

Study on the Rapid Detoxification of Tank-cleaning Oily Sludge by *Pseudomonas aeruginosa* NY3

Nie Hongyun^{1a}, Nie Maiqian^{1b*} and Wan Yan^{1c}

¹School of Environmental and Municipal Engineering , Xi'an University of Architecture and Technology , No. 13 Yanta Road, Xi'an 710055, Shaanxi Province , the People's Republic of China

^anie0212@126.com, ^bniemaiqian@xauat.edu.cn, ^c900smile@163.com

Keywords: *Pseudomonas aeruginosa* NY3; tank-cleaning oil sludge; degradation.

Abstract. Tank-cleaning oil sludge containing about 68.59 % saturated hydrocarbon and 14.93 % aromatic hydrocarbon was degraded by *P. aeruginosa* NY3 in this study. The results showed that, the degradation of oil sludge by *P. aeruginosa* NY3 was preferred under aerobic conditions, and sufficient dissolved oxygen could greatly enhance the degradation efficiency of oil sludge. Under conditions that 10% oil sludge (equivalent to 25g/l oil) were added in biodegradation medium, and supplemented with proper concentration of RL, pyruvic acid or Mg^{2+} , the degradation and transformation efficiency of oil sludge could be greatly accelerated. Under optimal conditions, the degradation rate of hydrocarbons in oil sludge could reach above 90% within 7days. The results of the present study showed the excellent ability of *P. aeruginosa* NY3 in degrading oils contained in tank-cleaning oily sludge under optimal conditions.

Introduction

Oily sludge, generated from the oil industry during oil exploration, production, transportation, storage, and refining processes, contains various amounts of hydrocarbons, water and suspended solids [1-3]. Oily sludge is a recalcitrant residue because of an emulsion of water, oil, fats, solids, organic compounds and metals. Many components of the oil sludge produced from petrochemical enterprise have been listed as priority pollutants by the United States Environmental Protection Agency as their potential toxicity, mutagenic and carcinogenic towards all ecology system [4]. According to the reports, the annual production of oily sludge from petrochemical industry in China is estimated to be 3 million tons [5]. And the total oily sludge yield is still increasing as a result of the ascending demand on refined petroleum products worldwide [6]. Confronting the environmental problems arose from the high production of such toxic oil sludge; the effective treatment method for oil sludge is urgently needed.

In the early stage of petrochemical enterprises development, it was common to dump the small amount of produced oil sludge into large sedimentation ponds or barren lands, where they have been settling for decades. But the way of such disposal presented a danger to the environment. So, the treating methods of physical chemistry (chlorination, and ozonation and wet air oxidation), physics (thermal combustion and incineration) and biology (land farming, composting, and soil/slurry treatment), have been studied and applied in oil sludge treatment [7]. Biodegradation/biotreatment is a nature compatible method character as effective, cheap, and easy to adopt in comparison to physicochemical and thermal methods [8]. However, there are also some limitations, such as deficiency of nutrients (except carbon) and poor bioavailability of hydrocarbons, which hindered the effectiveness of biodegradation of the oil sludge. The purpose of this study was to develop an effective and environmentally friendly method for purifying the hydrocarbons and other toxic organic compounds in tank-cleaning oil sludge by strain *Pseudomonas aeruginosa* NY3. Strain NY3 has been proved to be high-oil-tolerance bacteria which could remove 80% of all kinds of hydrocarbons of the petroleum within 240h when the amount of petroleum in the degradation systems reached to 20% [9]. In order to

improve the efficiency of purification, this paper focused on the actions of such factors of oxygen, rhamnolipid and special metal ion and cometabolic carbon during oil sludge degradation by strain NY3.

Materials and Methods

Chemicals. Crude oil sludge, containing 68.59% saturated hydrocarbon, 14.93% aromatic hydrocarbon, 1.56 % sulphaltene and 13.76% non-hydrocarbon, was kindly supplied by PetroChina Changqing Oilfield Company (Shaanxi, China). Unless otherwise stated, the organic solvents, media, salts and acids were purchased from various sources (Sigma, VWR and Fisher in USA or China).

