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Enhanced Culture in Static Batch Systems for Benzo[a]pyrene Degradation by Bjerkandera Adusta SM46 under Acidic and Saline Conditions

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Abstract

Biodegradation of high-molecular weight polycyclic aromatic hydrocarbons (PAHs) is biologically difficult to degrade due to its recalcitrant characteristics such as low solubility in water and inhibition effect. In the present study, we used an enhanced culture system (ECS) for *Bjerkandera adusta* SM46 on benzo[a]pyrene (BaP) degradation under acidic (pH 4.5) and saline conditions (pH 8.2). The ECS using Tween 80 (T-80) and $MnSO_4$ enhanced both the ligninolytic enzyme activities and degradation rate by the fungus. The optimum condition was obtained at a concentration for $MnSO_4$ of 0.4–0.9 mM and for T-80 of 0.45–0.70%, which increased degradation more than 87% at day 30 (pH 4.5). The ECS could also be applied under a saline condition for up to 82% degradation. Mangan peroxidase (MnP) and lignin peroxidase (LiP) showed high activities at pH 4.5 (110.9 and 79.9 U/L, respectively). Moreover, laccase and MnP showed higher activities (75.6 and 130.0 U/L, respectively) at saline pH 8.2 indicating stress condition stimulation. Using rice straw also enhanced the BaP degradation rate by the fungus via cell growth promotion. This study suggests that an ECS treatment and rice straw co-substrate system could potentially enhance PAH degradation for a static batch system under both an acidic and a saline-alkaline stress condition.

***Keywords*:** benzo[a]pyrene; *Bjerkandera adusta* SM46; enhanced culture system

Abbreviations:

BaP	:	Benzo[a]pyrene
ECS	:	Enhanced culture system
PAHs	:	Polycyclic aromatic hydrocarbons
WRF	:	White rot fungi

1. Introduction

Benzo[a]pyrene (BaP), comprising five fused benzene rings, is one of the most toxic and hard-to-degrade PAHs. There have been many studies of this compound because of its carcinogenicity, teratogenicity, and toxicity on the marine and terrestrial environments [1][2]. BaP is a high-molecular weight (HMW) PAH and has very low solubility in water due to its hydrophobicity [3]. Solubility of BaP in water is only 0.003 mg/L, lower than other PAHs such as naphthalene (31.7 mg/L), anthracene (0.07 mg/L), phenanthrene (1.3 mg/L), and pyrene (0.14 mg/L) [1]. BaP is also not efficiently degraded by some microorganisms because they do not induce the enzymes necessary for catabolism [3]. These characteristics make it difficult to remove BaP from the environment. Accumulation of PAH compounds, including BaP, have been reported in many studies [2][5][6].

Among the microorganisms used as biodegraders for bioremediation, white rot fungi (WRF) have shown the ability to degrade various pollutant PAHs, including BaP [3][7][8]. White rot fungi can produce extracellular ligninolytic enzymes such as laccase (Lac), mangan peroxidase (MnP), and lignin peroxidase (LiP) [1][2][3][4][8]. These enzymes have an important role in initializing the BaP cleavage process through BaP-quinone formation as an intermediate product [8]. In this study, we used a new isolated white rot fungus, namely *Bjerkandera adusta* SM46, as a biodegrading agent of BaP. Based on our previous screening studies, this fungal strain showed the highest potential for degrading BaP compared with other screened WRF such as *Trametes versicolor*, *Ganoderma gibossum*, and *Microporus subaffinis* [9]. The investigation of PAH degradation using these genera has also been previously reported [10][11]. However, the enhanced culture system (ECS) for BaP degradation by *Bjerkandera adusta* has

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not been tested to date. Using ECS for the WRF could increase BaP biodegradability and induce the ligninolytic system during the degradation process. This system could also be used in various extreme conditions such as saline-alkaline stress to alleviate PAH contamination in seawater.

In the present study, we investigated whether BaP degradation could be enhanced by *Bjerkandera adusta* SM46 using non-ionic surfactant Tween 80 (T-80) and an enzyme inducer ($MnSO_4$) under an acidic and saline condition. Three ligninolytic enzymes produced by the fungus were analyzed during incubation. In addition, we compared the enhanced culture system (ECS) and carbon substitution treatment using several lignocellulosic biomasses to determine the differences in the effects of chemical and natural inducers in the degradation by the fungus. Concentrated crude enzymes under non-immobilized and immobilized conditions were also used to investigate the biodegradation of BaP via an enzymatic process.

2. Materials and Method

2.1. Chemicals

Benzo[a]pyrene was obtained from Tokyo Chemical Industry Co. Ltd. (Tokyo, Japan). Agar, malt extract, glucose, silica gel C-200, polypeptone, and other solvents for extraction analysis were purchased from Wako Pure Chemical Industries, Ltd. (Japan). Artificial seawater powder was obtained from Delphis (Japan).

