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Enhancing the Biosorption Potential of *Pichia kluyveri* FM012 for 4-Bromophenols

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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, yield the best results, treating these contaminants requires a high cost and expertise. Therefore, this study focuses on bioremediation recovery, which is more efficient, economical, and safer for removal. In this case, the newly isolated potential of *Pichia kluyveri* FM012 in degrading 4-bromophenol was investigated. The impact of optimized parameters such as agitation, pH, nitrogen, and carbon source were also studied. After extensive testing, the best optimal degradation occurred at pH 5 with a stirrer speed of 150 rpm. Glucose and yeast performed the best compared to other carbon and nitrogen sources. The Langmuir model predicted the maximum biosorption capacity ($q_m = 38.46$ mg/g biomass), but the Freundlich model provided a better value of $R^2 = 0.999$. The pseudo-second-order kinetic model was fitting for the study of biosorption kinetics. The FTIR spectrum revealed the presence of asymmetric and symmetric vibrations of the aromatic ring and was assigned to C=C or C=O. Fungi showed biosorption ability across broad functional groups. These results provide interesting information about the ability of *Pichia kluyveri* FM012 and its potential applications for remediating resistant pesticides.

Novelty Statement In literature, there has no report on 4-bromophenol degradation by using *Pichia kluyveri* FM012 biosorbent. The general aim of this study is to examine the adsorption process of 4-bromophenols on *Pichia kluyveri* FM012.

Highlights
- The *Pichia kluyveri* FM012 is effective biosorbent for 4-bromophenols.
- Microorganisms on the cell surface contribute to increased biosorption.
- 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 removal (96%) with yeast for 15 days of incubation.

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1 Introduction

The problem at hand revolves around the widespread presence and environmental impact of bromophenols (BRPs), a category of brominated phenolic compounds derived from both natural and synthetic processes. These compounds find extensive applications across diverse industries, including the chemical sector, flame retardant production, polymer manufacturing, synthesis of resorcinol precursors, and formulation of pharmaceuticals and pesticides for agricultural use (Hadibarata et al., 2019; Khori et al., 2020; Rhee et al., 2003; Uhnáková et al., 2009). Additionally, the combustion of leaded petrol further contributes to the production of bromophenols, accentuating the anthropogenic sources of these substances (Howe et al., 2005; Uhnáková et al., 2009).

The problem is compounded by the contribution of marine organisms, such as sea sponges, algae, and bacteria, to the production of brominated compounds, indicating that these substances are not only human-made but also occur naturally in various ecosystems (Gribble, 2003; Lim et al., 2023; Malmvärn et al., 2005; Whitfield et al., 1999). However, the physicochemical properties of bromophenols, encompassing their toxicity, persistence, and bioaccumulation potential, pose significant environmental threats (Commandeur & Parsons, 1994; Berg et al., 1998; Muir & Howard, 2006; Schwarzenbach et al., 2010). Consequently, regulatory bodies, including the US EPA, have designated bromophenols as priority pollutants due to their harmful effects on the environment and living organisms (Dell'Erba et al., 2007).

The severity of the problem is underscored by the reported high levels of bromophenols in various environmental matrices. Concentrations reaching up to 187, 1140, and 3690 mg/L have been documented in photographic industrial wastewater, river water, and estuarine sediments, respectively (Bursey & Pellizzari, 1983; Nomani et al., 1996; Tolosa et al., 1991). These concentrations indicate the widespread contamination of different environmental compartments, emphasizing the urgent need for the development of efficient methods to remove pollutants containing 4-bromophenols before their release into the environment.

In essence, the problem statement emphasizes the multifaceted sources and applications of bromophenols, their adverse effects on the environment and living organisms, and the pressing need for effective remediation strategies to mitigate their contamination in diverse environmental settings. The high reported concentrations further highlight the urgency of addressing this environmental concern to prevent further ecological harm. The existing arsenal of environmental remediation techniques encompasses various chemical and physical methods, including photodegradation, photocatalysis, volatilization, advanced oxidation, and physical processing treatments. These methods have been proposed and implemented for treating pollutants containing phenolic compounds, specifically bromophenols. However, despite their effectiveness, these conventional approaches encounter challenges that make them less than ideal. Chemical methods, such as advanced oxidation with photocatalysts, metal oxides, biomimetic catalytic systems, and permanganate, have demonstrated positive results in degrading bromophenols (Lin et al., 2009; Xu et al., 2011; Zhong et al., 2012; Guo et al., 2012; Yu et al., 2012; Ding et al., 2013; Miró et al., 2013; Pang et al., 2013; Sun et al., 2013; Zhu et al., 2013a, b; Díez-Mato et al., 2014; Gao et al., 2014; Zhou et al., 2014; Zhu et al., 2014, 2015; Ng and Elshikh, 2021; Salman et al., 2022; Rayhan et al., 2022). Physical processing treatments, including UV/Fenton degradation, direct UV irradiation, and UV–vis/BiOBr, have also been employed (Anipsitakis & Dionysiou, 2004; Miró et al., 2013; Xu et al., 2011; Zhong et al., 2012). While these methods yield appropriate results, they face challenges related to the debromination efficiency for brominated aromatics, driven by the complexity of the aromatic reaction mechanism (Wentworth et al., 1967). Moreover, the generation of toxic by-products during these processes raises environmental concerns.

