

Application of a biofilm formed by a mixture of yeasts isolated in Vietnam to degrade aromatic hydrocarbon polluted wastewater collected from petroleum storage

Le Thi Nhi Cong, Cung Thi Ngoc Mai, Vu Thi Thanh, Le Phi Nga and Nghiem Ngoc Minh

ABSTRACT

In this study, three good biofilm-forming yeast strains, including *Candida viswanathii* TH1, *Candida tropicalis* TH4 and *Trichosporon asahii* B1, were isolated from oil-contaminated water and sediment samples collected in coastal zones of Vietnam. These strains were registered in the GenBank database with the accession numbers JX129175, JX129176 and KC139404 for strain TH1, TH4 and B1, respectively. The biofilm formed by a mixture of these organisms degraded 90, 85, 82 and 67% of phenol, naphthalene, anthracene and pyrene, respectively, after a 7-day incubation period using an initial concentration of 600 ppm phenol and 200 ppm of each of the other compounds. In addition, this biofilm completely degraded these aromatic compounds, which were from wastewater collected from petroleum tanks in Do Xa, Hanoi after 14 days of incubation based on gas chromatography mass spectrometry analysis. To the best of our knowledge, reports on polycyclic aromatic hydrocarbon and phenol degradation by biofilm-forming yeasts are limited. The results obtained indicate that the biofilm formed by multiple yeast strains may considerably increase the degradation efficiency of aromatic hydrocarbon compounds, and may lead to a new approach for eliminating petroleum oil-contaminated water in Vietnam.

Key words | aromatic hydrocarbon, biodegradation, biofilm, marine yeast, phenol, wastewater treatment

Le Thi Nhi Cong (corresponding author)
Cung Thi Ngoc Mai
Vu Thi Thanh
Nghiem Ngoc Minh
Department of Environmental Biotechnology,
Institute of Biotechnology,
Vietnam Academy of Science and Technology,
18 Hoang Quoc Viet,
Cau Giay,
Ha Noi,
Vietnam
E-mail: lenhicon@gmail.com

Le Phi Nga
University of Sciences,
Vietnam National University Ho Chi Minh City
(VNU-HCM),
227, Nguyen Van Cu Street, District 5,
Ho Chi Minh City,
Vietnam

INTRODUCTION

Petroleum hydrocarbon wastewater is considered a serious hazardous pollutant, especially when introduced into aquatic environments, because they are highly toxic to aquatic organisms and can destroy the ecology of beach areas (Mendiola *et al.* 1998). Aromatic hydrocarbons are the main constituents of petroleum oil, and are highly toxic to human health and ecosystems due to their carcinogenic and mutagenic properties (Haritash & Kaushik 2009). These aromatic compounds were traditionally treated physically and chemically, which is expensive and can transfer the pollutant from one phase to another in many cases. Therefore, the use of microorganisms for treatment and bioremediation purposes provides a very efficient tool for removing contaminants from effluents and natural water (Ward *et al.* 2003). Biodegradation using planktonic yeasts to remove or detoxify aromatic

hydrocarbons has been reported in the last few years. Hammer *et al.* (1998) reported that the *Trichosporon mucooides* SBUG 801 strain was capable of not only degrading monoaromatic compounds, but also degrading biaryl ether substrates by cleaving the aromatic structure of dibenzofuran. Awe *et al.* (2009) reported that the *Trichosporon asahii* SBUG-Y 833 yeast could assimilate phenylalkanes with alkyl chain lengths of seven to twelve carbon atoms, and this strain has the potential to form different hydroxylated coumarines from alkyl aromatics resulting in the biotechnological production of coumarine structures with the potential for anticoagulative and antitumor properties. Sietmann *et al.* (2010) reported that the *Arxula adenivorans* yeast is able to perform three different reactions (i.e., oxidative decarboxylation, nonoxidative decarboxylation and methoxylation) on one

hydroxylated aromatic acid, whereas other organisms use only one transformation route.

Recently, degradation by immobilized yeasts that occur as biofilms in the environment has become of interest. A biofilm is a biologically active matrix of cells and extracellular products attached to a solid surface or an interface (O'Toole *et al.* 2000). The ability of a biofilm to tolerate environmental stresses could be used to increase biodegradation. Once these cells are firmly bound, the activity of the community is dependent on the metabolism and growth of each member species under local surface conditions. Furukawa *et al.* (2011) reported the co-aggregation properties of the *Saccharomyces cerevisiae* BY4741 yeast and a lactic acid bacterium (i.e., *Lactobacillus plantarum* ML11-11) to demonstrate how co-aggregation of these two organisms played an important role in the formation of mixed-species biofilms. Such metabolic activities can include substrate consumption, cellular growth and replication and synthesis of exopolymers (Watnick & Kolter 2000). Biofilm systems have been shown to play an important role in the removal of contaminated industrial wastewater (Characklis 1981; Gehara 1999). Krallish *et al.* (2006) reported three separated strains of *Cryptococcus terreus* (strain PB4) and *Rhodotorula creatinivora* (strains PB7, PB12) that could degrade phenol and form biofilms. However, to the best of our knowledge, few studies have investigated the application of mixed-yeast biofilms in aromatic hydrocarbon treatment. In the current study, we studied the degradation of aromatic compounds contained in wastewater that was collected from petroleum storage by a mixed-species biofilm consisting of three yeast strains isolated from hydrocarbon-polluted areas. The secondary purpose of this study was to prove the potential of model mixed-species biofilms for treatment of petroleum-contaminated wastewater.

