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Pseudomonas aeruginosa NR.22 – local hydrocarbon degrader species originated from crude oil non-exploration site: isolation, survivability and hydrocarbon degradation study

Badrul Hizam Syuhadah, Nik Him Nik Raikhan*

School of Chemical Engineering, College of Engineering, Universiti Teknologi MARA, Selangor, Malaysia, emails: nikraikhan7952@uitm.edu.my (N.H.N. Raikhan), bhzam.syuhadah93@gmail.com (B.H. Syuhadah)

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ABSTRACT

Pseudomonas aeruginosa as a hydrocarbon degrader is not a something new. In fact, there were numerous published papers reported the excellent performance of this species as a hydrocarbon degrader. However, most of the previous study used *P. aeruginosa* that was isolated directly from the contaminated site. This however resulting a gap of information in understanding the relationship between species growth environmental condition on their hydrocarbon degrading ability. Therefore, in this study, enrichment method was used to isolate *P. aeruginosa* NR.22 (Ps.NR.22) from crude oil non-impacted recreational lakes in Section 7, Shah Alam, Selangor, Malaysia. The survivability and impact of crude oil concentrations (2% (w/v), 5% (w/v) and 7% (w/v)) on the kinetic growth of free moving Ps.NR.22 throughout the fermentation period were monitored through the changes of cell density till constant dropped of cell density obtained. 5% (w/v) was the ideal concentration to support the growth of Ps.NR.22 as specific growth rate, percentage of overall crude oil degraded by Ps.NR.22 and decay coefficient of cells in this culture medium recorded the highest (0.4787 d^{-1}, 94.1%, 0.0097 h^{-1}) followed by 7% (w/v) (0.3863 d^{-1}, 67.17%, 0.0016 h^{-1}) and 2% (w/v) (0.3325 d^{-1}, 50.02%, 0.003 h^{-1}). High decay coefficient value of cells in 5% (w/v) might be linked with active and fully utilization of crude oil during exponential phase. Next, potential of free moving Ps.NR.22 as a biodegradation tool was further expanded by evaluating its compatibility with membrane separation technology for removal of hydrocarbons. This was done by introducing 5% w/v crude oil water mixture to the immobilized Ps.NR.22 in a filtration cell through dead end filtration method. Accumulation of crude oil molecule on/within the surface of porous support ceramic activated the Ps.NR.22 hydrocarbon degrading mechanism. As a result, it was found out that, support ceramic with presence of Ps.NR.22 demonstrating de-fouling potential at minute 125 while with absence of Ps.NR.22 the flux rate kept on declining till the filtration ended. This showed that by Ps.NR.22 have the potential to enhance the performance of support ceramic in removing hydrocarbon.

Keywords: Pseudomonas aeruginosa NR.22; Crude oil non-impacted site; Crude oil; Kinetic growth study; Support ceramic

1. Introduction

Bioremediation in combating hydrocarbon pollutant – bioremediation of petroleum hydrocarbon has been in use since 1940 but gained popularity after the Exxon Valdez spill in 1980 [1]. Bioremediation which is a highly attractive, pioneering, cost-effective and environmentally friendly technology is based on the use of microorganism to remove hydrocarbon pollutants from soil or water [2]. This is an innovative technique in which microorganism

* Corresponding author.

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mitigate, degrade or reduce hazardous organic pollutants to harmless compounds such as CO_2, CH_4, H_2O and biomass without adversely affecting the environment [3]. Biodegradation is one of the primary mechanisms for bioremediation and here, the metabolic potentials, as well as enzyme produced by microorganisms, are utilized to rapidly degrade the contaminants [4]. Microorganism involved in the bioremediation processes include primarily bacteria, fungi and yeasts. However, bacteria are the most effective and reported petroleum hydrocarbons degrading microbial community [5].

Pseudomonas aeruginosa as a hydrocarbon degrader species is not a something new. In fact, among bacterial strains used for bioremediation, this species is one of the species that constantly gained a lot of attention from many researcher owed to its exceptionally high metabolic versatility in utilizing numerous organic compounds and their excellent adaptability to various conditions allowing them to survive in terrestrial, air and freshwater environments [6]. These make *P. aeruginosa* is an undeniable excellent hydrocarbon degrader. However, insufficient attention has been paid to *P. aeruginosa* that was raised and growth in pristine environment [7] as most of the studies reported and focused on the performance and efficiency of *P. aeruginosa* that was originated from the hydrocarbon petroleum contaminated site. This is expected as Varjani and Upasani [8] have mentioned that, employing native bacterial strain isolated from petroleum contaminated sites ensures higher tolerance of microorganism towards hydrocarbon toxicity and better resistance to the environmental variations as compared to other viable microorganisms present in non-polluted sites. Therefore, by using hydrocarbon degrader species that was already exposed to the pollutant not only can guarantee its survivability but also their ability in resisting, degrading and utilizing hydrocarbon pollutant as their source of carbon. Consequently, the adaptation period needed by the isolate can be shortened and negative impact of toxic nature of the pollutant on cell metabolism of isolate also can be minimized.

This however creating a gap of information in understanding the impact of difference growth condition environment on survivability and hydrocarbon degrading ability of *P. aeruginosa*. Thus, the goal of this study is to isolate local strains of *P. aeruginosa* from crude oil non-impacted (pristine environment) recreational lake in Shah Alam, Malaysia using the simplest yet promising enrichment method and test the isolate survivability and capability in degrading and utilizing crude oil as their sole source of energy to grow and reproduce. At the end of this study, a deeper understanding on *P. aeruginosa* degradation and adaptation abilities especially for *P. aeruginosa* that originated from pristine environment can be discover. It is a worthwhile approach to help in developing a better understanding and maximized the application of this species in bioremediation.