Furthermore, conventional methods often require significant financial investment and specialized expertise for effective implementation. The high costs associated with these treatments, coupled with the need for expert oversight, pose practical barriers to widespread application. Given these challenges, a biological approach emerges as a promising strategy for degrading brominated phenolic compounds, particularly bromophenols. Biosorption, a natural process, offers several advantages over chemical and

physical methods. Biological methods, facilitated by microorganisms, can provide a cost-effective and environmentally benign alternative (Hadibarata et al., 2011). Microorganisms, including bacteria and fungi, have the ability to adapt and efficiently degrade aromatic compounds, overcoming some of the limitations of conventional treatments (Dos Santos et al., 2007; Sen et al., 2016).

Biosorption stands out as the natural physiochemical processes taking place in specific biomass enable it to passively accumulate and attach contaminants onto its cellular structures (Sponza & Uluköy, 2005). Despite the advantages of biosorption, a notable research gap persists in comprehending the intricate dynamics, particularly in the context of brominated phenolic compounds. The current body of knowledge acknowledges the versatility of biosorption, demonstrating its potential effectiveness in both aerobic and anaerobic conditions. Various microorganisms, such as algae (El-Sheekh et al., 2009; Hadibarata et al., 2018), actinomycetes (Badis et al., 2010), bacteria (Ayed et al., 2017; Buntić et al., 2017), yeast (Singh et al., 2012; Song et al., 2017), and fungi (Kristanti et al., 2016; Lai et al., 2017), have been employed in studies exploring the biosorption of aromatic compounds. While recent bioremediation research has predominantly focused on bacteria and fungi, revealing their remarkable adaptability, high activity, and widespread distribution (Dos Santos et al., 2007), a critical research gap emerges.

The limitations and challenges observed in bacterial activity during the biosorption of aromatic amines (Qu et al., 2010) underscore the need for a deeper understanding of the complex microbial interactions within biosorption processes. Additionally, the demonstrated ability of fungi to effectively biosorb complex organic materials without relying on extracellular ligninolytic enzymes (Sen et al., 2016) remains an underexplored aspect, particularly in the context of efficient systems such as solid–liquid separation methods (Mishra & Malik, 2013). However, the presence of a halogenated group in the aromatic ring increases resistance to microbial attack (Uberoi & Bhattacharya, 1997). Therefore, the removal of halogenated aromatic compounds by microorganisms has attracted the attention of researchers, and some bacteria and fungi have been shown to enhance the removal process (Purnomo et al., 2008, 2010). Some microorganisms such as *Achromobacter piechaudii* strain TBPZ (Ronen et al., 2005) and *Ochrobactrum* sp. strain TB01 (Yamada et al., 2008) are said to eliminate bromophenol. In addition, *Sphingopyxis chilensis* S37 and *Sphingopyxis*-like strain S32 have been reported to degrade tribromophenol (Aranda et al., 2003). *Arthrobacter chlorophenolicus* has been shown to complete the removal of 4-chlorophenol within 24 h by using chlorophenol as a carbon and energy source (Westerberg et al., 2000). 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 the following: (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 removal 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 and Culture Condition