MATERIALS AND METHODS

Microorganism strains

The yeast strains used in this study were isolated as previously described from hydrocarbon-contaminated water and sediment samples in coastal areas of Vietnam, such as Quang Ninh, Hai Phong and Thanh Hoa (Nhi-Cong *et al.* 2014). Isolation and enrichment procedures were carried out at 30 °C. Assimilation tests and sequencing of the ribosomal internal transcribed spacer (ITS) region revealed that all three strains were basidiomycetous yeasts. Strains TH1, TH4 and B1 were identified as *Candida viswanathii*, *Candida*

tropicalis and *Trichosporon asahii*, respectively (Nhi-Cong *et al.* 2014). All three strains were able to grow in minimal salt medium supplemented with phenol. These strains utilized a number of hydrocarbons as the sole carbon source, such as phenol, tetradecane and diesel oil (Table 1). The strains were maintained at 4 °C on malt agar plates in the strain collection at the Environmental Biotechnology Laboratory, Institute of Biotechnology, Vietnam Academy of Science and Technology. The sequences obtained in this study were deposited in the GenBank database. The accession numbers are JX129175, JX129176 and KC139404 for strains TH1, TH4 and B1, respectively.

Culture media

Yeast minimal medium (YMM): 5.0 g l⁻¹ NH₄H₂PO₄, 2.5 g l⁻¹ KH₂PO₄, 1.0 g l⁻¹ MgSO₄·7H₂O, 20 mg l⁻¹ Ca(NO₃)₂·H₂O, 2.0 mg l⁻¹ FeCl₃·6H₂O, 0.5 mg l⁻¹ H₃BO₃, 0.4 mg l⁻¹ MnSO₄·5H₂O, 0.4 mg l⁻¹ ZnSO₄·H₂O, 0.2 mg l⁻¹ Na₂MoO₄, 0.1 mg l⁻¹ CuSO₄·H₂O, 0.1 mg l⁻¹ CoCl₂, 0.1 mg l⁻¹ KJ, supplemented with 2% (w/v) glucose and 1% (v/v) vitamin solution (20.0 mg l⁻¹ p-aminobenzoic acid, 0.2 mg l⁻¹ biotin, 0.2 mg l⁻¹ folic acid, 200 mg l⁻¹ myo-inositol, 40.0 mg l⁻¹ nicotinic acid, 40 mg l⁻¹ pantothenic acid, 40.0 mg l⁻¹ pyridoxine hydrochloride, 20.0 mg l⁻¹ riboflavin, 100.0 mg l⁻¹ thiamine hydrochloride), according to Hammer *et al.* (1998).

Malt agar (MAg) for storage and cultivation of yeasts on solid medium: 18 g l⁻¹ agar-agar and 25 g l⁻¹ bio malt. For the biofilm test, malt medium containing 25 g l⁻¹ bio malt was used (Hammer *et al.* 1998).

Table 1 | Utilization of carbon substrates by the TH1, TH4, B1, QN1 and QN2 strains

Substrate	TH1	TH4	B1	QN1	QN2
Anthracene	++	+	++	+	+
Diesel oil	++	++	++	+	+
Naphthalene	++	+	++	-	-
<i>n</i> -Undecane	+	++	+	+	+
<i>n</i> -Dodecane	+	++	+	+	+
<i>n</i> -Tridecane	+	++	+	+	+
<i>n</i> -Tetradecane	+	++	+	+	+
<i>n</i> -Pentadecane	+	++	+	-	-
<i>n</i> -Hexadecane	++	++	+	-	-
Phenol	++	+	++	+	+
Pyrene	++	++	++	+	+

-: no growth; +: growth; ++: good growth.

Biofilm formation test

The experiment was performed using the method described by O'Toole *et al.* (2000) and Shimada *et al.* (2012). An overnight culture was diluted to $OD_{600} = 0.3$ and inoculated (1%) into 300 μL of malt medium in a 1.5 ml micro-centrifuge tube (TC131615, Nippon Genetics, Tokyo, Japan). The tube was allowed to stand at 30 °C for 2 days, and then the medium was removed. Next, the tube was gently rinsed with distilled water and filled with 500 μL of a 1% crystal violet (CV) solution. After 25 min, the CV solution was removed, and the tube was washed with distilled water again. The CV attached to the biofilm was dissolved in 400 μL of acetone and quantified by measuring its absorbance at 570 nm. The experiment was performed in triplicate, and the results were produced with standard deviation. The *Acinetobacter calcoaceticus* P23 was used as a positive control, and the tube without any strain was used as a negative control (Yamaga *et al.* 2010). The best biofilm forming strain was selected for further experiments. Each data point is the average of triplicate experiments.