2. Methodology

2.1. Crude oil samples

For crude oil biodegradation related study, it was crucial to use crude oil sample collected from the same reservoir. This was due to the fact that hydrocarbon recovered from different reservoirs varies widely in compositional and physical properties [9]. Therefore, crude oil sample used was collected from Dulang Oil Field that located 130 km offshores Kuala Terengganu, Peninsular Malaysia in the South China Sea in a water depth of about 76 m.

2.2. Microbial culture and inoculum preparation

This method was adapted from Nik Raikhan et al. [10]. Lake water sample was collected from a recreational lake in Section 7, Shah Alam, Selangor, Malaysia. Few drops of water sample were then dropped on a sterilized solidified cetrimide agar (CA). Spreader was used to evenly distribute the water sample on the entire surface of agar. Then, the agar was incubated at 37°C. After 24 h, bacterial colonies with rapid growth and large diameter were picked and purified on a new CA [11]. The strain later on was identified as *P. aeruginosa* NR.22 (referring to *P. aeruginosa* species that was originated from crude oil non-exploration site) was preserved on slants of *Pseudomonas* agar with 5% (w/v) glycerol at –20°C [10]. For routine experiments, Ps.NR.22 was maintained on CA at 4°C in a refrigerator and sub-cultured once in every 3 d. Ps.NR.22 was activated and microbial inoculum was prepared by transferring culture from CA into nutrient broth (NB) medium and incubated at 37°C at 200 rpm for 24 h.

2.3. Morphological characterization

Morphological characteristics were studied by performing Gram staining of Ps.NR.22 pure culture suspension of Ps.NR.22. Type of pigmented coloration from growth on CA plate were also observed and recorded.

2.4. Biodegradation studies

2.4.1. Biodegradation of crude oil by free moving Ps.NR.22

Volume of the inoculum that was added into the crude oil culture medium was 10% (v/v) from the total of working volume used. Therefore, for biodegradation study, 25 mL of Ps.NR.22 inoculum was poured into crude oil culture broth that contained 250 mL of sterilized NB and crude oil at different concentration (2% w/v, 5% w/v and 7% w/v) [12].

2.4.2. Optical density and cell dry weight analysis

Optical density (OD) was determined spectrophotometrically at 560 nm. 3 mL of sample collected regularly within the fermentation period to measure the bacteria growth in the crude oil culture broth. The average from three readings of absorbance value will be recorded and from the data obtained, bacteria growth curve can be plotted and bacteria kinetic growth can be determined. OD vs. cell dry weight graph was plotted for growth performance.

2.4.3. Kinetics of Ps.NR.22 growth

Growth kinetics of Ps.NR.22 were determined as per described by Sakthipriya et al. (2015) and [10].

The specific growth rate (μ, h^{-1}) of free Ps.NR.22 was calculated from Eqs. (1) and (2).

$$\frac{dX}{dt} = rX = \mu X \tag{1}$$

where X is the biomass dry weight at time t (h)(g L^{-1}), rX is biomass production rate (g L^{-1} h^{-1}) and μ was calculated by integrating Eq. (2) with conditions, $X = X_0$ at $t = t_0$.

$$\mu = \frac{\ln X - \ln X_0}{t - t_0} \tag{2}$$

Eq. (2) was used to calculate specific growth rate values. It has been presumed that the aeration provided was sufficient for growth. Hence, oxygen was not considered as the growth limiting factor [13]. μ also determined from graph of $\ln(X/X_0)$ against degradation time (until stationary phase end) (h). Doubling time (t_d), a time requisite for Ps.NR.22 to double from its initial count was calculated by using Eq. (3).

$$t_d = \frac{\ln 2}{\mu} \tag{3}$$

Death coefficient was determined after Ps.NR.22 enter dead phase. Thus, the measurement of OD was continued even after complete utilization of crude oil by Ps.NR.22 (even after stationary phase end). During this dead phase, some of the cells become the carbon and energy source for other cells. Decay coefficient of the growth curve in a batch scale reactor was determined using Eq. (4).

$$\frac{dX}{dt} = \left(k_d\right)dX \tag{4}$$

where k_d is the decay coefficient (h^{-1}) and this value was obtained from graph of ln(OD) against degradation time (h).

2.4.4. Extraction of residual oil

Extraction of residual oil was carried out as per the method described by Varjani et al. (2016) and Sakthipriya, et al. (2018). In brief, crude oil culture broth was mixed with equal volume of *n*-hexane in a separating funnel and was shaken vigorously for few seconds to maximize the contact between broth and hexane. Then, the mixture was left for an hour to let the separation process to occur. The bottom layer containing mixture of crude oil and *n*-hexane was taken out in a round bottom flask. *n*-hexane and moisture were evaporated by heating in a water bath. Overall percentage of crude oil degradation was obtained by calculating the difference between the initial weight of crude oil and residual oil extracted [14].

$$3.0\%\ \text{of HC degraded} = \frac{\left(\text{Initial} - \text{final}\right)\text{of HC}}{\text{initial weight of HC}} \times 100\% \tag{5}$$

2.5. Potential of Ps.NR.22 to be integrated with another crude oil wastewater treatment method

In this part of study, all the filtration procedure were carried out in Amicon® stirred cells (filtration cell) in unstirred dead end microfiltration mode. Experimental set up for filtration procedure and view of membrane installment in the membrane holder as being illustrated in Figs. 1 and 2. Meanwhile, Table 1 listed the dimension of the filtration cell and support ceramic used. Based on Table 1, it can be seen that the filtration size of filtration cell was wider than the size of support ceramic. Hence, as shown in Fig. 2, impenetrable gel layer was placed around support ceramic to cover the exposed filtration area of the filtration cell so that the feed solution only passing through the installed support ceramic.