2.2. Fungal strain

Bjerkandera adusta SM46, a white rot fungus (WRF), was used in this study. The WRF was isolated from Saragamine Mountains, Matsuyama, Japan. The sequence of SM46 ribosomal DNA including the ITS region showed 99–100% identity with *Bjerkandera adusta*. *Bjerkandera adusta* SM46 was selected based on its ability to degrade BaP and produce extracellular ligninolytic enzymes. The fungus has saline-alkaline tolerant characteristics determined by screening under several seawater concentrations [9]. The mycelia of *B. adusta* SM46 was maintained in malt extract agar (MEA) medium (malt extract 20 g/L, glucose 20 g/L, polypeptone 1 g/L, agar 20 g/L) in a disposable plastic Petri dish at 4°C prior to use.

2.3. Experimental design of enhanced culture system for BaP degradation

BaP degradation was enhanced in 100 mL-Erlenmeyer flasks containing 20 mL of malt extract liquid medium (malt extract 20 g/L, glucose 20 g/L, and polypeptone 1 g/L). The medium was sterilized for 20 min at 121°C and then three plugs of each fungus were inoculated into the Erlenmeyer flask and pre-incubated for 7 d. The effect of $MnSO_4$ and T-80 on biodegradation was examined using a factorial design with concentrations of $MnSO_4$ of 0, 0.5, and 1.0 mM, and T-80 of 0, 0.25, 0.50, and 1%. The solution was added at day 6 after fungal inoculation. The result was presented using contour plot analysis MINITAB 14. The optimum condition was also applied to a saline condition at pH 8.2 and salinity 35 g/L. Each of the inoculated flasks was supplemented with BaP solution (final concentration 20 ppm) then incubated for 15 and 30 days in dark conditions at 25°C. A positive control (autoclaved control) was used in this experiment to quantify the adsorption losses of BaP-caused mycelium in a liquid medium. The positive control was sterilized using an autoclave before adding BaP. The degradation rate and extracellular ligninolytic enzymes expressed during incubation were measured during the incubation process.

2.4. Selection of lignin-based co-substrate for *Bjerkandera adusta* SM46 during BaP degradation

To investigate a suitable natural co-substrate for *B. adusta* SM46 in BaP degradation, five substrates were used: wood meal (5 g/L), rice straw (5 g/L), waste pulp (5 g/L), kapok (2.5 g/L), and ligninesulphonic acid (5 g/L). The co-substrates were added to the 100 mL-Erlenmeyer flasks containing 20 mL of malt extract liquid medium (without glucose). Enzyme activities (laccase/Lac, mangan peroxidase/MnP, and lignin peroxidase/LiP), degradation rate, and fungal biomass were measured on day 15 and day 30. The contents of lignin, hemicellulose, and cellulose of natural biomasses were also measured (by Research Centre of Chemistry- Indonesian Institute of Sciences).

2.5. Free-cell crude enzyme production

Rice straw, as the most suitable substrate for *B. adusta* SM46, was used as a pre-grown medium for ligninolytic enzyme production. Furthermore, 10 g of rice straw (w/w), 10% of glucose, 15% of *shiitake no sato* (a kind of nutrient for mushroom), and 60% of distilled water were added to the autoclavable-plastic tray and then autoclaved for 3 h at 121°C. Several 5 mm disks of fungal mycelia *B. adusta* were added to the medium and then incubated for approximately one month. After incubation, white fungal mycelia appeared in the medium and the ligninolytic enzymes were ready to be extracted. Crude enzyme was extracted from the pre-grown fungus using a homogenizer at 10000 rpm for 10 minutes with malonate buffer pH 4.5. After filtration, filtrate was centrifuged at 8000 rpm and 4°C for 20 min. Clear supernatant was collected and placed in a glass beaker. Ammonium sulfate (75%) was added to beaker and stirred for approximately 1–2 h in an ice bath, then centrifuged at 8000 rpm and 4°C for 20 min. A pellet was collected and diluted in the malonate buffer. Crude enzyme powder was obtained after freeze-drying for approximately 3 d [12]. The enzyme activity of the crude enzyme powder was analyzed and ready to be used for enzymatic hydrolysis of BaP.

2.6. Immobilization of crude enzyme

Crude enzyme was immobilized using alginate beads. Crude enzyme (0.34 U/mL) and sodium alginate (1.5% w/v) were mixed and stirred for approximately 2 h. The enzyme-alginate solution was then added to the $CaCl_2$ solution (1 mM) by pipette, drop by drop. Alginate bead-immobilized enzyme was formed as indicated by the appearance of granules at the solution surface. The immobilized enzyme was keep at 4°C prior to use.

2.7. Enzymatic hydrolysis of BaP

For enzymatic degradation, free-cell crude enzyme and immobilized enzyme using alginate beads were used. The experiments were conducted in 100 mL-Erlenmeyer flasks containing 20 mL malonate buffer (pH 4.5) for acidic conditions and 20 mL seawater (pH 8.2, salinity 35 g/L) for saline-alkaline conditions. The required amount of BaP (20 ppm) was added to the solution. In this study, the effect of ECS using a mixture of T-80 and $MnSO_4$ was also investigated. The experiment was conducted for 2, 4, 6, 8, 15, and 30 d.