Observation of biofilm by scanning electronic microscopy (SEM)

Mixed-species biofilms were formed in a 1.5 ml polypropylene tube at 30 °C for 48 h in MAg medium. After 24 h and 48 h, the biofilms were washed twice with a 0.85% sodium chloride solution and allowed to dry at room temperature for 30 min. The resulting biofilms were fixed stepwise with 5% OsO_4 and 2% glutaraldehyde in 0.1 M phosphate-buffered saline (pH 6.3). After fixation, the samples were dehydrated by increasing the concentration of ethanol and sputter coated with gold. The samples were observed by a Model S-2400 SEM (Hitachi, Tokyo, Japan).

Analysis of sustainable biodegradation of phenol by high performance liquid chromatography (HPLC)

For the degradation test using the yeast, 6.3×10^3 cells (equivalent to 1% of dilution with $OD_{600} = 0.3$) of each strain were inoculated separately into 20 mL of malt medium, and 1.2×10^2 cells of these yeasts were cultivated into the same medium volume at 30 °C for 2 days. Next, the medium was removed, and the resulting biofilms were rinsed twice with distilled water. Then, the biofilms were filled with 50 mL of YMM supplemented with 600 ppm phenol and allowed to stand at 30 °C. A control experiment was conducted to inoculate the boiled cells of yeasts. At different times (i.e., 0.5, 1, 3, 5 and 7 days), whole culture medium was collected and

centrifuged at 6,000 g for 15 min at 4 °C. The supernatants were subjected to HPLC analysis. Each sample (100 μL) was separated on a Cosmosil 5C₁₈-AR II column (4.6 \times 150 mm; Nacalai Tesque, Kyoto, Japan) using a flow rate of 0.5 mL min⁻¹. The mobile phase contained 63% (v/v) water and 37% (v/v) acetonitrile. The detector wavelength was set to 254 nm to detect phenol. All of the samples were prepared in triplicate.

Analysis of polycyclic aromatic hydrocarbon (PAH) degradation by gas chromatography (GC)

Naphthalene, anthracene and pyrene degradation tests using biofilms formed by mixture species (mixed-species biofilms) were performed with a amount of 200 ppm in the same manner with a phenol test. At different times (i.e., 0.5, 1, 3, 5 and 7 days), the whole culture in the bottle was directly extracted with an equal volume of a solvent mixture containing hexane and acetone (1:1) with 80 ppm biphenyl as an internal standard. The mixtures were shaken vigorously for 3 min to extract naphthalene, anthracene or pyrene and were centrifuged (6,000 g, 20 min at room temperature) to separate the aqueous and solvent layers. The GC analysis of PAHs was carried out using a GC system HP6890 (Agilent, Palo Alto, California, USA) equipped with a 340 $\mu\text{m} \times 30$ m non-polar capillary HP-1 column and flame ionization detector (FID). Briefly, the oven temperature was increased linearly from 80 °C to 300 °C at a rate of 10 °C min⁻¹. The temperature of the injector and detector were set to 250 °C. The flow velocity of the He carrier gas was 30 cm min⁻¹. These experiments were performed independently at least three times, and standard deviations were calculated.

All of the chemicals were of analytical grade and obtained from Sigma (USA) or Fluka (Japan).

Degradation of PAH components contained in petroleum wastewater

A similar amount of yeast biomass in the phenol degradation test was incubated in 20 mL of malt medium at 30 °C for 2 days. Then, the medium was removed, and the biofilms were rinsed twice with distilled water. Next, the biofilms were filled with 100 mL of petroleum wastewater supplemented with a 1% (v/v) vitamin solution. The natural petroleum wastewater without yeast biomass was used as a control experiment. After 14 days, the culture was extracted and analyzed with the same procedure as the PAH degradation tests.

Viable cell counts of the mixed-species biofilm

At different times (i.e., 0.5, 1, 3, 5 and 7 days) during the biodegradation tests, viable cells were counted. The experiment was conducted using the method described by Furukawa *et al.* (2011). The mixed-species biofilms that formed in the micro-centrifuge tube were washed twice with a 0.85% sodium chloride sodium, and the residual biofilm was removed by repeated pipetting. The removed biofilm was transferred to a tube that was then vortexed. The culture media used in this study were malt agar with chloramphenicol (100 mg/l) and cycloheximide (30 mg/l). Cultivation was performed at 30 °C for 48 h.