2.5.1. Preparation of Ps.NR.22 bacterial suspension

Few loops of Ps.NR.22 was transferred into 50 mL of sterilized 1/10 diluted NB in a 150 mL sterilized conical flask [15,16]. The flask was then incubated for 24 h at 37°C with shaking rate 150 rpm in an incubator shaker. The growth was maintained at the exponential growth phase. Then, cells were harvested by centrifugation at 10,000 rpm for 20 min, washed three times with deionized water and then re-suspended in sterilized phosphate buffer saline solution (PBS).

2.5.2. Immobilization of Ps.NR.22 on support ceramic

50 mL of 10^6 CFU/mL Ps.NR.22 suspension was fed into the filtration cell. Then, pressure of 1 bar was introduced into the cell to drive the feed solution passed through the porous support ceramic. Due to larger size of Ps.NR.22, this cell was retained and adsorbed on the surface of support ceramic. The number of cells immobilized on support ceramic was measured by comparing the absorbance value and colony counting of Ps.NR.22 in feed and permeate samples [17]. The absorbance value was measured using a UV-Visible Spectrophotometer with wavelength of 560 nm [12].

2.5.3. Microfiltration of crude oil water mixture

5% (w/v) of crude oil concentration was prepared by mixing 9.49 g of crude oil with distilled water. Due to the hydrophobic nature of crude oil, ultrasonic homogenizer was used for 1 h to obtain a homogeneous crude oil water mixture. The preparation was carried out in room temperature [18]. The prepared crude oil water mixture was stable in terms of homogeneity for 5 d (crude oil was not separated from water). All the experiments were conducted in the stabilization period of the emulsion [19].

As soon as the immobilization procedure of Ps.NR.22 ended, 50 mL of 5% (w/v) of crude oil water mixture was fed into the filtration cell. Pressure of 1.5 bar was then introduced into the cell to drive the feed solution passing through BACM (CM with immobilized Ps.NR.22). Time taken to collect every 5 mL of permeate were recorded. This recorded data was then used to calculate the flux rate based on Eq. (6). The filtration continues until a constant permeate flux reading obtained (repeated

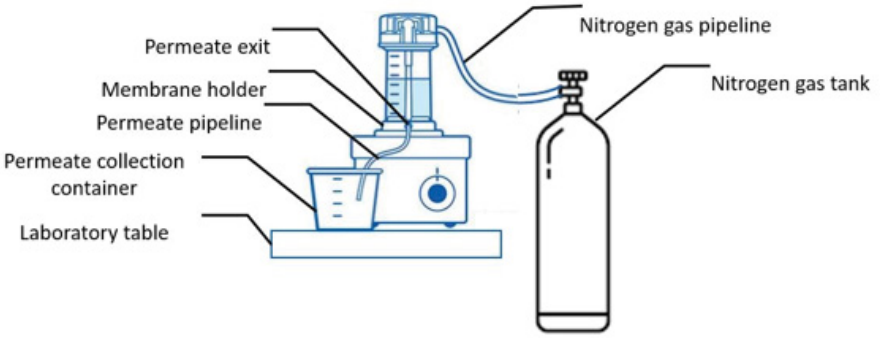

Fig. 1. Experimental set-up for filtration procedure.

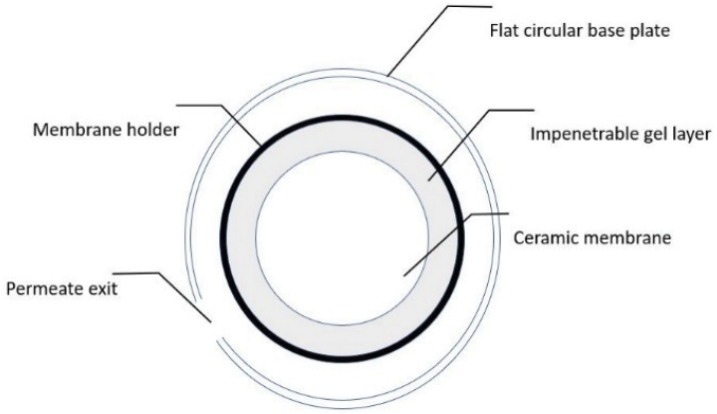

Fig. 2. View of membrane holder of the filtration cell.

Table 1
Listed the dimension of the filtration cell and support ceramic used

Height	11.7 cm
Filtration area	13.4 cm^2
Process volume	50 mL
Filter diameter	44.5 mm
Support ceramic effective area	5.73 cm^2
Membrane material	Ceramic alumina oxide (purchased from China)

batch operation mode). This procedure was repeated by replacing BACM with CM (ceramic membrane with no presence of immobilized Ps.NR.22).

2.5.4. Permeation rate test

Permeation rate was measured by using Eq. (6) [20].

$$J = \frac{dV}{A\,dt} \tag{6}$$

where V is the water permeate volume (L), A is membrane surface effective area (m^2) and t is permeation time (h).