Pichia kluyveri was collected from the tropical rainforest by the Forest Research Institute Malaysia (FRIM) in Selangor, Malaysia. The screening methodology involved incubating the Petri dish at room temperature for a period ranging from 7 to 15 days. Following this incubation period, the strain demonstrating potential was transferred to fresh agar to obtain a pure culture. Notably, the

selection criterion extended beyond RBBR, as the strain showcased the ability to induce a color change in the dye, transitioning from blue to yellow during the incubation period. This screening process offers valuable insights into the strain's efficacy in handling not only RBBR but also its potential for interacting with other compounds, such as 4-bromophenol, contributing to a more comprehensive understanding of its biosorption capabilities. The selected fungi were screened in liquid media to identify the optimal medium for removal of 4-bromophenol. The liquid medium consisted of 1% (w/v) glucose, 1% (w/v) yeast, and 0.2% chloramphenicol. The experiments were conducted in 100-mL Erlenmeyer flasks, each containing 20 mL of liquid medium and 40 ppm of 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. For inoculation, three mycelial plugs of the fungus were extracted using a cork borer from the outer rim of an actively growing culture on an inoculum plate. These plugs were then inoculated into the Erlenmeyer flask. The duration of the pre-incubation was varied between 7 and 15 days to achieve a consistent radial growth.

Each liquid medium was supplemented with 40 ppm of 4-bromophenol and then incubated for 7-day intervals in the dark at a temperature of 25 °C. To establish a baseline for comparison, a liquid medium with 4-bromophenol but without inoculum was also incubated to determine the loss of initiation. Throughout the incubation process, the concentration of 4-bromophenol was monitored. This investigation was conducted three times to ensure reliability and consistency of the results.

2.3 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) was examined.

2.4 Analytical Methods

The liquid medium was mixed with ethyl acetate to extract the fungal enzyme. The medium underwent separate extractions using funnel separation three times (200 mL each). The liquid medium and mycelia were separated through filtration and subsequently extracted with ethyl acetate. All extract solutions underwent evaporation using a rotary evaporator to remove the ethyl acetate solvent. The extracts were then pooled for each culture, purified through column chromatography, and loaded onto a silica gel column before eluting with 150 mL of dichloromethane. Subsequently, the extract solution was evaporated to approximately 2 mL using a rotary evaporator and then dissolved in 10 mL of toluene for the subsequent analysis.

Gas chromatographic analysis of the extract solution was conducted using an Agilent 7820A instrument equipped with a split/splitless injector and coupled to an ionization flame detector (FID). The compounds were separated on an HP-5 capillary column (30 m, 0.32 mm i.d., 0.25-m film thickness). The oven's temperature was initially 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 maintained at 330 °C. Nitrogen was used as the carrier gas, with an injection volume of 1 μL and a flow rate of 3 mL/min. By contrasting the retention times between the sample and the control, the peak of the sample was identified.

The sorption of 4-bromophenol was evaluated by examining its removal from the liquid medium and its uptake by the fungal biomass. To assess the efficiency of the biosorption process, the concentration of 4-bromophenol in both the liquid media and the fungal biomass was monitored and compared to the initial concentration. The experimental procedure involved inoculating fungi, such as *Pichia kluyveri*, into a specially formulated liquid medium containing 4-bromophenol. Following an incubation period of 7 to 15 days, the concentrations of 4-bromophenol in both the liquid medium and the fungal biomass were determined. The comparison between the initial concentration and the concentrations observed after the biosorption process provides insights into the extent of 4-bromophenol removal and the effectiveness of the fungal biomass in sorbing this particular contaminant.

2.5 Biosorption Isotherm

Two isotherm models, namely Langmuir and Freundlich, were used to describe the biosorption equilibrium. The Langmuir model assumes that there is no interaction between adsorbent and pollutant. In addition, the revisable adsorption or desorption process takes place on a homogeneous surface upon formation of a saturated monolayer. The Langmuir isotherm model can be expressed mathematically as: (Dash & Das, 2015)

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

where K_L is the Langmuir adsorption constant (L/mg) with respect to the adsorption energy, q_m (mg/g) is the maximum adsorption capacity, and q_e and C_e are the biosorption capacity (mg/g) at equilibrium and equilibrium concentrations of pollutants (mg/L).

A Freundlich model is proposed based on the heterogeneous surface adsorption. The model is given as:

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

where K_F (L/mg) and n are Freundlich constants related to the capacity of the adsorbent-biosorbent and the adsorption constant of the intensity of the biosorbent, respectively. q_e is the equilibrium biosorption capacity (mg/g) and C_e is the equilibrium concentration of the pollutant (mg/L).

2.6 Biosorption Kinetics

In order to determine the kinetic behavior of 4-bromophenol biosorption, two pseudo-first-order and pseudo-second-order kinetic models were used. The adsorbent was mixed with concentrations (30 mg/L) of 4-bromophenol solution in 250 mL of an Erlenmeyer flask. A total of 34 flasks were prepared to perform this experiment in duplicate, maintained at constant stirring speed (150 rpm) and temperature (25 °C). Control flasks without biosorbents were also prepared and these experiments were performed. The sample was collected at different time points to determine the concentrations of 4-bromophenol residues. The biosorption capacity (q) was determined by following the following pseudo-first-order equation: (Taşar et al., 2014)

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

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 (Dash et al., 2014):

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

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.