Microorganism and medium. *Pseudomonas aeruginosa* NY3 (accession number: GU377209) used in this study was isolated and identified as previously reported [10].

The mineral salt medium I (MSM I) consisted of (per liter): 1.0g NH_4NO_3 , 0.1ml 1M $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$ solution, 0.05ml 1M $\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$ solution, 25ml phosphate buffer solutions (PBS). The composition of PBS was (g/l): 42g $\text{K}_2\text{HPO}_4 \cdot 3\text{H}_2\text{O}$, 28g $\text{NaH}_2\text{PO}_4 \cdot 2\text{H}_2\text{O}$, 1.0ml trace element solution. The trace element solution contained 4.5g $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$, 0.148g $\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$, 0.3g $\text{MnCl}_2 \cdot 4\text{H}_2\text{O}$, 0.024g $\text{CoCl}_2 \cdot 6\text{H}_2\text{O}$, 0.024g $\text{NiCl}_2 \cdot 6\text{H}_2\text{O}$, 0.017g $\text{CuCl}_2 \cdot 2\text{H}_2\text{O}$, 0.109g $\text{Na}_2\text{MoO}_4 \cdot 2\text{H}_2\text{O}$ and 0.062g H_3BO_3 . The pH of the media was adjusted to 7.0-7.5 using 1M NaOH and autoclaved at 121°C for 30min.

The mineral salt medium II (MSM II) consisted of (per liter): 5.0g NH_4NO_3 , 1ml trace element solution, 0.1ml 1M $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$ solution, 0.05ml 1M $\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$ solution, 100ml phosphate buffer solution (PBS).

Preparation of bacteria suspensions. To achieve enough amounts of cells, *P. aeruginosa* NY3 from a slant was inoculated into a 250ml flask containing 100ml autoclaved sterilization preculture medium, which contained per liter distilled water, 3.0g beef extract, 10.0g peptone and 5.0g NaCl, while the pH was adjusted to 7.2-7.4 before autoclaving at 121°C for 30min, and cultured until the absorption around 550nm was 1.65 ± 0.08 . Subsequently, inocula from beef extract peptone were transferred to 100ml of freshly prepared minimal salts medium (MSM I) supplemented with 5ml celery seed oil as the sole carbon and energy source. All growth experiments were cultured at 30°C on a rotary shaker at 160rpm for 72h, and then adjust the absorption around 600nm (OD600nm) of the culture to 1.57 ± 0.05 for use.

Biodegradation of oily sludge. Biodegradation experiments were performed in 50ml Erlenmeyer flasks containing 10ml MSM II and 50% inoculum size of strain NY3, supplemented with 2g crude oil sludge as the sole carbon and energy source. To study the influence factors, oxygen concentration, rhamnolipids (RL), and other accessory factors were taken into account. The culture was incubated in the flasks on a rotary shaker (30°C and 160rpm) for a certain time and then poured into a separating funnel. The flasks were washed with 10ml petroleum ether (boiling range: 30~60°C). Shaking the separating funnels thoroughly, and then separated the organic phase. Repeated this procedure of extraction for two times and then collect the organic phase to centrifuge tube and centrifuged at 8000rpm and 4°C for 5min. The upper organic phase was collected into a weighing bottle which was previously weighed and air dried to constant weight. The degradation rate was calculated as the following equation (1):

$$\text{Degradation rate} = (A-B)/(A) \times 100 \quad (1)$$

A: Initial weight of oil sludge

B: Final weight of oil sludge

Gas chromatography analyses. The n-alkanes fraction was analyzed by gas chromatography (GC) using a FID detector (Agilent Technologies 6890N). The column was a HP-5 capillary column (5% phenyl Methyl Siloxane, 30mm×320μm×0.25μm) with nitrogen as a carrier gas. The column temperature was held at 50°C for 7min, raised from 50°C to 100°C with 20°C min⁻¹ increment and then increased from 100°C to 290°C with 5°C min⁻¹ increment. The injector and transfer line temperature

were 300°C. Individual components present in the n-alkanes fraction were determined by matching the retention time with authentic standards.