3. Results and discussion

3.1. Isolation and morphology of Ps.NR 22

As being mentioned earlier, the aim of this study is to evaluate the hydrocarbon degrading ability of *P. aeruginosa* that growth in crude oil non-impacted site. Therefore, in attempting to achieve the first objective of this study, enrichment method was used to isolate and select the desired species which is *P. aeruginosa* from recreational lake in Section 7, Shah Alam, Selangor, Malaysia. An enrichment method according to Pasumarthi et al. (2013), has long been the method of choice for isolating bacteria expressing specific phenotypes. The enrichment culture was evaluated for its ability to resists the toxicity of the cetrimide substance that has detergent like qualities that was inhibitory to most bacterial species except for *P. aeruginosa* and also ability in producing pigmented colonies as one the special characteristics of this species is the production variety of pigments [22]. Pigment production was determined through visual examination using naked eyes on coloration of colonies that was formed on CA after 24 h of incubation at 37°C. Up till today, there was no research reported on the relation between type of pigmentation produced by *P. aeruginosa* with their ability as a hydrocarbon degrader, but for consistency purposes, the isolate only used when they produced green yellowish

fluorescent colonies coloration (Fig. 3c). Fig. 3a shows a variety of pigmentation produced by Ps.NR.22 throughout the study. This secretion of pigmentation confirming that the colonies growth on CA was no other than *P. aeruginosa*. In addition to that, type of Gram staining of the isolate bacterial suspension was also observed. From Fig. 3b, it can be clearly seen that Ps.NR.22 is a Gram-negative where this is commonly reported for *P. aeruginosa* [23].

3.2. Crude oil biodegradation study by free moving Ps.NR.22

Since Ps.NR.22 was isolated from crude oil non-impacted/non-exploration site, survivability of the isolate in a culture medium that supplemented with different crude oil concentrations need to be studied. The survivability of Ps.NR.22 was

Crude oil served as the sole carbon source needed by *P. aeruginosa* to support their cell growth. Inability to gain this nutrient restrained the growth of this species. As crude oil was not the common carbon source that Ps.NR.22 has been living with, there was possibility that this species might does not possess the right chemical and enzymatic metabolites to assist them in degrading and utilizing crude oil. Apart from that, the toxic nature of the crude oil itself might also contributing in affecting the survivability of Ps.NR.22 in the crude oil culture medium.

3.2.1. Growth of Ps.NR.22 in the crude oil culture medium

Growth of Ps.NR.22 in each of the culture mediums were monitored through the changes of the cell density throughout the fermentation medium. Through the data obtained, growth curve of Ps.NR.22 in all culture mediums were plotted as shown in Fig. 3c. Since Ps.NR.22 never deal with crude oil before, it was expected that this isolates required longer adaptation period or lag phase for them to exploit new environmental conditions or to synthesis cellular components necessary to support their growth [24]. But Fig. 3c revealed that regardless of the crude oil concentration used, Ps.NR.22 managed to adapt almost instantaneously and can be considered as fast growth species as within 24 h as soon as the fermentation started, the exponential phase already begun. These were based on the increased of cell density from 0.1486 to 0.2627 g mL^{-1} in 2% w/v, 0.1473 to 0.8046 g mL^{-1} for 5% w/v and 0.2012 to 0.3612 g mL^{-1} in 7% w/v and the cell density kept on increased till the exponential phase ended at day 4. The increased of cell density also served as evident that Ps.NR.22 capable to utilize crude oil as sole source of carbon and energy [25]. Then, the growth was continued with stationary phase until day 17 and fell into the decline or death phase until day 21. The experiments were ended once all the cells in every medium reached their death phase (21 d).