2.7 Characterization

The surface morphology of the sample was investigated with scanning electron microscopy (SEM) using the HITACHI S-3400N instrument. SEM is a powerful imaging technique that allows for detailed observation of surface structures at high magnifications. In this study, magnifications ranging from 5000× to 10,000× were employed to capture fine details and provide a comprehensive analysis of the sample's surface features. To analyze the functional groups present in the sample, Fourier transform infrared (FTIR) spectroscopy was employed using the Perkin Elmer Spectrum One instrument. This analytical technique provides valuable information about the chemical composition of the sample by measuring the absorption of infrared radiation at different frequencies corresponding to molecular vibrations. The sample preparation for FTIR analysis involved mixing the dried sample with potassium bromide (KBr) in a specific weight ratio of 100:1. Subsequently, the mixture was compressed using a hydraulic piston to create a pellet with a standardized diameter of 10 mm.

This pelletization process ensures uniformity and facilitates consistent measurement during spectroscopic analysis. The FTIR spectra were recorded over a broad range of wave numbers, spanning from 400 to 4000 cm^{-1}, with a spectral resolution of 4 cm^{-1}. This range encompasses various vibrational modes associated with different functional groups in the molecules present in the sample. The obtained spectra serve as a fingerprint that reveals information about the chemical bonds and functional groups within the material. Comparative analysis was performed by examining the spectra of treated and untreated samples.

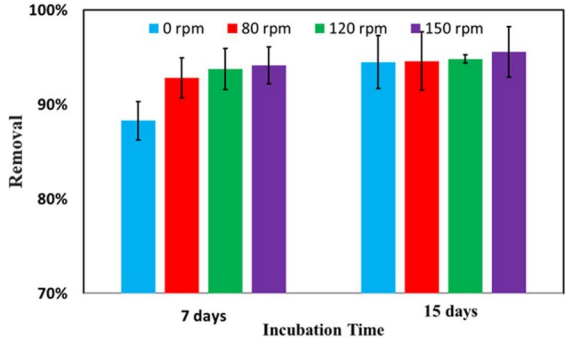

Fig. 1 Effect of agitation on biosorption of 4-bromophenol by *Pichia kluyveri* sp. FM012. Condition: glucose = 10 ppm, yeast = 10 ppm, pH = 5, temperature = 25 °C

3 Results and Discussion

The removal of 4-bromophenols by *Pichia kluyveri* FM012 primarily involves adsorption rather than degradation processes. In this context, adsorption refers to the physical and chemical interactions that result in the binding of 4-bromophenols onto the surfaces of *Pichia kluyveri* FM012 cells or its extracellular structures, without undergoing metabolic breakdown or transformation. The adsorption process relies on the affinity of the microbial biomass for 4-bromophenols, facilitated by factors such as surface charges, functional groups, and the composition of the cell wall. As 4-bromophenols come into contact with *Pichia kluyveri* FM012, they adhere to the microbial surfaces through electrostatic interactions, hydrogen bonding, or other attractive forces. This adsorption-based approach offers a means of achieving comprehensive elimination of 4-bromophenols from the environment without necessarily involving the degradation of the pollutant. The advantage of this method lies in the ability of *Pichia kluyveri* FM012 to act as a sorbent, concentrating and immobilizing 4-bromophenols, thus reducing their presence in the surrounding medium. Understanding the intricacies of this biosorption process is crucial for developing effective strategies for the remediation of environments contaminated with 4-bromophenols.

3.1 Effect of Agitation

The effect of agitation on the biosorption of 4-bromophenol by *Pichia kluyveri* FM012 is elucidated in Fig. 1, revealing the dynamic influence of agitation on the sorption process. The impact of agitation manifests in two primary ways, significantly influencing fungal incubation. Firstly, higher agitation speeds contribute to the adequate oxygenation of the culture, ensuring a well-oxygenated environment for the fungal cells. Simultaneously, the elevated energy generated by increased agitation imparts substantial shear stress on the compromised culture cells and mycelium, potentially enhancing the accessibility of sorption sites.