2.8. Analysis method for BaP degradation

After 15 and 30 d of incubation, the culture medium was filtered to separate fungal mycelia and liquid medium. The liquid was extracted using funnel separation with 50 mL ethyl acetate three times. The fungal mycelia were extracted using an ultrasonic extraction method with ethyl acetate for 24 h. Each extract was collected and NaCl solution (5%) was added to remove fat. The collected extract was filtered with anhydrous sodium sulphate and then evaporated to obtain concentrated extracts. Both extracts from the above were combined and concentrated to 2 mL for GC-FID. The residue was dissolved in toluene and anthracene (20 ppm) was added as an internal standard and then analyzed using gas chromatography (GC-FID Shimadzu 2014), with a TC-5 capillary column (30 m, id x 0.25×0.25 mm). The helium carrier gas was delivered at a constant flow rate of 1.5 mL/min with a column pressure of 100 kPa and interface temperature of 280°C. The temperature was started at 60°C, increased at 10°C/min to 280°C, and maintained at 280°C for 10 min. The injection volume was 2 µL and the injector temperature was maintained at 280°C. The degradation rate was measured using a standard calibration curve. For metabolite detection, a similar method following previous studies was used [9].

2.9. Enzyme activity analysis

Laccase (Lac) activity was measured using the reaction of syringaldazine and extracellular enzymes in a sodium acetate buffer [13]. Manganese peroxidase (MnP) activity was measured as described by Takano et al. (2004) [14]. The reaction mixture contained 20 mM 2,6-dimethoxyphenol as a substrate, 50 mM malonate buffer (pH 4.5), 20 mM $MnSO_4$, and 2 mM H_2O_2. Lignin peroxidase (LiP) activity was determined from the reaction of 2 mM H_2O_2 and LiP buffer at 310 nm [15]. One unit (U) is defined as the amount of enzyme oxidizing 1 µmol of substrate per minute. The measurements were conducted using a UV-vis spectrophotometer.

3. Results and Discussion

3.1. Enhanced culture system for BaP degradation using MnSO4 and Tween 80

Enhanced biodegradability of BaP can be conducted using two methods. The first method is by fungal induction using a specific mineral solution for ligninolytic enzyme enhancement, which has a more important role in the degradation. Based on our previous studies [9], $MnSO_4$ was revealed as a suitable inducer among several ligninolytic inducers such as $CuSO_4$ and veratryl alcohol for enhancing the degradation rate by the fungus. Production enhancement of MnP by some white rot fungus with $MnSO_4$ has also been reported in some studies. However, each reported WRF has a different concentration tolerance in the presence of mineral inducer [7][16].

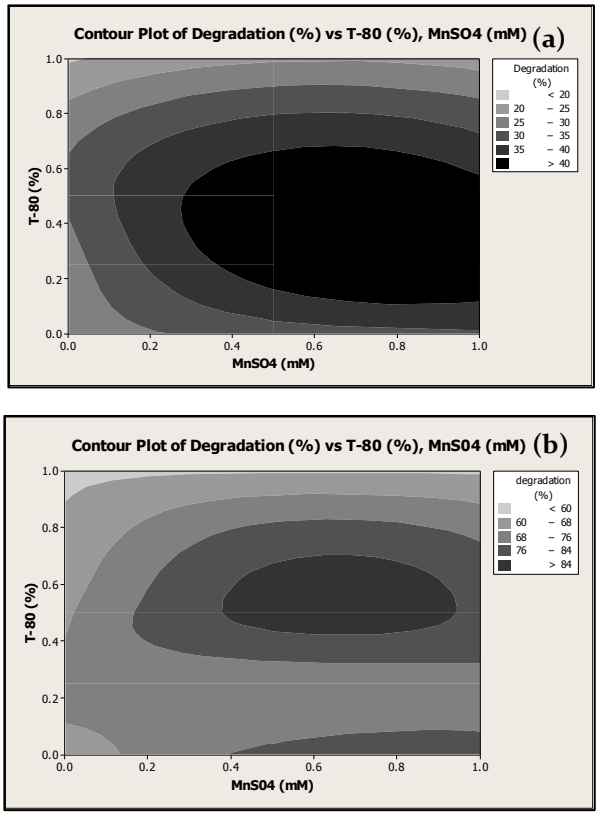

Figure 1 Contour plot of the biodegradation of BaP versus $MnSO_4$ and T-80 for 15 days (a) and 30 days (b)