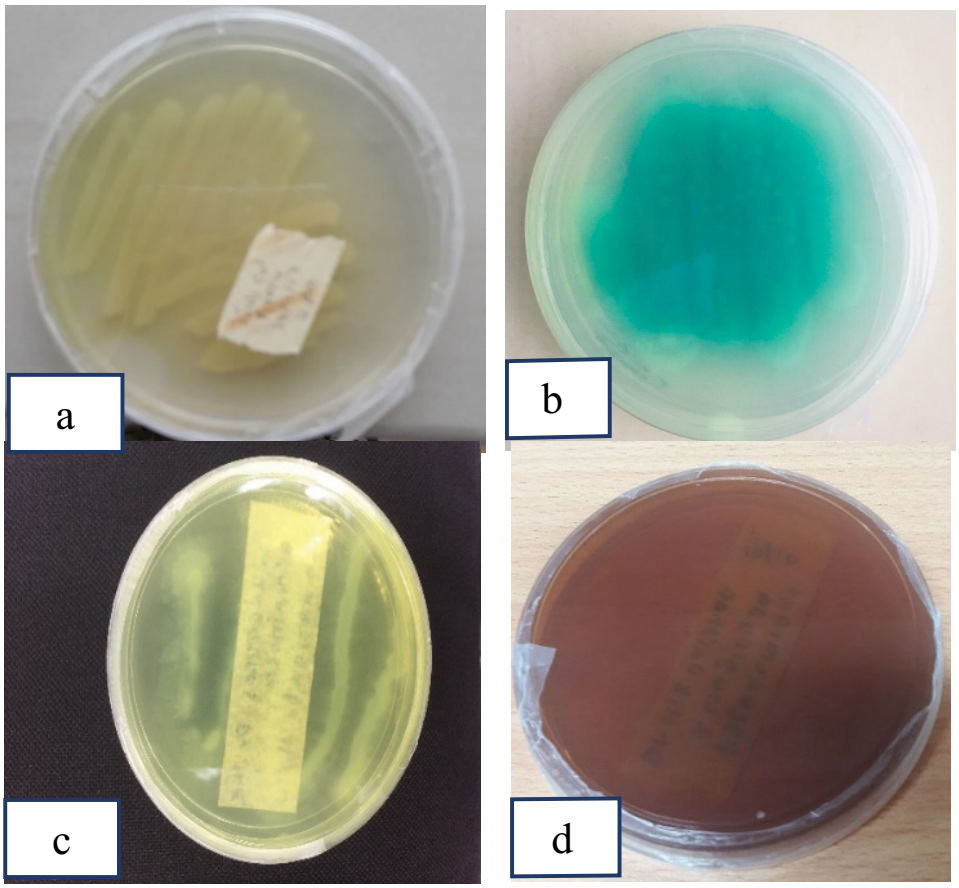

Fig. 3. Variety of colonies coloration due to the growth of Ps.NR.22 on cetrimide agar, (a) non-pigmented, (b) pyocyanin, (c) pyoverdine and fluorescein and (d) pyorubin.

3.2.2. Kinetic growth of Ps.NR.22

The growth kinetic of Ps.NR.22 was studied during the exponential phase and the results are showed in Fig. 3d.

Subject to Fig. 3d, the specific growth rate observed were 0.3325, 0.4787 and 0.3863 d^{-1} when crude oil concentration added into the culture medium was 2% w/v, 5% w/v and 7% w/v. Therefore, the doubling time were 2.0847, 1.4480 and 1.7943 d^{-1} respectively. These findings showed that the growth of Ps.NR.22 directly proportional to the crude oil concentration added and the ideal crude oil concentration for Ps.NR.22 was 5% w/v as the growth rate recorded was the highest as they took the shortest time for them to double in size. On the other hand, 2% w/v was the least ideal concentration as the growth rate recorded was the lowest and longer time taken by Ps.NR.22 to increase in size.

Meanwhile, *P. aeruginosa* DKB1 and *P. aeruginosa* strain ASW-2 showed a contradicted response. It was reported by Chen et al. (2020), that the degradation efficiency of free *P. aeruginosa* strain ASW-2 that was isolated from Zhejiang Coast in China reached 58.6% at the crude oil concentration of 2% (w/v). When concentration of crude oil increased to 4% (w/v), the degradation efficiency decreased by 3.9% and significantly reduced when the concentration increases to 10% (w/v). Growth of *P. aeruginosa* DKB1 isolated from hydrocarbon contaminated site in India decreased when the crude oil concentration used above 1% (v/v). Besides that, this species also considered as slow growth as it took 5 d for this species to start increase in size [27]. According to both authors, this reaction might be due to the toxicity of the crude oil [26,27]. Therefore, Ps.NR.22 can be considered have high tolerant towards high toxicity of crude oil. Varjani and Upasani [8] once mentioned that one of the reasons isolating hydrocarbon degrader species from the contaminated site was to ensures higher tolerance of microorganism towards hydrocarbon toxicity and better resistance to the environmental variations as compared to other viable microorganisms present in non-polluted sites, but Ps.NR.22 proved that *P. aeruginosa* that originated from crude oil non-polluted site also have the high tolerance towards toxic crude oil.

The experiment ended when cells showed a constant dropped and it took 21 d for Ps.NR.22 reached the last growth phase which is death or decline phase. Referring to Fig. 3e, decay coefficient for Ps.NR.22 in the culture medium supplemented with 2% w/v, 5% w/v and 7% w/v crude oil concentration were observed to be 0.003, 0.0097 and 0.0016 h^{-1}. This demonstrated that the number of Ps.NR.22 cells decayed per hour in 5% w/v was the highest followed by 7% w/v and 2% w/v. This might be linked with active and fully utilization of crude oil during exponential phase.

3.2.3. Crude oil biodegradation by free moving Ps.NR.22

According to Gao et al. [11], survival and growth of microbes in medium supplemented with hydrocarbons after their inoculation period is a key deciding factor for the rate of their biodegradation. As growth of cell in 5% w/v was the highest, indirectly this will contribute to highest percentage of crude oil degraded as cells gain enough nutrient from the surrounding to support their cell growth [12]. Thus, data presented in Table 2 relevant with the growth rate findings of Ps.NR.22 as the highest percentage of crude degraded was in vulture medium supplemented with 5% w/v of crude oil concentration followed by 7% w/v and 2% w/v. *P. aeruginosa* was known for its abilities in degrading wide range of hydrocarbon compounds [28]. Therefore, achieving percentage of crude oil degraded more than 90% was possible.

Table 2
Percentage of crude oil degraded by Ps.NR.22

Concentration of crude oil (w/v)	μ (h^{-1})	Percentage of crude oil degraded (%)
2	0.3325	50.02
5	**0.4787**	**94.1**
7	0.3863	67.17

3.3. Potential of Ps.NR.22 to be integrated with membrane separation technology in treating crude oil water solution

Next, potential of free moving Ps.NR.22 as a biodegradation tool was further expanded by evaluating its compatibility with membrane separation technology for removal of hydrocarbons. The compatibility was evaluated only based on the ability of Ps.NR.22 to degrade crude oil hydrocarbon after being immobilized on the surface of porous ceramic support material. The impact of the presence of Ps.NR.22 on the performance of membrane in treating crude oil water solution will not be discussed.