During the initial static phase, characterized by minimal agitation, a lower efficiency in 4-bromophenol removal (88%) is observed over a 7-day period. This phase represents suboptimal conditions for sorption, as the limited agitation restricts both oxygen availability and the physical interactions between the fungus and the contaminant. As the agitation speed and duration are increased, the removal of 4-bromophenol exhibits a general upward trend, culminating in optimal elimination at a speed of 150 rpm, where 96% removal is achieved. This peak efficiency can be attributed to the synergistic effects of enhanced oxygenation and increased shear stress, facilitating improved substance exchange between the culture medium and the fungal cells.

The efficacy of sorption is closely tied to the interface between the fungus's surface and the supplied oxygen, showcasing the importance of these factors in fostering an environment conducive to efficient biosorption. Furthermore, lower rotation speeds are associated with reduced biosorption, resulting in insufficient contact between adsorbent particles in the aqueous solution. This observation aligns with the findings of previous studies (Hadibarata et al., 2007; Parvathi

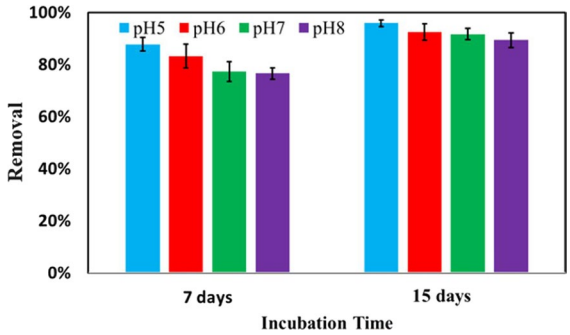

Fig. 2 Effect of pH on biosorption of 4-bromophenol by *Pichia kluyveri* sp. FM012. Condition: glucose = 10 ppm, yeast = 10 ppm, agitation speed = 150 rpm, temperature = 25 °C

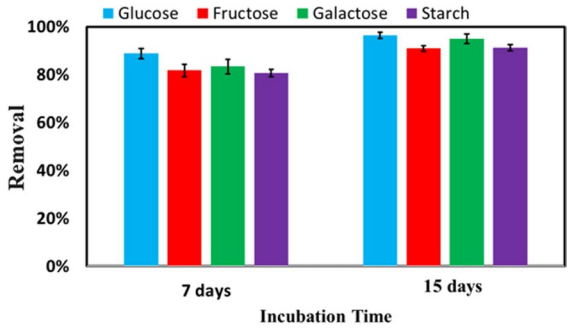

Fig. 3 Effect of carbon source on biosorption of 4-bromophenol by *Pichia kluyveri* sp. FM012. Condition: yeast = 10 ppm, agitation speed = 150 rpm, pH = 5, temperature = 25 °C

et al., 2007; Rajesh et al., 2014), underlining the significance of agitation in promoting effective biosorption. Overall, the study emphasizes the dynamic relationship between agitation and the sorption process, shedding light on the critical factors influencing the removal of 4-bromophenol by *Pichia kluyveri* FM012.

3.2 Effect of pH

Figure 2 shows the influence of adsorption 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 biosorption of 4-bromophenol. At lower pH, protonation of H^+ can combine with the surface of the fungi to increase biosorption (Commandeur & Parsons, 1994). However, as pH continues to increase, 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 adsorption (Argun & Dursun, 2006; Jalali et al., 2002; Kalac et al., 1996; Putra et al., 2014).

3.3 Effect of Carbon Sources

Figure 3 shows the effects of different carbon sources on the biosorption 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 removal 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 removal of 4-bromophenol with a value of 96%. In the same period, the removal 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 consumable carbon source such as glucose can stimulate the sorption of pollutants (Östberg et al., 2006) 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 biosorption of 4-bromophenol (Khelifi et al., 2009). Due to the more easily degradable carbons, they discovered that the relationship between the microbial response and organic additions increased the biosorption of 4-bromophenol.

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 removal of 4-bromophenol in shake flask liquid cultures observed with yeast 88 and 96% for 7- and 15-day incubation, respectively. On the other hand, cultures supplemented with peptone and

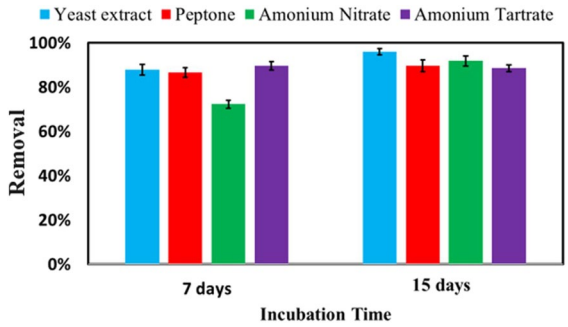

Fig. 4 Effect of nitrogen source on biosorption of 4-bromophenol by *Pichia kluyveri* sp. FM012. Condition: glucose = 10 ppm, agitation speed = 150 rpm, pH = 5, temperature = 25 °C