Results and Discussion

Influences of oxygen content on the degradation efficiency of oil sludge. Culture medium containing 10% oil sludge (equivalent to 25g/l oil) was treated by *P. aeruginosa* NY3 under different concentrations of dissolved oxygen, the degradation rate of oil contained in oil sludge at different incubation time was presented in Fig. 1. Oil removal rates were time-dependent and also closely related to amount of dissolved oxygen (DO) in cultures. Under condition of 5.2mg/l DO, the NY3 strain showed high capacity of oil metabolization, and about 30%~40% removing rate of oil higher than that under the condition of the lower amount of DO (2.4mg/l). With 5.2mg/l dissolved oxygen, the oil removal rate by strain NY3 within 96h incubation exceeded 80% which was much higher than other bacteria reported previously [11].

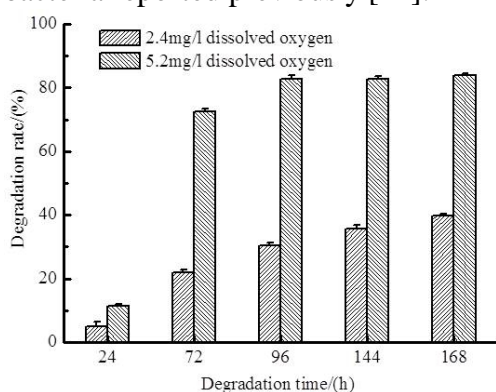


Fig. 1 The influence of oxygen on the degradation rate of oily sludge. Data are expressed as mean value and standard deviation of independent triplicates.

Influences of RL concentrations on oil sludge degradation. By addition of RL ranged from 0mg/l to 205mg/l (see 2.4) into degradation liquids, the oil removing results by strain NY3 within 168h were showed in Fig. 2. Obviously, about 40mg/l RL could augment the degradation rate of the total oil. And increase the amount of RL could not result in higher oil removal rate. Under the condition of 40mg/l RL, the degradation rates of all kinds of n-alkanes in the oil sludge were shown in Fig. 3. Comparing to the condition without RL, the degradation rates of all kinds of n-alkanes, especially C34, were higher than that under the condition without RL. These experiments showed that the proper concentration of RL could enhance the degradation of oil sludge. Some reports explained that RL was functioned as a bio-surfactants in the degradation system, but others thought that RL may change the surface of cells which lead to the variation of oil metabolization [12-13]. Anyhow, the results presented here proved that RL could accelerate the degradation of oil sludge by *P. aeruginosa* NY3 which was consistent with previous reports.

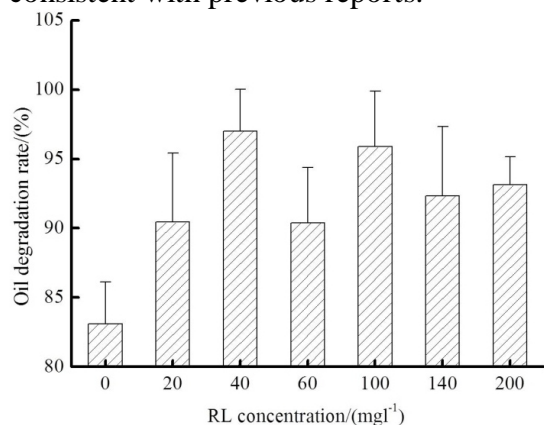


Fig. 2 Influences of RL concentration on oil degradation rate. Data are expressed as mean value and standard deviation of independent triplicates.

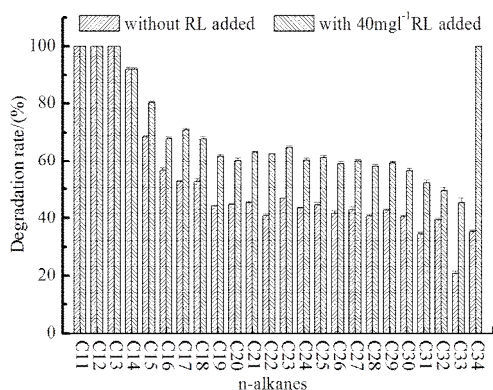


Fig. 3 Degradation rates of n-alkanes after cultured for 24h: n-alkanes were presented by the number of carbon contained, for example, undecane in the Fig. 3 was expressed as C11, and so on. Data are expressed as mean value and standard deviation of independent triplicates.