Ceramic was selected as a solid support material for Ps.NR.22 immobilization owed to its inert properties. This will prevent the formation of secondary pollutant. Besides that, compared to organic material, ceramic was known for its microbial resistant properties [29].

3.3.1. Immobilization of whole cell Ps.NR.22 on solid support ceramic

Immobilization of whole cell Ps.NR.22 on support ceramic (SC) was achieved through dead end microfiltration of 50 mL (2.7×10^6 CFU mL^{-1}) of Ps.NR.22 suspension. After the filtration ended, 1.7×10^4 CFU mL^{-1} of cells calculated in the permeate sample. Decreased of colony counting in permeate in compared to feed sample indicated that there were Ps.NR.22 immobilized on SC surface [30]. Cell immobilized support ceramic (CISC) was a term used in this study to represent support ceramic with presence of immobilized Ps.NR.22.

3.3.2. Influence of immobilized Ps.NR.22 on the adsorbed crude oil

Another properties of support ceramic is an excellent material in promoting adsorption of crude oil [31–34]. Hence, the influence of immobilized Ps.NR.22 on crude oil that was adsorbed and accumulated on the surface of support ceramic will be tested. For that purpose, in order to stimulate the adsorption of crude oil on support ceramic surface, 50 mL of 5% (w/v) crude oil water mixture was

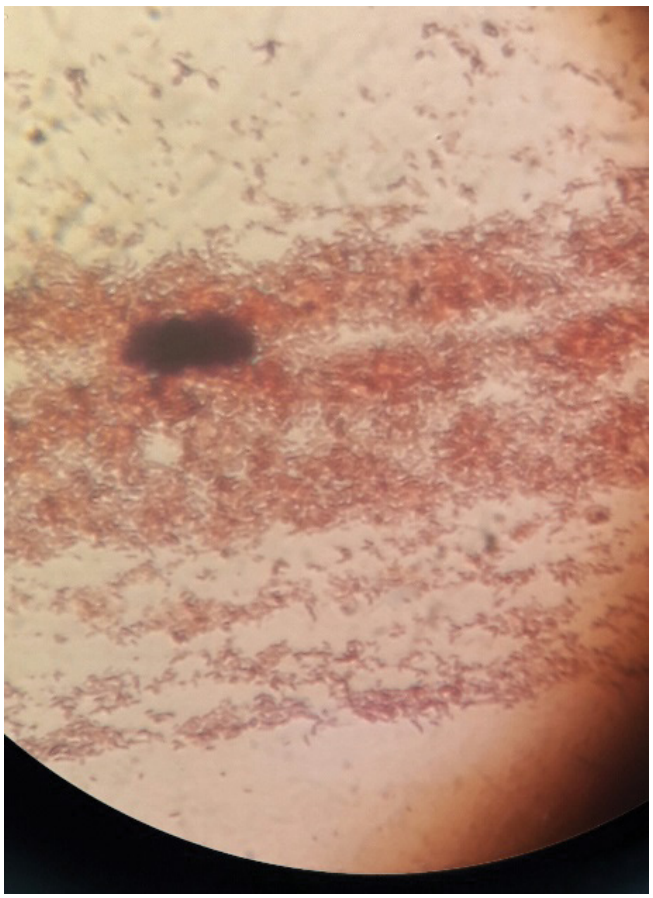

Fig. 4. Gram-negative Ps.NR.22.

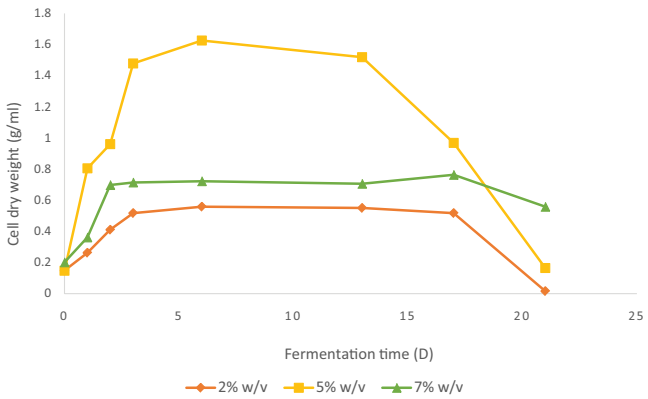

Fig. 5. Growth curve of free moving Ps.NR.22 in the crude oil culture medium throughout 21 d of fermentation period.

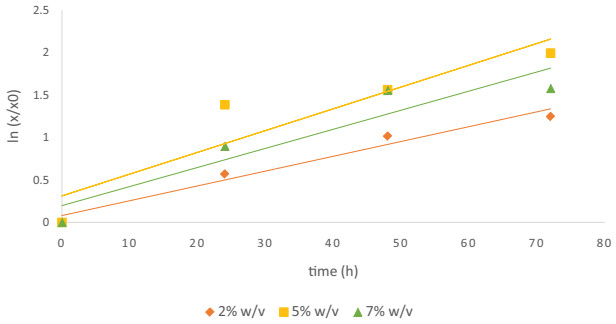

Fig. 6. Specific growth rate of free moving Ps.NR.22 in the crude oil culture medium during the exponential phase.