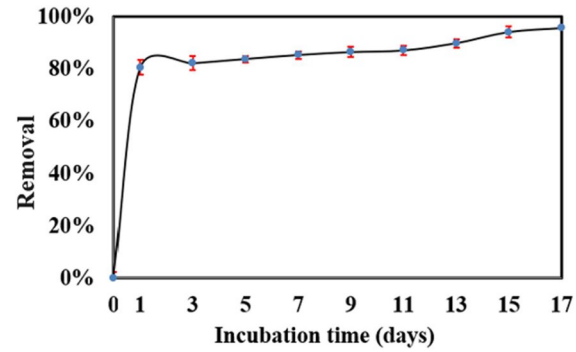

Fig. 5 Effect of contact time on biosorption of 4-bromophenol by *Pichia kluyveri* sp. FM012. Condition: glucose = 10 ppm, yeast = 10 ppm, agitation speed = 150 rpm, pH = 5, temperature = 25 °C

ammonium tartrate reached 90 and 89% sorption after 15 days of incubation. Nitrogen is an essential component of amino acids, which are the building blocks of proteins. Proteins are crucial for various cellular functions, including the synthesis of structural components. Adequate nitrogen supply supports the synthesis of proteins that may be involved in the biosorption process, such as those located on the microbial cell surface (Krysenko, 2023). Specific nitrogen is also present in molecules like ATP (adenosine triphosphate), a primary energy carrier in cells. Adequate nitrogen supports cellular respiration and energy production, influencing overall metabolic activity. Improved energy levels may contribute to increased microbial activity and biosorption capacity (Ene et al., 2014).

Meanwhile, the performance of ammonium nitrate in biosorpt 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 eliminate 4-bromophenol.

3.5 Effect of Contact Time

The contact time has a parallel correlation with the biomass surface area in adsorption studies (Huang et al., 2013). It is important to design batch adsorption studies by monitoring the percentage of 4-bromophenol removal. 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.

3.5.1 Isotherm 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 (Babalola et al., 2009; Onyancha et al., 2008). 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.

3.6 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 (Hameed et al., 2008). 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

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

Biomass	Langmuir parameters			Freundlich parameters		
	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

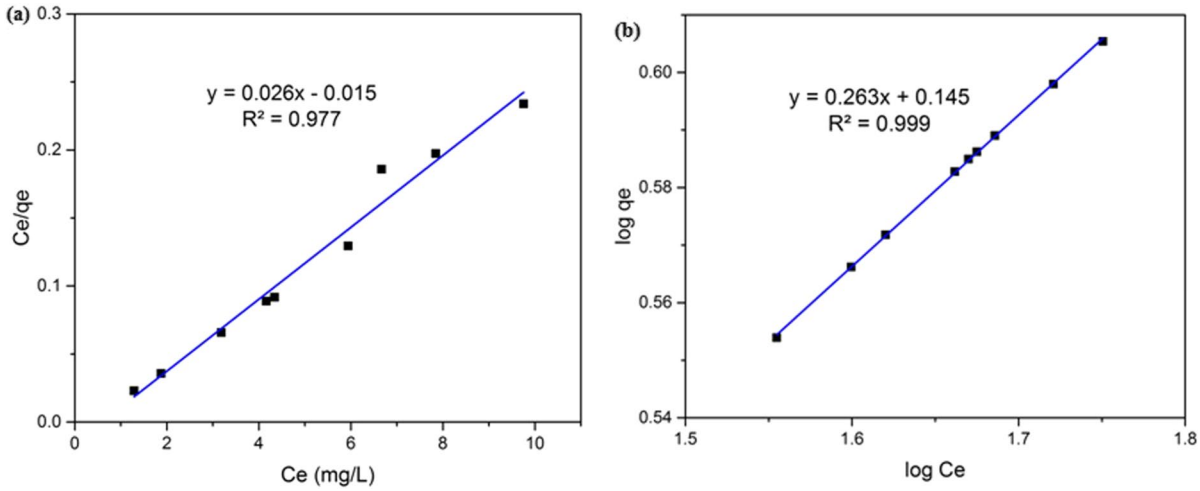

Fig. 6 Adsorption isotherms: (**a**) Langmuir and (**b**) Freundlich isotherm for the adsorption of 4-bromophenol