RESULTS AND DISCUSSION

Biofilm formation

Five different hydrocarbon degrading yeast strains isolated from petroleum-contaminated water and sediment samples in coastal areas of Vietnam were evaluated for their biofilm forming ability (Figure 1(a)). Among these tested yeast strains, *Candida viswanathii* TH1, *Candida tropicalis* TH4 and *Trichosporon asahii* B1 formed robust biofilms. Although the other yeasts formed marginal biofilms, these strains exhibited the best biofilm formation ability among the tested strains. *Acinetobacter calcoaceticus* P23 was used as a positive control (Yamaga *et al.* 2010). For our purpose, a mixed-species biofilm consisting of TH1, TH4 and B1 was formed and investigated using SEM (Figure 1(b) and 1(c)). The results demonstrated that the mixed-species biofilms of these yeasts are not flat but highly structured. This result also indicated that co-aggregation among these organisms rapidly occurred in the log phase. Furukawa *et al.* (2011) tested the co-aggregation between a bacterium (i.e., *Lactobacillus plantarum* ML11-11) and a yeast (i.e., *Saccharomyces cerevisiae* BY4741) and demonstrated that co-aggregation occurred in both the log and stationary phases. Our experimental result is consistent with this result. Therefore, we chose this mixed-species yeast biofilm as a model for further experiments.

Phenol degradation

The efficiency of the selected yeasts for phenol degradation was individually evaluated by HPLC (Yamaga *et al.* 2010). The capacity of the selected collection of yeast to degrade phenol was first assessed by separately cultivating them as

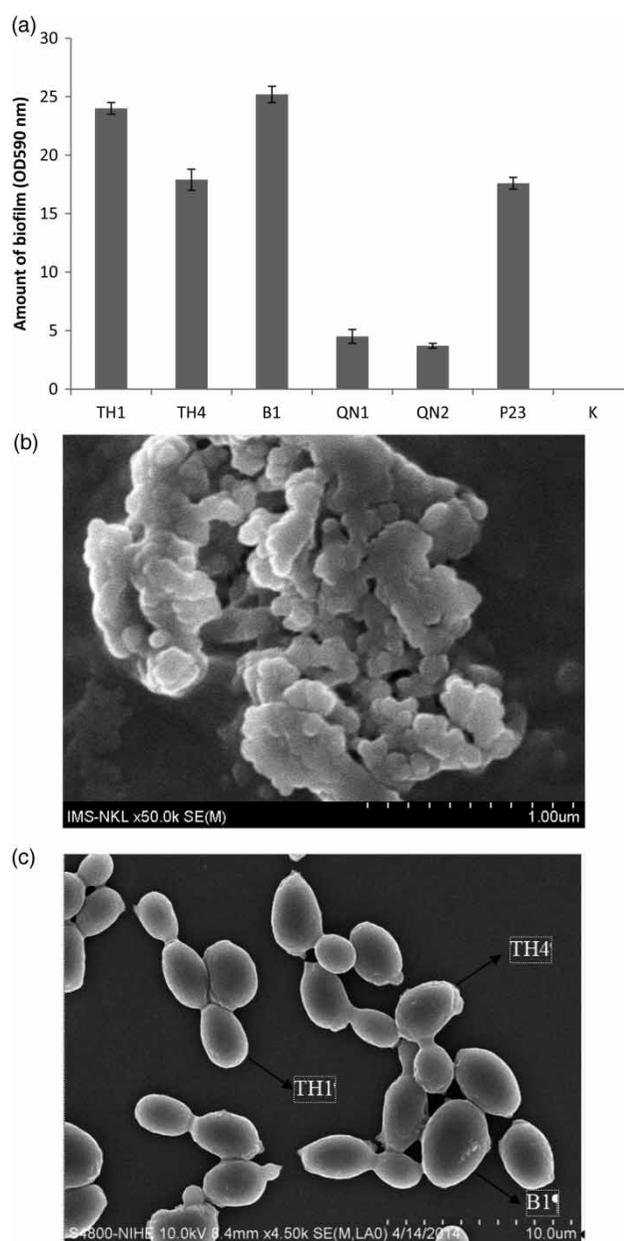


Figure 1 | (a) Amount of biofilm formed by hydrocarbon-degrading yeast strains (TH1, TH4, B1, QN1, and QN2) and *Acinetobacter calcoaceticus* P23 measured by using the CV staining method. *Acinetobacter calcoaceticus* P23 was used as a positive control, and K is negative control (without inoculation) (Yamaga *et al.* 2010). Error bar represents standard deviation ($n = 3$). (b) Image of SEM of a mixed-species biofilm formed by yeast strains TH1, TH4 and B1. (c) Image of SEM of TH1, TH4 and B1 cells.

biofilms of each strain. The results of HPLC analyses demonstrate that biofilms formed by the TH1 and B1 strains degraded 56% of the phenol after 7 days using an initial concentration of 200 ppm (Figure 2). As shown in Figure 2, all three studied strains possessed different levels of growth and phenol degradation. These results indicated that the