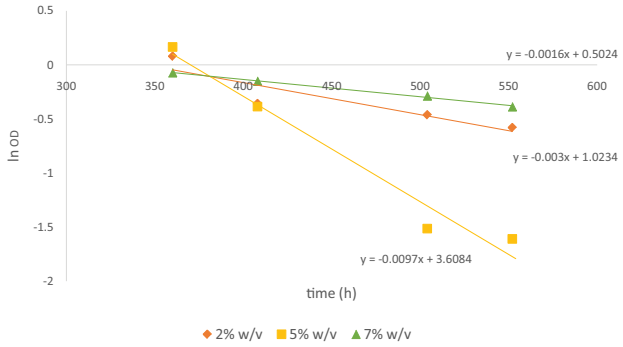

Fig. 7. Decay coefficient of free moving Ps.NR.22 in the crude oil culture medium during death/decline phase.

fed into the filtration cell and force to pass through the porous support ceramic by introducing pressure of 1.5 bar into the system. SC (with no presence of cells) act as control in this test. Pressure of 1.5 bar was applied to prevent damaged done to the immobilized Ps.NR.22. High pressure might cause burst of immobilized cell membrane [20]. Based upon Fig. 8, it can be seen that as soon as the feed sample begun to flow passed through both SC and CISC, the permeate rate declined almost instantaneously. This due to adsorption of crude oil molecules on the surface of support ceramic that interrupt and blocked the passage. This proves that support ceramic material indeed a good material in adsorbing crude oil.

Without living Ps.NR.22 on the support ceramic, the flux rate of SC kept on declining till the end of the filtration. But for CISC, it can be seen that after 125 min of filtration, CISC started to show de-fouling properties as the constantly declined flux rate begun to increase. Activation of crude oil degradation mechanism explained the de-fouling properties of CISC as the accumulated adsorbed crude oil triggered the hydrocarbon degrading mechanism of immobilized Ps.NR.22. As soon as the immobilized Ps.NR.22 started to degrade crude oil, the blocked passage started to unblock and this improve the permeability of CISC. There was a possibility that the de-fouling potential was due leaking of immobilized cell or desorption of crude oil. Hence, permeate sample was collected at 125 min of filtration time and was analysed by Fourier-transform infrared spectroscopy (FTIR) to detect any changes of functional group in permeate in compared to feed sample.

FTIR result analysis shown in Fig. 9 showed difference of peak pattern of feed and permeate sample collected. This proved the occurrence of degradation of adsorbed crude oil and at the same time prove the presence of immobilized Ps.NR.22 on CISC. According to Varjani and Upasani [8], when pollutants of oily waste has been used by *P. aeruginosa* through metabolic processes, it leads to formation of

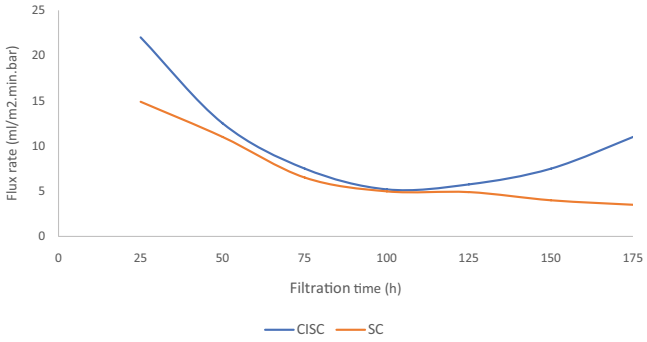

Fig. 8. Effect of oil adsorption on the permeate rate of SC and CISC.

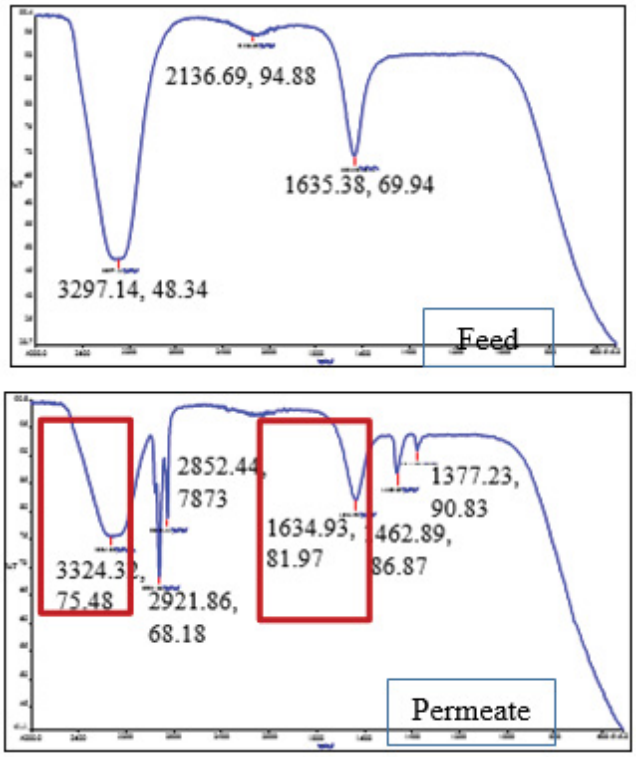

Fig. 9. Formation of new functional groups in permeate showing the influence of immobilized cell on adsorbed crude oil on CISC.

intermediates such as primary or secondary alcohol, aldehyde, fatty acids, acetate, acetyl esters and methyl ketone. Besides that, degradation of long chain crude oil also led in formation of shorter chain of hydrocarbon compounds. As a consequence, it causes feed and permeate solution having different chemical properties. This explained the formation of new functional groups in permeate sample as demonstrated in Fig. 9. Aliphatic of alkyne and alkenes groups that detected in the feed sample at wavelength of 2,136.69 and 1,635.38 cm^{-1} had been degraded into simpler alkenes groups as there were more functional groups of alkenes detected in permeate sample. This group was detected at peaks 1,634.93; 1,462.89 and 1,377.23 cm^{-1}.