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

Parameters	Pseudo-first order			Pseudo-second order			Intra-particle diffusion		
	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

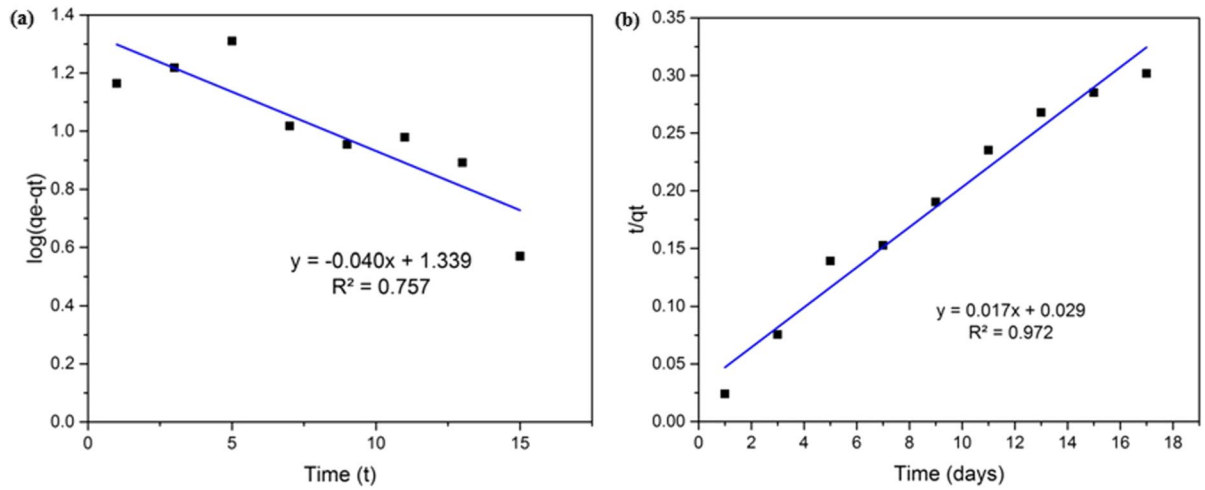

Fig. 7 Adsorption dynamics: (**a**) pseudo-first-order and (**b**) pseudo-second-order kinetics plots for the adsorption of 4-bromophenol by *Pichia kluyveri* sp. FM012

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.

3.7 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, Fig. 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 removal and increase the biosorption capacity.

The FTIR 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 (Ramrakhiani et al., 2011). The second peak may be due to disulfide or nitro groups (Ramrakhiani et al., 2011; Zhao et al., 2015) and bioligands (Akar et al., 2009). 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 (Wei et al., 2011). All of these findings indicated that adhesion of 4-bromophenol to the cell