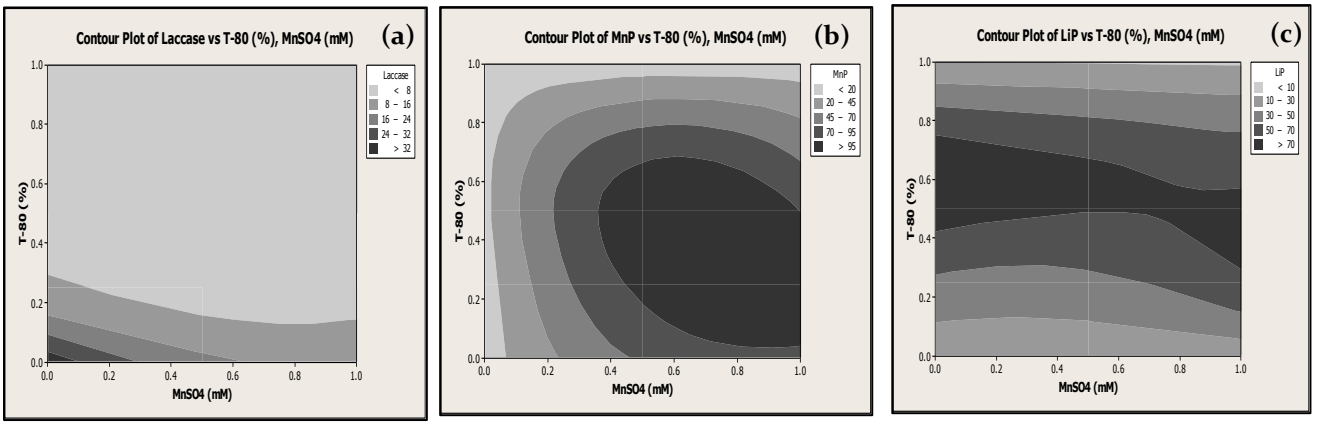

Figure 2 Contour plot of laccase (a), MnP (b), and LiP (c) versus $MnSO_4$ and T-80 for 15 days

The second method is by increasing the solubility of BaP in the water based-liquid medium. Surfactant is commonly added to a culture to enhance the solubility of PAHs. Synthetic surfactants such as T-80, Tween-40, and NAR-111-2 are some potential surfactants that can increase the biodegradability of hydrophobic pollutants such as petroleum crude oil and PAHs [17][18][19]. However, a combination of these two factors in a culture of *B. adusta* SM46 to enhance BaP biodegradation has not been tested to date, and is expected to result in a high biodegradation rate by the fungus.

Based on contour plot analysis of $MnSO_4$ and Tween 80 in biodegradation, the optimum condition (degradation rate more than 40%) at 15 days was obtained at a concentration for $MnSO_4$ of >0.25 mM and for Tween 80 of 0.10–0.65% (Fig. 1a). The optimum condition (degradation more than 84%) at 30 days was obtained at a concentration for $MnSO_4$ of 0.4–0.9 mM and for T-80 of 0.45–0.70% (Fig. 1b). The condition could improve the degradation up to 87% in 30 days. Adding $MnSO_4$ and T-80 increased BaP degradation 24% compared with control. A high concentration of Tween 80 (more than 0.8%) seems to have decreased degradation to less than 60%.

Ligninolytic enzymes produced during degradation was measured at day 15. The high activity of laccase was only detected in the culture treatment without adding $MnSO_4$ and T-80 (Fig. 2a). MnP activity was significantly affected by adding $MnSO_4$. A concentration of more than 0.4 mM enhanced MnP production more than 10-fold (Fig. 2b). High LiP was obtained at a concentration for Tween 80 of 0.4–0.7% and was not affected by the $MnSO_4$ concentration (Fig. 2c). The highest enzyme activity detected was 37.5 U/L for laccase, 110.9 U/L for MnP, and 80.9 U/L for LiP. MnP was revealed as the predominant enzyme produced during the degradation process by the fungus.

In the culture treatment, $MnSO_4$ has two important roles during the degradation process by the fungus. The first is to induce the catabolic system of the fungus to produce a more specific ligninolytic enzyme such as MnP. For the second, MnP functions as a redox mediator in the enzymatic reaction of BaP and MnP. T-80 is a non-ionic surfactant and emulsifier derived from polyethoxylated sorbitan and oleic acid and is often used in foods. T-80 has been reported not only as an enhancer of hydrophobic-pollutant solubility but can also increase LiP production by WRF [17][19]. Adding T-80 can increase the oxygen mass transport coefficient between the growth medium and the interior of the fungal cell [16]. Behnood et al. (2014) also found that adding T-80 can reduce interfacial tension (IFT) and increase the solubility of hydrophobic pollutants such as crude oil [19].

3.2. Comparison of BaP degradation under an acidic and a saline-alkaline stress condition

In this study, we applied ECS for BaP degradation under saline-alkaline stress. Several studies reported that some PAHs such as BaP were found in seawater and some coastal areas that have similar saline-alkaline characteristics [20][21]. However, using WRF such as *B. adusta* for contaminated seawater is rarely reported due to limited white rot fungi that are salt tolerant [22]. Two types of liquid medium were used in the present study: malt extract liquid medium at pH 4.5 (ME) and seawater-liquid medium at pH 8.2 with salinity 35 g/L (SME). At pH 4.5, ECS significantly enhanced (at a 95% level of significance) MnP and LiP production. MnP and LiP increased 13-fold and 5-fold, respectively, at pH 4.5 under ECS (Fig. 3a). Enhancing the production of these enzymes stimulated more BaP removal in the culture system. BaP degradation improved 87% at 30 days. ECS improved BaP removal by about 25% at pH 4.5. However, ECS inhibited laccase production 8-fold on day 15. This phenomenon may be due to interaction of the $MnSO_4$ and Tween 80 with a self-inhibiting laccase in the medium. Under a saline condition (pH 8.2), the BaP degradation rate increased significantly up to 82% at day 30 (Fig. 3b). Moreover, production of ligninolytic enzymes under saline alkaline stress (non-ECS) was lower than under

an acidic condition. The condition affected reduction of BaP removal by the fungus. However, adding $MnSO_4$ and T-80 to the culture reduced the inhibition effect of saline-alkaline stress. The degradation of BaP under ECS conditions was 44% higher than the control treatment (non-ECS). Laccase and MnP increased 37-fold and 43-fold at day 15, respectively (Fig. 3b). However, there was no significant enhancement of LiP under the saline condition. The ECS treatment under a saline condition (pH 8.2) had a greater effect than under an acidic condition (pH 4.5). The fungal intracellular mechanism with $MnSO_4$ and Tween 80 under saline-alkaline stress by *B. adusta* SM46 requires further investigation. Kamei et al. (2008) reported that the ability of a fungus to produce ligninolytic enzymes under a saline condition was affected by specific saline-dependent regulation of the enzymes [23].