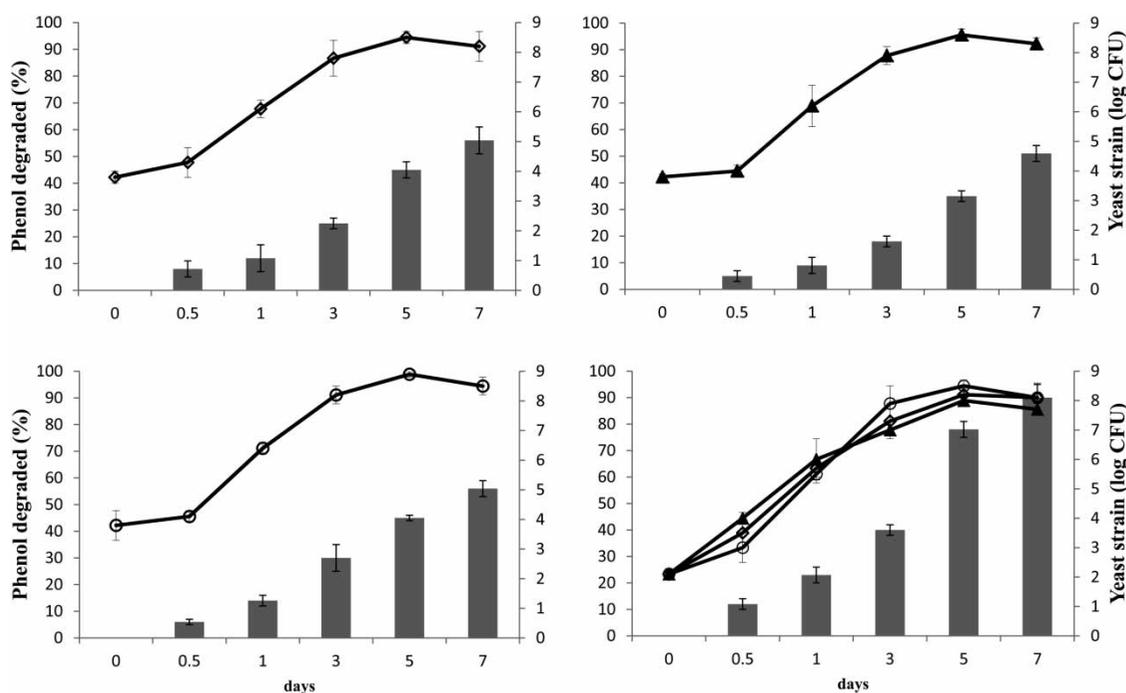


Figure 2 | Time course change in percentage of phenol degraded (bar, left axis) and viable count (line, right axis) of yeast strain TH1 (open diamond), TH4 (closed triangle), and B1 (open circle) in single- or mixed-species biofilm. Error bar represents the standard deviation ($n = 3$).

characteristics of each strain affected phenol removal and the ability of the cells to grow and produce biomass. Using a mixed-species biofilm, 78 and 90% of the phenol was degraded after 5 and 7 days, respectively. This result is in agreement with previous reports by *Zhong et al.* (2011), who determined that the interactions between *Mycobacterium* sp. and *Sphingomonas* sp. in mixed cultures increased the removal of PAHs. Most of the previous studies on the degradation of phenol were performed with suspended bacterial strains. Some investigations have also been performed on biofilm-forming yeast strains that were able to utilize phenol (*Godjevargova et al.* 2003; *Perron & Welander* 2004). However, the concentrations of phenol that were used were lower than the concentration used in this study. *Krallish et al.* (2006) used 200 ppm phenol and reported that the phenol degradation by immobilized yeast strains was always higher on zeolites compared to filter sand under normal osmotic growth conditions. This report is the first application of a mixed-species biofilm formed by *C. viswanathii*, *C. tropicalis* and *T. asahii* for the removal of phenol. In addition, the mixed-yeast biofilms have been shown to be more efficient than single-species biofilms. Therefore, these biofilms were used for further experiments with more persistent compounds than phenol.

PAH degradation

Mixed-yeast biofilms were tested for their ability to degrade different PAH contaminants, such as naphthalene, anthracene and pyrene, which acted as carbon sources. The degradation efficiency of these PAHs was independently evaluated by GC (*Shimada et al.* 2012). Different profiles of degradation were detected, as shown in *Figure 3*. Although the naphthalene and anthracene degradation reached 35% and 34% on day 3, respectively, a lower amount of pyrene (26%) was degraded after 3 days of cultivation; 85, 82 and 67% of naphthalene, anthracene and pyrene, respectively, were removed on day 7. *Shimada et al.* (2012) reported that the bacterial strain *Pseudomonas stutzeri* T102 biofilm-associated cells used 48% of 100 ppm naphthalene after 36 h. However, the research was not conducted with a higher concentration of naphthalene, and the studies did not involve mixed-species biofilm as in our study. In comparison to the degradation rates obtained in studies using a combination of bacteria (*Zoogloea* sp.) and fungi (*Aspergillus niger*) by *Jia et al.* (2011), a much lower value was reported (100 ppm pyrene), and 80% of the pyrene was degraded after 14 days. In addition, an increase in colony forming units of the viable cell numbers was only observed