Influences of pyruvic acid on the efficiency of oil sludge degradation. The influence of pyruvic acid on oil sludge degradation was carried out by adding various amounts of pyruvic acid ranged from 0mg/l to 30mg/l (see 2.4) into degradation liquids, the degradation rate of oil within 168h by strain NY3 was presented in Fig. 4. It could be seen that the removing rate of the oil was increased from 74% to 95% by 6mg/l pyruvic acid. More amount of pyruvic acid could not result in higher oil removal rate. The experimental phenomenon was easily and frequently observed in the laboratory which could show the promoting effects of the added pyruvic acid in the biodegradation medium (Fig. 5). Under the same cultural conditions, the biodegradation medium with a certain amount of pyruvic acid after degrading for 168h became sticky and turned to brownish red. And obviously, the original added oil sludge was disappeared and there were large amount of biomass, especially biopolymers, produced in the cultural medium by strain NY3 (Fig. 5 c).

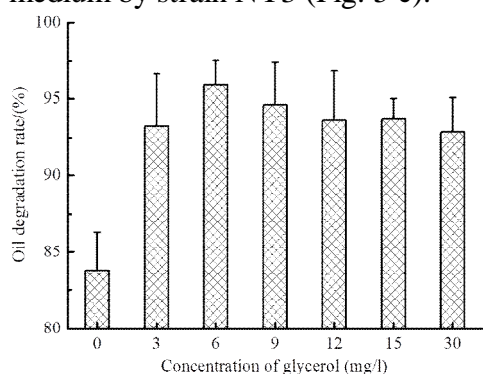


Fig. 4 The influence of the concentration of pyruvic acid on oil degradation rate. Data are expressed as mean value and standard deviation of independent triplicates.



Fig. 5. Pictures of oil sludge degradation system: the degradation conditions were the same as that referred in 2.4, and the degradation time was 168h. (a) control I: only with oil sludge in MSM II, (b) control II: strain NY3 was inoculated in control I, (c) 6mg/l glycerol was added control II

Influence of Mg^{2+} on oil sludge degradation. The influence of Mg^{2+} on oil sludge degradation was carried out by adding different amounts of Mg^{2+} ranged from 0.02mg/ml to 0.12mg/ml (see 2.4). For controls, there was no extra $MgSO_4 \cdot 7H_2O$ added in MSM I and MSM II. The results proved that total oil degradation rate within 168h was much higher with 0.06mg/ml Mg^{2+} in the medium (Fig.6). The results of oil residue in medium under existence of 0.06mg/ml Mg^{2+} analysed by GC have been showed in Fig.7. Comparing to the blank control, almost all the n-alkanes in oil sludge have been removed except for polycyclic aromatic compounds-phenanthrene and pyrene in which the removal rates were about 23%. According to the report of N. Grossowicz et al., the production of pyocyanine by *p. aeruginosa* was promoted by the additional Mg^{2+} , which in turn may increase the degradation rate of alkanes [14].

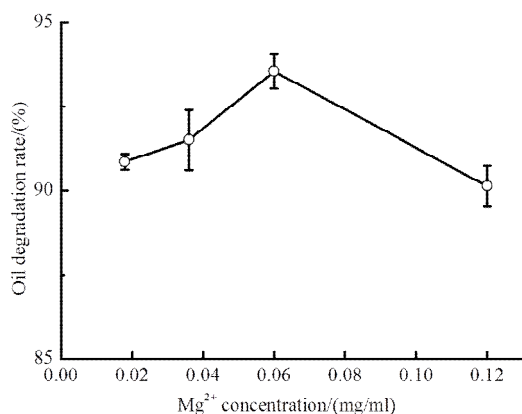


Fig. 6 Influence of the concentration of Mg^{2+} on oil degradation efficiency. Strain NY3 suspensions was inoculated in MSM II supplemented with 10% oily sludge and certain amount of Mg^{2+} , cultured for 168h at 30 °C, and the inoculums size was 50%. Data are expressed as mean value and standard deviation of independent triplicates.