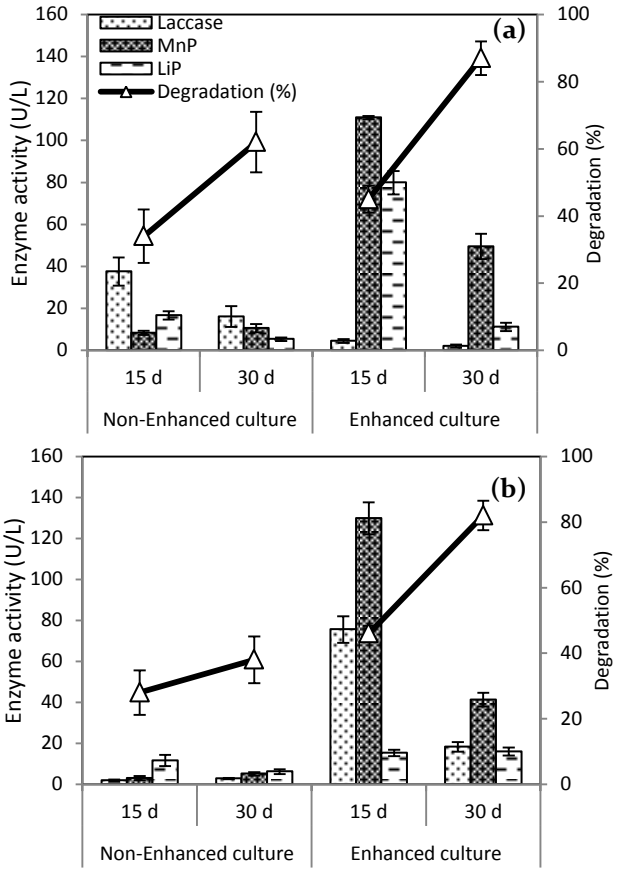

Figure 3 Comparison of degradation rate and ligninolytic enzyme production at pH 4.5 (a) and saline pH 8.2 (b)

3.3 Effect of lignocellulosic-based co-substrate on enhancement of BaP degradation

Figure 4a shows the effects of five lignocellulosic-based co-substrates on the biodegradation of BaP by the white rot fungus *B. adusta* SM46. BaP was degraded efficiently when several substrates such as rice straw, pulp, and glucose (as a comparison) were added to the culture medium. The highest degradation rate was obtained by adding rice straw to the system. The degradation rate improved by 32% compared with control (24%). Adding pulp, ligninesulfonic acid, and glucose increased the BaP degradation rate by 14%, 12%, and 10%, respectively. Wen et al. (2011) reported that enhanced degradation efficiency by WRF with co-substrates is related to the fungal co-metabolism system [23]. Several studies have also reported the same result [3][25][26]. Mesyami and Baheri (2003) reported that pine wood chips, peat moss, and Kellogg's Bran Flakes could be the bulking agents and solid amendments for degrading crude oil by white rot fungi [25]. Sari et al. (2014) also reported that oil palm empty fruit branch (OPEFB) can be used as an alternative co-substrate for white rot fungi to degrade organopollutants in batches and a bioreactor [26].

Kapok and wood meal are lignocellulosic sources that are abundant in nature and rarely studied. However, adding wood meal and kapok as co-substrates to a culture system do not increase the degradation efficiency by the fungus. These two co-substrates may be unsuitable for *B. adusta* SM46 fungal growth in inducing the ligninolytic system [24]. Lignin content in biomass (Table 1) did not correlated with the ligninolytic enzymes production .

Figure 4b shows ligninolytic enzyme production during BaP degradation using several co-substrates at day 15. The results showed that additional co-substrates could stimulate the secretion of the specific ligninolytic enzymes, except for wood meal and kapok. The maximum production of Lac (65 U/L) and MnP (104 U/L) was reached when adding rice straw, and the degradation efficiency of BaP was also the highest. The maximum production of LiP (41 U/L) was obtained when adding ligninesulfonic acid as a co-substrate. However, adding wood meal and kapok did not significantly enhance the ligninolytic system. Lac was not detected in these two culture systems. MnP was only 1 and 18 U/L, respectively, while LiP was only 10 and 18 U/L, respectively. The low enzyme production positively correlated with the low BaP degradation efficiency. The degradation efficiencies of BaP with wood meal and were also lower than control.