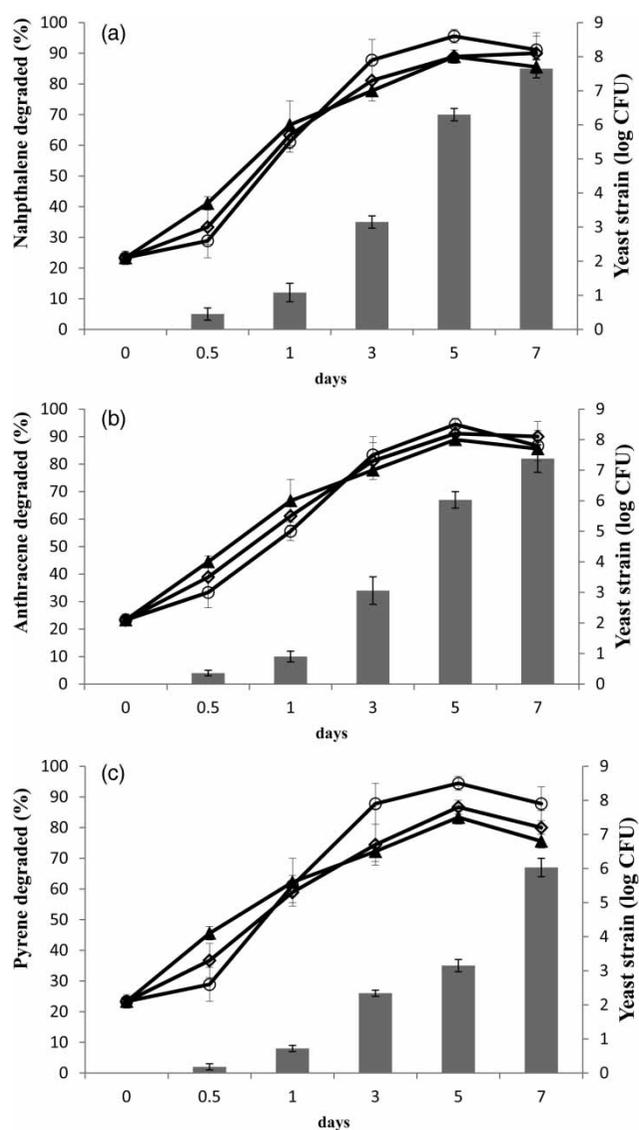


Figure 3 | Time course change in percentage of naphthalene (a), anthracene (b), or pyrene (c) degraded (bar, left axis), and viable count (line, right axis) of TH1 (open diamond), TH4 (closed triangle), and B1 (open circle) in mixed-species biofilm. Error bar represents the standard deviation ($n = 3$).

after 3 days, which also resulted in a higher PAH removal. After 5 days, the cell numbers tended to decrease, which was related to the dead-phase of microorganisms due to a long incubation period. These results indicated the ability of the cells to utilize these PAHs as the sole carbon and energy source to produce biomass.

The above data are consistent with previous results where mixed-species biofilms are typically more tolerant to environmental stresses than their individual components. This ability may be due to both specific gene expression and physicochemical toughness resulting from densely packed cells encased in extracellular matrices (Gehara

1999; Jeswani & Mukherji 2012). In addition, the mixed-species biofilms are consistent with natural biofilms, which provide favorable niches (Singh *et al.* 2006). Next, we further examined the degradation of PAHs contained in natural petroleum-contaminated wastewater using mixed-yeast biofilms.

PAH polluted wastewater degradation

The reduction in various hydrocarbon constituents of the wastewater was determined after 14 days (Figure 4). The results showed that in the biofilm experiment, a maximum removal of 95% was observed for heptadecane, and the lowest removal was observed for pyrene (75%); the reduction in the amounts of PAH compounds including anthracene, naphthalene, 1,2,3,4-tetrahydro-6-methylnaphthalene, decahydro-4,4,8,9,10-pentamethylnaphthalene, phenanthrene and pyrene ranged from 75 to 81%; the reduction in the amounts of *n*-alkane compounds, such as hexadecane and hexacosane, was 92 and 94%, respectively. This result was in agreement with the fact that PAHs are more persistent in microorganisms than *n*-alkanes. In addition, the amount of these PAHs estimated by GC was 5,000 ppm. Therefore, this reduction by this type of biofilm was very effective. Meanwhile, in control experiments with only natural wastewater, these components decreased from 3 to 16%. Through denaturant gradient gel electrophoresis-polymerase chain reaction analysis, several DNA bands observed in control samples after 14 days mean the viable cells are contained in natural wastewater (data not shown). This result was explained by the existence of native microorganisms in wastewater and these organisms slowly degraded these PAH compounds.

Although there are no studies on the simultaneous removal of phenolic, PAH and heterocyclic compounds with mixed-yeast-species biofilms, a few researchers have reported the removal of phenolic and PAH compounds by biofilm-forming bacteria and yeasts attached to carriers. Jeswani & Mukherji (2012) reported the removal of phenolic, heterocyclic and PAH compounds from synthetic biomass gasifier wastewater using a three-stage rotating biological contactor using biofilm forming bacteria (i.e., *Exiguobacterium aurantiacum*) and activated sludge (1:3 v/v). This method resulted in 85–96% removal of phenol, naphthalene, phenanthrene, fluoranthene and pyrene. Moscoso *et al.* (2012) reported the capacity of a collection of bacteria consisting of two strains (i.e., *Staphylococcus warneri* and *Bacillus pumilus*) for the degradation of three PAHs including phenanthrene, pyrene and benzo[*a*]anthracene. Ninety