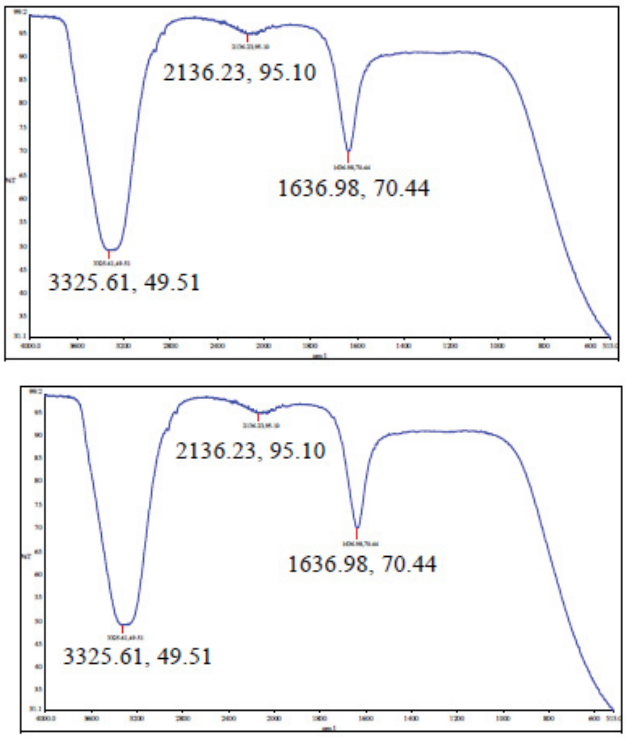

Fig. 10. FTIR result analysis of feed and permeate sample when crude oil water mixture filtered with BCM.

On the other hand, Fig. 10 demonstrated no occurrence of degradation process of adsorbed crude oil on SC as there was no presence of living Ps.NR.22. This was proven as there was no formation of new functional groups in permeate in compared to feed samples as the result of degradation products by cells.

Fig. 11 shows the difference of permeate sample turbidity collected from SC and CISC. By using naked eyes, it can be clearly seen that presence of immobilized Ps.NR.22 gave a great influence in producing better quality of water permeate in term of appearance. This might be linked to excellent degradation mechanism by immobilized Ps.NR.22 supported by great crude oil adsorbing abilities performed by support ceramic. Hence, the influence of presence of Ps.NR.22 in enhancing the performance of porous ceramic membrane in treating oil wastewater should be tested in future work.

4. Conclusion

Ps.NR.22 proved that *P. aeruginosa* species from crude oil non-contaminated site have the great crude oil degrading potential as compared to other microbes that isolated directly from the contaminated site. Ps.NR.22 demonstrated a rapid growth as the exponential phase begun in less than 24 h after the inoculation period end regardless the crude oil concentration used. Besides that, this species also has high tolerant towards toxic crude oil as increasing the crude oil concentration resulting in increasing of Ps.NR.22 growth rate. Among the crude oil concentration

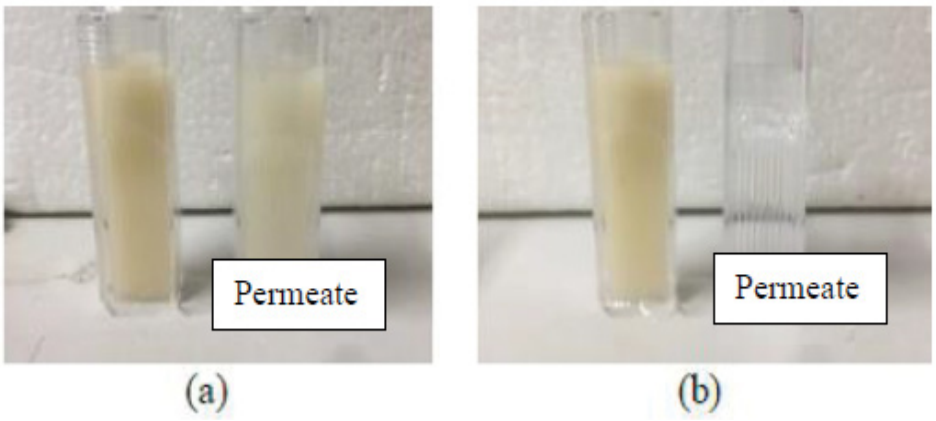

Fig. 11. Difference of turbidity of permeate water collected from: (a) SC and (b) CISC.

used, 5% w/v was the optimum concentration for P.NR.22 as the cells in this fermentation medium gave the highest growth rate and percentage of overall crude oil degraded followed by 7% w/v and 2% w/v. Ps.NR.22 also showed its ability in degrading wide range of hydrocarbon compounds as 94% of crude oil managed to be degraded by Ps.NR.22 in 21 d.

Ps.NR.22 not only capable in degrading crude oil in a liquid medium but also degrading adsorbed crude oil on a solid support ceramic. With the presence of living cells on surface of support ceramic, de-fouling potential was noticed as the constant declined permeation rate of support ceramic started to increase as the result of activation of degradation of adsorbed crude oil by Ps.NR.22. In future work it was recommended to study the potential of this species to improve conventional method of membrane cleaning (hybrid cleaning) for oily wastewater treatment.

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