Table 1 Content of lignocellulosic-based co-substrates

No	Co-substrate	Cellulose [%]	Hemicellulose [%]	Lignin [%]
1	Pulp	38.9	15.2	29.6
2	Kapok	34.3	17.3	17.9
3	Rice straw	35.4	13.6	14.2
4	Wood meal	38.0	12.0	30.0
5	LSA	-	-	-

Figure 5a and 5b show the comparison of the degradation efficiency of BaP and ligninolytic enzymes produced during culture treatment with and without rice straw as a co-substrate. The significant enhancement of both degradation rate and enzyme activity occurred not only under a non-saline condition but also a saline-alkaline

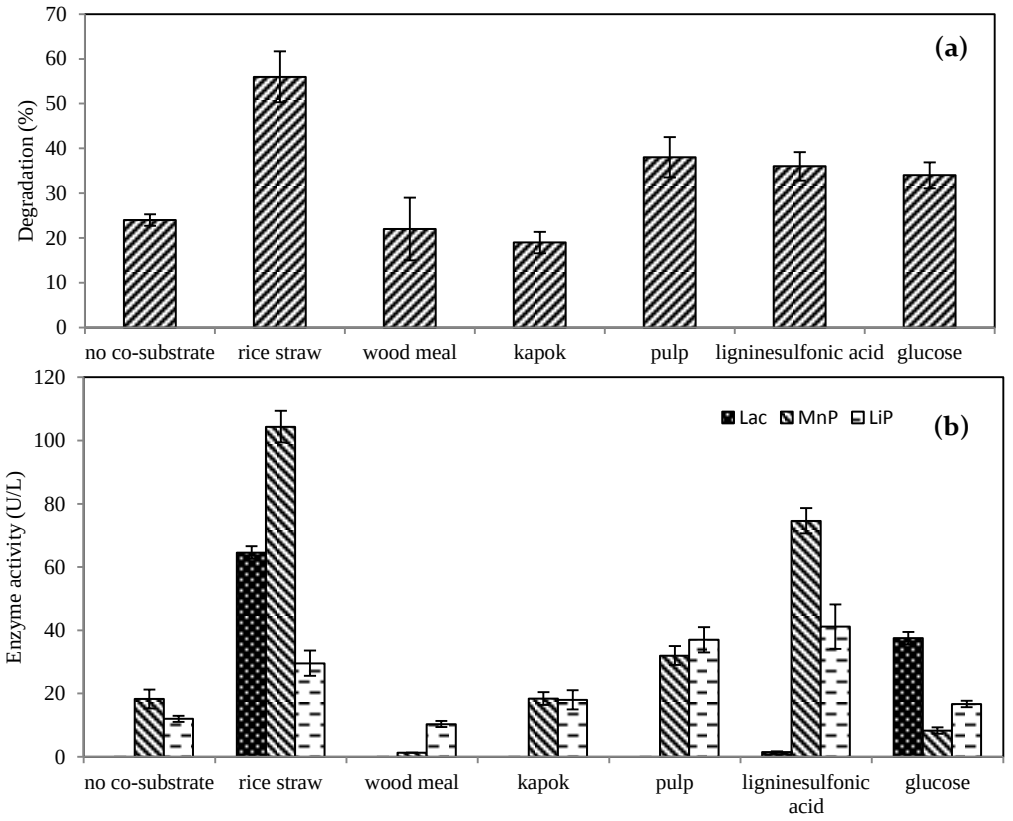

Figure 4 The degradation of benzo[a]pyrene (a) and production of ligninolytic enzyme (b) with and without co-substrate

condition. Adding higher concentrations of rice straw improved ligninolytic enzyme production,especially for Lac and MnP under both conditions, but it did not significantly improve degradation efficiency. Adding rice straw (2.5% and 5%) increased the degradation rate up to 76.8% and 78.7% at pH 4.5, respectively. Therefore, under the same condition, the degradation rate increased up to 78.3% and 82% at saline pH 8.2, respectively.

There was a different mechanism of inducing the catabolic system under the ECS condition using rice straw as a co-substrate. In the ECS, there was no enhancement of fungal growth under both conditions (Fig. 6a). Meanwhile, with rice straw, fungal growth increased by 62.3% at pH 4.5 (non-saline) and 64.8% at pH 8.2 (saline) (Fig. 6b). Based on these results, we can summarize that ECS enhanced the induction of the fungal catabolic system with no cell growth promotion. Moreover, enhancing the catabolic system with rice straw as a co-substrate involved cell growth promotion. To our knowledge, this report is the first to describe a mechanism of co-metabolism for BaP degradation by *B. adusta*, particularly under a saline-alkaline stress condition.