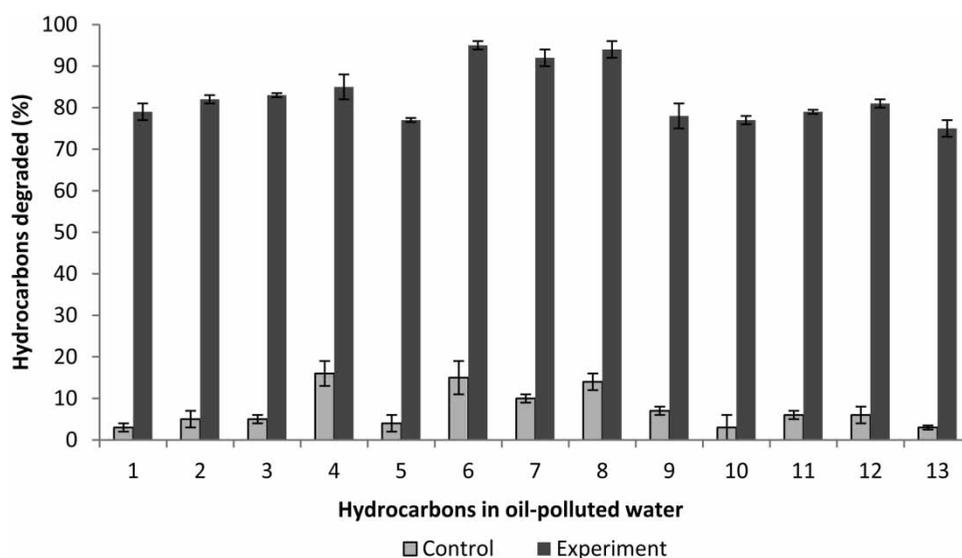


Figure 4 | Percentage of hydrocarbon components degraded contained in oil-polluted water collected from petroleum storage by mixed-species biofilm (dark grey) or oil-polluted water without biofilm (light grey) after 14 days of incubation. 1; anthracene; 2; benzene; 3; 4-(2-butanyl)-1,2-dimethylbenzene; 4; (1,4-dimethylpent-2-enyl)benzene; 5; 1-methyl-4-(1-methyl-2-propenyl)benzene; 6; heptadecane; 7; hexadecane; 8; hexacosane; 9; naphthalene; 10; 1,2,3,4-tetrahydro-6-methylnaphthalene; 11; decahydro-4,4,8,9,10-penta-methylnaphthalene; 12; phenanthrene; 13; pyrene. Error bar represents the standard deviation ($n = 3$).

percent removal of PAHs, which had an initial concentration of $100 \mu\text{M}$, was observed after only 3 days of cultivation on a bioreactor scale. Abu-salah *et al.* (1996) reported the bacterial degradation of aromatic compounds adsorbed on powdered activated carbon where the amounts of phenol, *p*-nitrophenol, and phenanthrene were decreased from $1,300$ to 300 mg l^{-1} , from 500 to 50 mg l^{-1} and from 300 to 50 mg l^{-1} , respectively. Krallish *et al.* (2006) reported the phenol degradation of single-yeast-species biofilm using *Cryptococcus terreus* and *Rhodotorula creatinivora* attached to solid carriers of zeolite or filter sand. Therefore, to the best of our knowledge, there are no reports on the direct use of mixed-yeast-species biofilms without carriers for the removal of PAHs contained in oil-polluted wastewater.

CONCLUSIONS

The results obtained confirm the ability of the *C. viswanathii* TH1, *C. tropicalis* TH4 and *T. asahii* B1 strains to degrade a wide range of toxic contaminants present in petroleum-polluted wastewater. These strains could be present simultaneously in the biofilm when they were mixed and cultivated. These results demonstrate the potential for developing a method for the decontamination of wastewater using a mixed-species yeast biofilm. In addition, the results of this study indicate that a number of PAH degrading yeasts and mechanisms exist in the natural environment.

Further detailed investigations of these organisms will provide useful information regarding the removal of PAHs contained in oil-polluted wastewater in petroleum storage in Vietnam.

ACKNOWLEDGEMENT

This research was funded by the Vietnam National Foundation for Science and Technology Development (NAFOSTED) under grant number 106.03–2011.53.

