilibrium concentration and the amount of adsorbate.

Table 1. Biosorption equilibrium parameters of the isotherm models by *Pichia Kluyveri sp.* FM012

	Langmuir parameters			**Freundlich parameters**		
Biomass	q_{max} (mg/g)	K_L (L/mg)	R^2	K_F (L/mg)	n	R^2
Pichia Kluyveri sp. FM012	38.46	-1.733	0.977	1.802	3.802	0.999

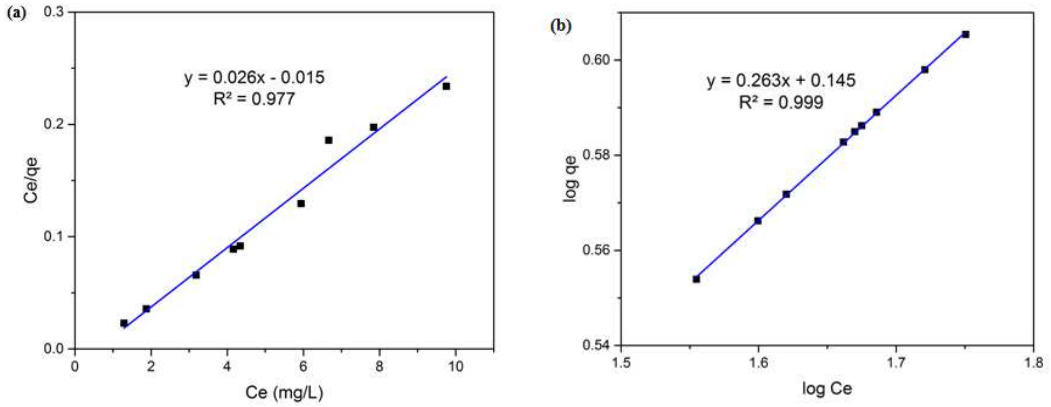

Fig. 6. Adsorption isotherms: (a) Langmuir and (b) Freundlich isotherm for the adsorption of 4-bromophenol

3.7 Biosorption kinetics

The studies of kinetics are important to determine the efficacy and effectiveness of biosorption. This study is quite significant for controlling the residence time of solute uptake at the solid solution interface of waste water treatment [67]. The kinetics of 4-bromophenol adsorption were determined in both models; pseudo-first-order and pseudo-second-order kinetic models. Table 2 shows the results of pseudo first order and pseudo second order values of biosorption by *Pichia kluyveri* FM012. The data were fitted to the linearized graph to obtain values of q_e, K_1 and K_2 through the slope and intercept calculations. The value of the correlation coefficient (R^2) of the pseudo-first-order kinetic model (R^2 = 0.757) was lower than that of the pseudo-second-order model (R^2 = 0.972) (Fig. 7). The pseudo-first-order reaction is more indicative of the reaction of the physisorption process, assuming that between the uptake rate and time is directly proportional to the amount of active site on the adsorbent surface. In contrast, the

second-order pseudo-model suggested a chemisorption process assuming that chemical adsorption between adsorbate and adsorbent has a short time span.

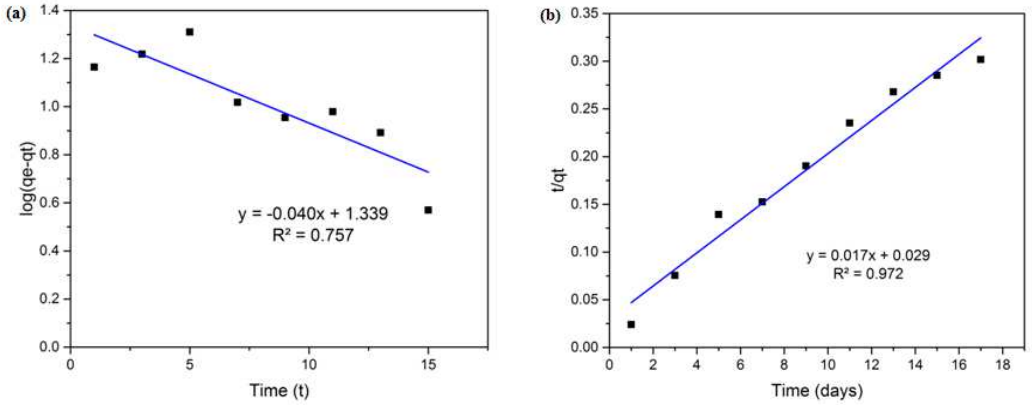

Fig. 7. Adsorption dynamics: (a) Pseudo first-order and (b) Pseudo second-order kinetics plots for the adsorption of 4-bromophenol by *Pichia Kluyveri sp.* FM012

Table 2. Integrated equations, boundary conditions and kinetic parameters of the biosorption of 4-bromophenol by *Pichia Kluyveri sp.* FM012

	Pseudo-first order			**Pseudo-second order**			**Intra-particle diffusion**		
Parameters	q_e (mg/g)	k_1	R^2	q_e (mg/g)	k_2	R^2	C	k_{diff}	R^2
	83.946	0.073	0.757	83.333	0.1519	0.972	36.85	2.05	0.793