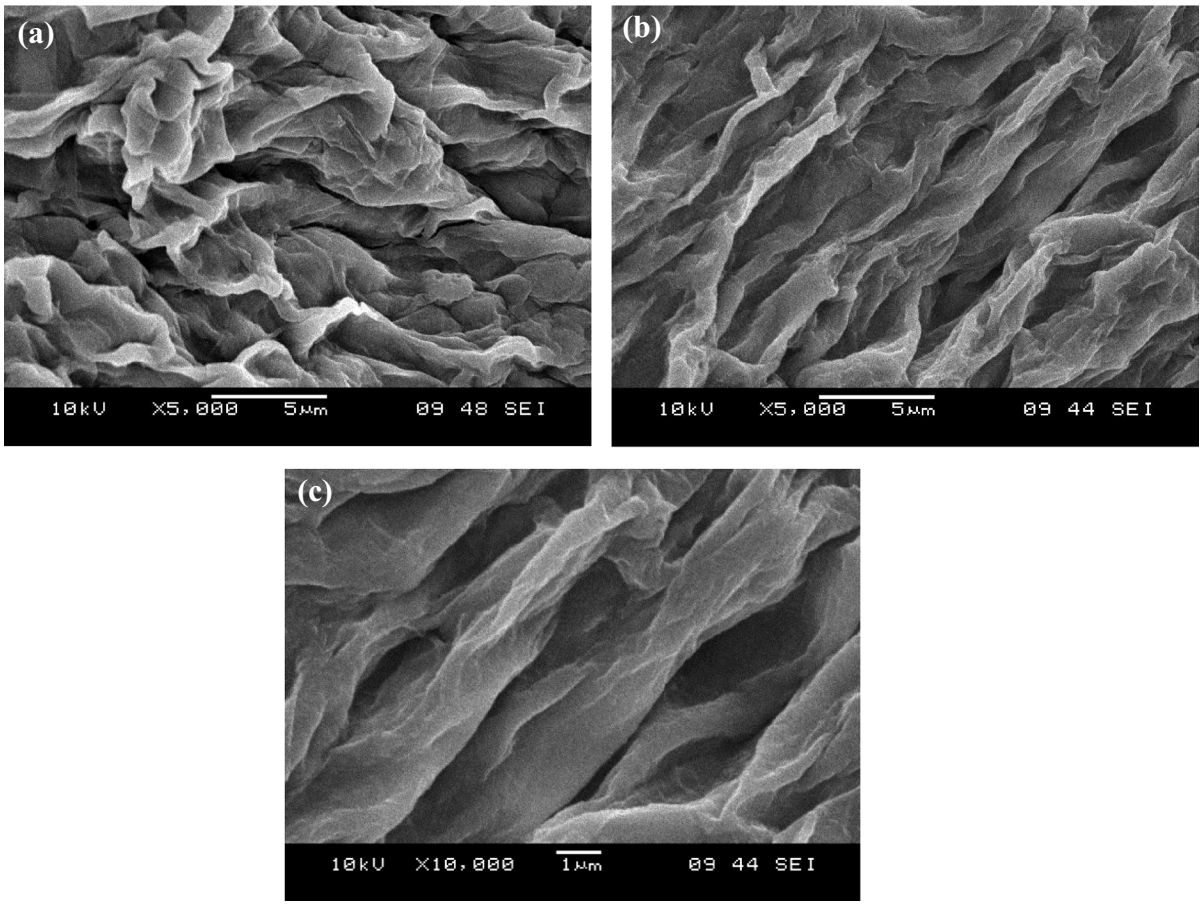

Fig. 8 Scanning electron microscopy (SEM) micrographs of the *Pichia kluyveri* before (**a**) and after (**b**, **c**) of biosorption

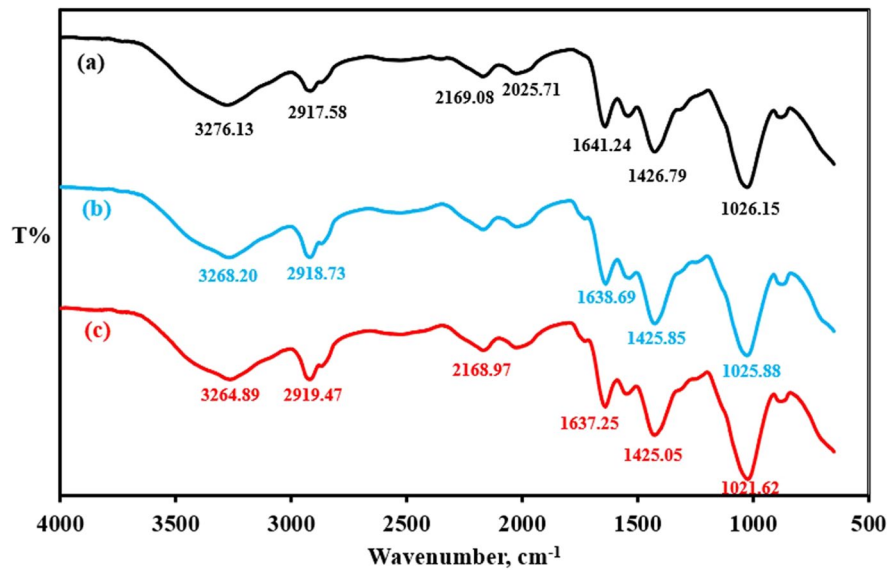

Fig. 9 FTIR spectra of *Pichia kluyveri* sp. FM012: (**a**) control, (**b**) 7 days, (**c**) 15 days

surface pores, and active binding sites resulted in adsorption.

4 Conclusions

The biosorption 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 biosorption of 4-bromophenol under aerobic conditions. The biosorption 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 biosorption. 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.

Author Contribution Ismallianto Isia: investigation, methodology, data formal analysis. Yudi Sukmono: investigation, methodology, data formal analysis, validation, writing—original draft, review and editing. Tony Hadibarata: investigation, conceptualization, writing, review and editing, supervision. Murat Yılmaz: writing—review and editing, visualization.

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Data Availability Data will be made available on request.

Declarations

Ethical Approval Not applicable.

Competing Interests The authors declare no competing interests.

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