REFERENCES

- Abu-salah, K., Shelef, G., Levanon, D., Armon, R. & Dosoretz, C. G. 1996 Microbial degradation of aromatic and polyaromatic toxic compounds adsorbed on powdered activated carbon. *Journal of Biotechnology* **51**, 256–272.
- Awe, S., Mikolasch, A. & Schauer, F. 2009 Formation of coumarines during the degradation of alkyl substituted aromatic oil components by the yeast *Trichosporon asahii*. *Applied Microbiology and Biotechnology* **84**, 965–976.
- Characklis, W. G. 1981 Bioengineering report: fouling biofilm development: a process analysis. *Biotechnology and Bioengineering* **23** (9), 1923–1960.
- Furukawa, S., Nojima, N., Yoshida, K., Hirayama, S., Ogihara, H. & Morinaga, Y. 2011 The importance of inter-species cell-cell co-aggregation between *Lactobacillus plantarum* ML11-11 and *Saccharomyces cerevisiae* BY4741 in mixed-species

- biofilm formation. *Bioscience Biotechnology and Biochemistry* **75** (8), 1430–1434.
- Gehara, F. 1999 Activated sludge biofilm wastewater treatment system. *Water Research* **33**, 230–238.
- Godjevargova, T., Ivanova, D., Alexieva, Z. M. & Dimova, N. 2003 Biodegradation of toxic organic components from industrial phenol production waste waters by free and immobilized *Trichosporon cutaneum* R57. *Process Biochemistry* **38**, 915–920.
- Hammer, E., Krowas, D., Schaefer, A., Specht, M., Franck, W. & Schauer, F. 1998 Isolation and characterization of dibenzofuran-degrading yeast: identification of oxidation and ring cleavage products. *Applied and Environmental Microbiology* **64** (6), 2215–2219.
- Haritash, A. K. & Kaushik, C. P. 2009 Biodegradation aspects of polycyclic aromatic hydrocarbons (PAHs): a review. *Journal of Hazardous Materials* **169**, 1–15.
- Jeswani, H. & Mukherji, S. 2012 Degradation of phenolics, nitrogen-heterocyclics and polynuclear aromatic hydrocarbons in a rotating biological contactor. *Bioresource Technology* **111**, 12–20.
- Jia, C., Li, P., Xiajun, L., Tai, P., Liu, W. & Gong, Z. 2011 Degradation of pyrene in soils by extracellular polymeric substances (EPS) extracted from liquid cultures. *Process Biochemistry* **46**, 1627–1631.
- Krallish, I., Gonta, S., Savenkova, L., Bergauer, P. & Margesin, R. 2006 Phenol degradation by immobilized cold-adapted yeast strains of *Cryptococcus terreus* and *Rhodotorula creatinivorans*. *Extremophiles* **10**, 441–449.
- Mendiola, S., Achutegui, J. J., Sanchez, F. J. & San, M. J. 1998 Polluting potential of wastewater from fish-meal and oil industries. *Grasa Aceites* **49**, 30–33.
- Moscoso, F., Tejjiz, I., Deive, F. J. & Sanroman, M. A. 2012 Efficient PAHs biodegradation by a bacteria consortium at flask and bioreactor scale. *Bioresource Technology* **119**, 270–276.
- Nhi-Cong, T. L., Ngoc-Mai, T. C., Morikawa, M. & Minh, N. N. 2014 Transformation of iso-pentylbenzene by biofilm-forming strain *Candida viswanathii* TH1 isolated from oil-polluted sediments collected in coastal zones in Vietnam. *Journal of Environmental Science and Health, Part A Toxic/Hazardous Substances and Environmental Engineering* **49**, 777–786.
- O'Toole, G. A., Kaplan, H. B. & Kolter, R. 2000 Biofilm formation as microbial development. *Annual Review of Microbiology* **54**, 49–79.
- Perron, N. & Welander, U. 2004 Degradation of phenol and cresols at low temperature using a suspended-carrier biofilm process. *Chemosphere* **55**, 45–50.
- Shimada, K., Itoh, Y., Washio, K. & Morikawa, M. 2012 Efficacy of forming biofilms by naphthalene degrading *Pseudomonas stutzeri* T102 toward bioremediation technology and its molecular mechanisms. *Chemosphere* **87**, 226–233.
- Sietmann, R., Uebe, R., Böer, E., Bode, R., Kunze, G. & Schauer, F. 2010 Novel metabolic routes during the oxidation of hydroxylated aromatic acids by the yeast *Arxula adenivorans*. *Journal of Applied Microbiology* **108** (3), 789–799.
- Singh, R., Paul, D. & Jain, R. K. 2006 Biofilms: implications in bioremediation. *Trends in Microbiology* **14**, 389–397.
- Ward, O. P., Singh, A. & Van Hamme, J. 2003 Accelerated biodegradation of petroleum hydrocarbon waste. *Journal of Industrial Microbiology and Biotechnology* **30**, 260–270.
- Watnick, P. & Kolter, R. 2000 Biofilm: city of microbes. *Journal of Bacteriology* **182**, 2675–2679.
- Yamaga, F., Washio, K. & Morikawa, M. 2010 Sustainable biodegradation of phenol by *Acinetobacter calcoaceticus* P23 isolated from the rhizosphere of duckweed *Lemna aoukikusa*. *Environmental Science and Technology* **44**, 6470–6474.
- Zhong, Y., Luan, T., Lin, L., Liu, H. & Tam, N. F. Y. 2011 Production of metabolites in the biodegradation of phenanthrene fluoranthene and pyrene by the mixed culture of *Mycobacterium* sp. and *Sphingomonas* sp. *Bioresource and Technology* **102**, 2965–2972.

First received 10 February 2014; accepted in revised form 7 May 2014. Available online 19 May 2014