3.8 Characterization of *Pichia Kluyveri* before/after biosorption

Scanning electron microscopy (SEM) is a tool used to examine the morphology of strand samples before and after biosorption of 4-bromophenol by *Pichia kluyveri* FM012. The SEM images observed in Fig. 8 showed detailed surface morphology and structure of the biosorbent between the control and the sample at different magnifications. The surface morphology of the untreated adsorbent differs significantly from that of the treated one. Before adsorption (Fig. 8a), the surface of the adsorbent was rough, uneven, streaky folds with many valleys and pores appeared. The correlation between pores and the number of available binding sites was the increase in biosorption capacity. In contrast, Figs. 8b and 8c show a morphological change on the surface of *Pichia kluyveri* FM012, the surface of the biosorbent was less streaky, wrinkles, valleys and pores appeared, which could be due to the adsorption of 4-bromophenol. In addition, the biosorbing surfaces become adhesive and thicker after the biosorption

process. This is evidence that *Pichia kluyveri* FM012. support the degradation and increase the biosorption capacity.

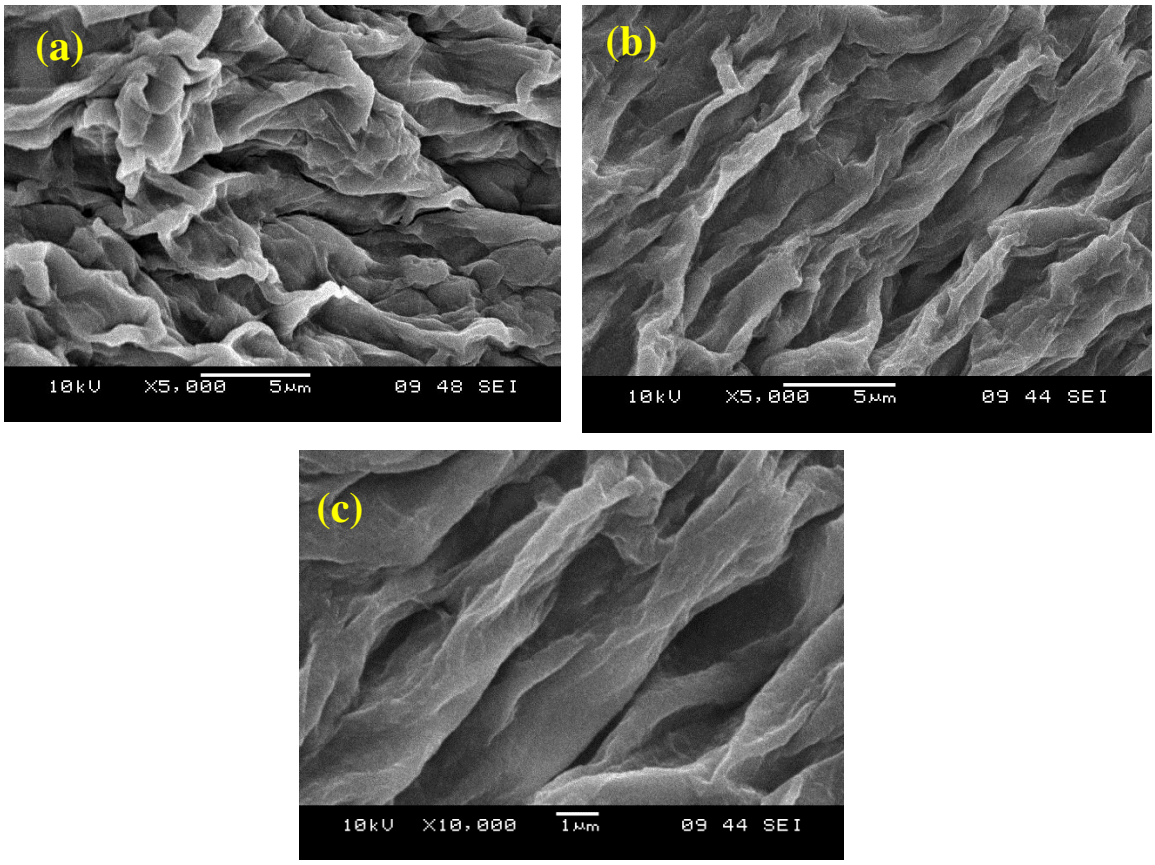

Fig. 8. Scanning electron microscopy (SEM) micrographs of the *Pichia Kluyveri* before (a) and after (b and c) of biosorption