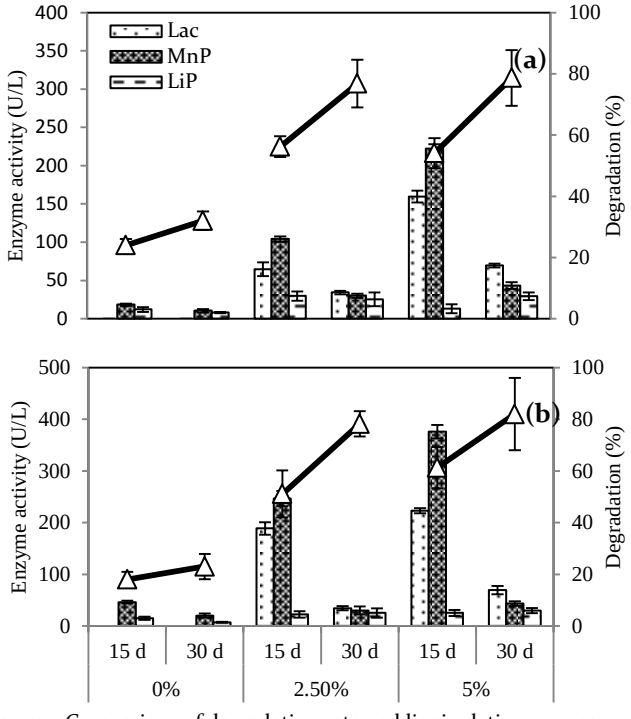

Figure 5 Comparison of degradation rate and ligninolytic enzymes with rice straw as a co-substrate at pH 4.5, non-saline (a) and pH 8.2, saline

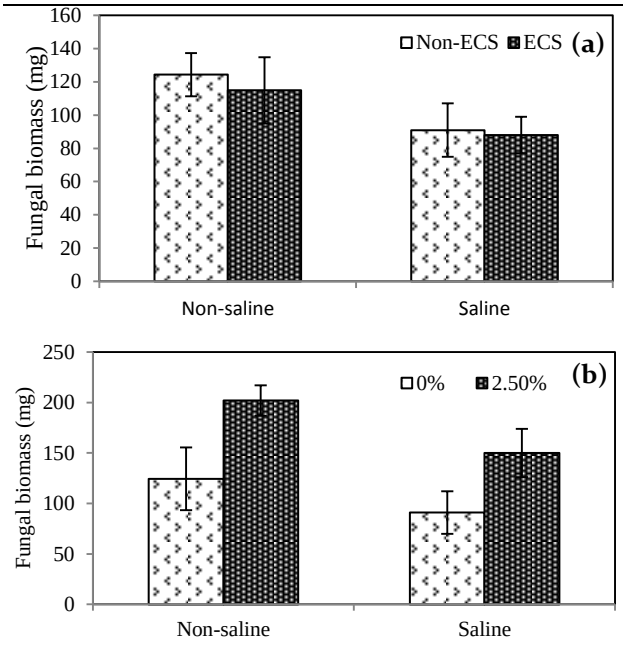

Figure 6 Comparison of fungal biomass under an ECS condition (a) and with rice straw as a co-substrate (b)

3.3. BaP degradation using free crude enzyme and alginate-bead immobilized enzymes

For enzymatic degradation, isolate *B. adusta* SM46 was pre-grown for one month in rice straw containing glucose (10%) and shiitake nutrient (15%) with a final water content of 60%. Concentrated crude enzyme was obtained using ammonium sulphate precipitation (75%). Each flask of treatment used 340 mU (MnP). Under ECS, the degradation rate exceeded 81% in two days at pH 4.5 (Fig. 7), increasing more than 3-fold compared with the non-ECS treatment under the same condition. However, under a saline condition, ECS treatment did not significantly enhance the degradation rate. Under both conditions, the biodegradation rate was stagnant after six days.

The immobilization process of an enzyme using alginate beads (1.5% w/w) can retain the degrading ability of the enzyme for longer incubation. The degradation rate was 96% at pH 4.5 and 78% at pH 8.2 at 30 days. Enzyme immobilization is a usable method for maintaining high percentage activity of an enzyme for long incubation, increases the thermostability of an enzyme, and avoids inactivity because of an imbalance in the chemical content in a fungal culture [27].

3.4. Metabolite detection during enzymatic degradation of BaP

Finally, we investigated the metabolite products of BaP in a cell-free reaction mixture with crude enzyme. The reaction mixture turned orange during incubation, and the color intensity increased with longer incubation. This phenomenon indicates that BaP changes into different compounds as metabolites. To verify the presence of metabolites, after the reaction the ethyl acetate extract was analyzed using a TLC, UV-spectrophotometer, and GC-mass spectrometer. In the course of BaP conversion, we found clear evidence for the intermediary formation of BaP-1,6-dione, which was concluded from the appearance of the TLC profile, UV and visible spectra (maximum absorbance), and fragment ion of GC-MS analysis, compared with a synthetic standard of BaP-1,6-dione (Fig. 8). Ring fission products of this compound were not found in the reaction mixture. A different result was obtained with degradation using the fungal cells. Previous studies reported that degradation of BaP using fungal cells of *B. adusta* SM46 [9] resulted not only in BaP-1,6-dione but also other ring fission products such as naphthalene acetic acid, *p*-hydroxybenzoic acid, benzoic acid, and catechol. Mineralization of PAHs is a complicated process involving many enzymes and different catabolic pathways [1][2][3][9]. MnP-crude enzyme itself