The FT-IR spectrum is a crucial instrument for demonstrating how macromolecules' functional groups are impacted. After 15 days of adsorption, the new peak at 3276.13 cm^{-1} which symbolizes the elongation of the hydroxyl groups shifted to 3264.89 cm^{-1} (Fig. 9), demonstrating that the combination of 4-bromophenol with the endophytic elongation is the bond length and the vibration brought on by stretching increases the -OH group. In addition, aliphatic CH stretching is attributed to the peaks of 2917.58 cm^{-1} and 1426.79 cm^{-1}. These peaks were moved to 291.47 cm^{-1} and 1425.06 cm^{-1} after adsorption. In Fig. 9, a shift peak was seen at 1637.25 cm^{-1} and 877.94 cm^{-1} of two additional peaks, which was attributed to the ester and amide functional groups' bond C-O stretch [68]. The second peak may be due to disulfide or nitro groups [68, 69], and bioligands [70]. The peak at 1641.24 cm^{-1} represents the asymmetric and symmetric vibration of the aromatic ring and is assigned C=C or C=O. The peak of the amide

bonds is at 1541.01 cm^{-1}. After the adsorption process, these peaks shift to 1537.01 cm^{-1}. In general, the bacteria and fungi showed biosorption ability in broad functional groups [71]. All of these findings indicated that adhesion of 4-bromophenol to the cell surface pores, and active binding sites resulted in adsorption.

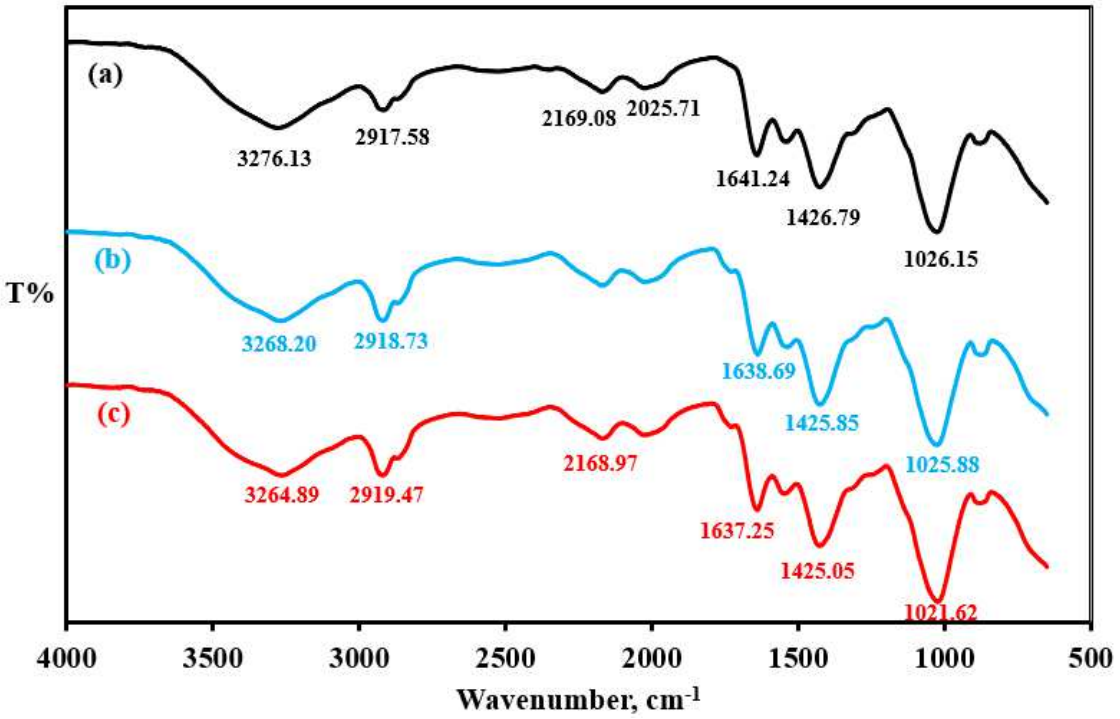

Fig. 9. FTIR spectra of *Pichia Kluyveri sp.* FM012: (a) control, (b) 7 days, (c) 15 days.

4. Conclusions

The biodegradation efficiency of brominated phenolic compounds is related to the halogen atom, the optimal conditions, and abilities of the microorganism to decompose pollutants in the experiments, as well as the cell surface properties of microorganisms. In the present study, the new potency of *Pichia kluyvery* FM012 demonstrated the degradation of 4-bromophenol under aerobic conditions. The degradation process followed Freundlich adsorption and pseudo-second-order kinetics and was enhanced under optimal conditions. In addition, microorganisms with a high level of hydrophobicity on the cell surface also contribute to increasing biodegradation. This is an interesting finding in the bioremediation of resistant pollutants that should be explored in future studies for other toxic and recalcitrant environmental pollutants.

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437
438 **Ethical Approval**
439 Not Applicable.
440
441 **Competing interests**
442 The authors declare that there is no conflict of interest.
443
444 **Authors' contributions**
445 **Ismallianto Isia**: Investigation, methodology, data formal analysis. **Yudi Sukmono**:
446 Investigation, methodology, data formal analysis, validation, writing-original draft,
447 review and editing. **Tony Hadibarata**: Investigation, conceptualization, writing, review
448 and editing, supervision. **Murat Yılmaz:** Writing - Review & Editing, Visualization.
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455 **Availability of data and materials**
456 Data will be made available on request.
457
458
459 **References**
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