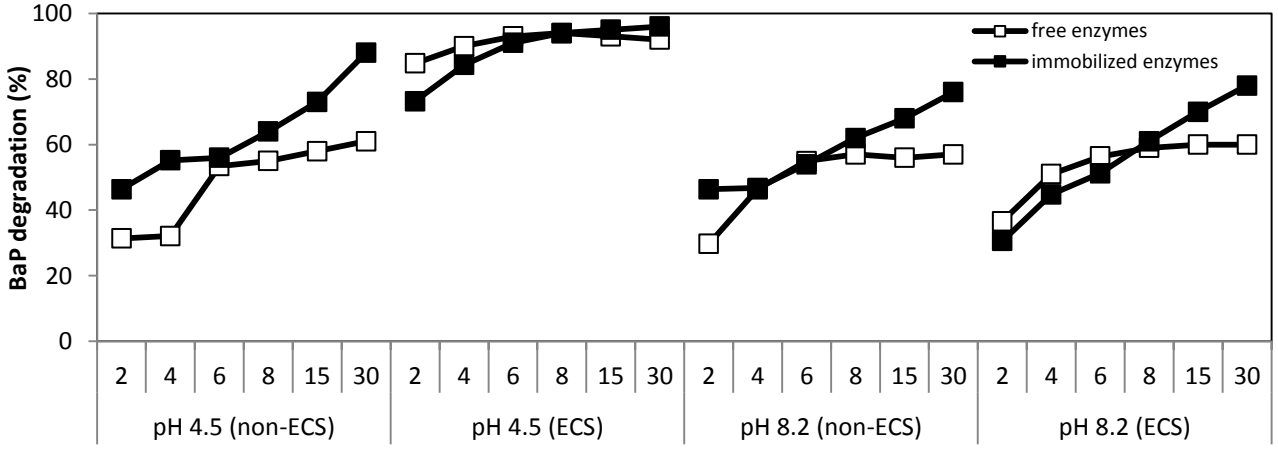

Figure 7 Degradation of BaP by free-cell crude enzymes and immobilized enzymes produced by rice straw and pre-grown *B. adusta* SM46

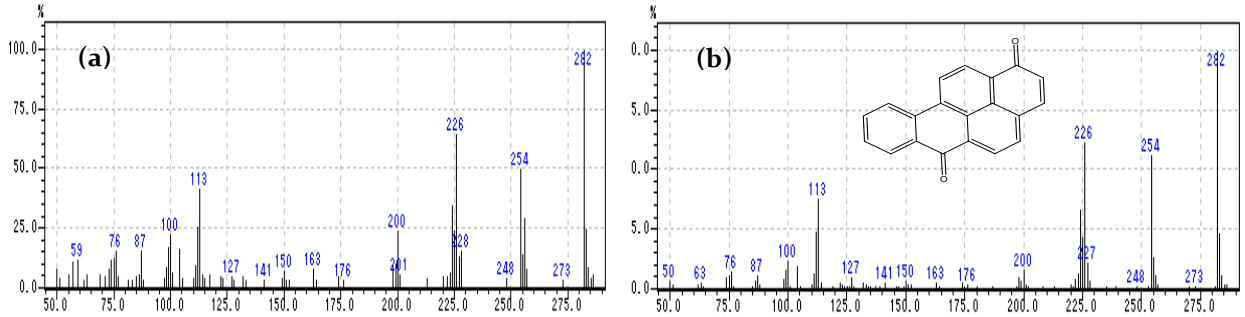

Figure 8 Mass spectra of standard (a) and sample (b) BaP-1,6-dione

appears to only initialize the process of degradation by quinone formation, but it cannot further catabolize into a lower molecular weight. However, extracellular MnP of *B. adusta* SM46 proved to be a stable enzyme for transforming BaP into intermediate compounds that can be easily catabolized by other enzymes produced by the fungi.

Based on our present and previous investigation, we propose that degradation using fungal cells is more effective than an enzymatic process to completely destroy BaP. WRF can produce various intracellular and extracellular enzymes that are involved in the ring fission process. As further investigation, we will focus on degrading various PAHs using ECS treatment in different media such as contaminated solid medium (sea sand and soil) and a vertical bioreactor system by fungal treatment with *B. adusta* SM46.

Environmental pollution caused by hazardous wastes containing recalcitrant xenobiotic chemicals such as benzo[a]pyrene has become a major problem that threatens the sustainability of ecosystems as well as human health. Bioremediation is one potential method for removing this toxic pollutant. As a further application of our research, *B. adusta* SM46 along with the ECS system can be used as an *ex situ* treatment, such as in a vertical bioreactor system for removing PAHs in contaminated seawater.

4. Conclusion

This study shows that a combined inducer of $MnSO_4$ and Tween 80 can be used to enhance BaP degradation by *Bjerkandera adusta* SM46 under two different conditions: an acidic and a saline-alkaline condition. Based on contour plot analysis, the highest degradation was achieved with a $MnSO_4$ concentration higher than 0.4 mM and Tween 80 of 0.4–0.7% at pH 4.5. Applying the optimum condition to the fungal culture system under saline-alkaline stress increased the degradation efficiency more than 44%. A substitute carbon source of rice straw enhanced fungal growth, ligninolytic enzymes, and BaP degradation under non-saline and saline conditions. ECS enhanced the induction of the fungal catabolic system without cell growth promotion. Moreover, enhancing the catabolic system with rice straw as a co-substrate involved cell growth promotion. Enzymatic hydrolysis of BaP under an ECS condition significantly increased degradation efficiency up to 3-fold compared with control.

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