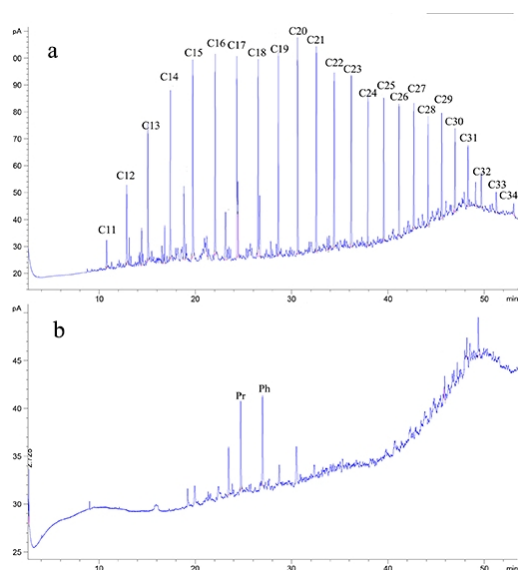


Fig. 7 GC spectra of the compounds in oil sludge after cultured for 168h at 30 °C. a: blank control (10ml strain NY3 cells from the MSM I seed liquid was inoculated into 10ml MSM II supplemented with 10% of oil sludge); b: degradation medium which contained the same blank control medium supplemented with 0.06mg/ml Mg^{2+} .

Conclusions.

Here in our experiment with the tank-cleaning oil sludge from Petrochemical Enterprise containing about 68.59% saturated hydrocarbon and 14.93% aromatic hydrocarbon. Nearly all of the hydrocarbons could be removed and transformed within 168h by *P. aeruginosa* NY3. Sufficient dissolved oxygen could greatly enhance the degradation efficiency of oil sludge. Under the existence of as high as 10% oil sludge (equivalent to 25g/l oil), with trace amount of other factors, such as RL, pyruvic acid and Mg^{2+} , oil degradation and transformation rate could also greatly augment. The results of the present study showed excellent ability of *P. aeruginosa* NY3 in degrading tank-cleaning oily sludge under optimal conditions.

Acknowledgement

This work was supported by a grant from the National Natural Science Foundation of China (NO. 51278405), Shaanxi Province Key Program for International S&T Cooperation Projects of China (2012KW-25) and 2011 Yulin City University-Industry Cooperation Program of Shaanxi province in China.

References

- [1] Wang, J., Yin, J., Ge, L., Shao, J.H., Zheng, J.Z, Wang, J., Yin, J., Ge, L., Shao, J.H., Zheng, J.Z, 2010. Characterization of oil sludge from two oil fields in China. *Energ. Fuel.* 24 (2010): 973-978.

- [2] Long, X.Y., Zhang, G.L., Shen, C., Sun, G.S., Wang, R.X., Meng, Q, Application of rhamnolipid as a novel biodemulsifier for destabilizing waste crude oil. *Bioresource Technol.* 131(2013): 1-5.
- [3] Zhang, J., Li, J.B., Thring, R.W., Hu, X., Song, X.Y., Oil recovery from refinery oily sludge via ultrasound and freeze/thaw. *J. Hazard. Mater.* 203–204(2012): 195-203.
- [4] Janbandhu, A., Fulekar, M.H., Biodegradation of phenanthrene using adapted microbial consortium isolated from petrochemical contaminated environment. *J. Hazard. Mater.* 187(2011), 333-340.
- [5] X. Wang, Q.H. Wang, S.J. Wang, F.S. Li, G.L. Guo, Effect of biostimulation on community level physiological profiles of microorganisms in field-scale biopiles composed of aged oil sludge, *Bioresour. Technol.* 111(2012): 308–315.
- [6] Guangji Hu, Jianbing Li, Guangming Zeng, Recent development in the treatment of oily sludge from petroleum industry: A review. *J. Hazard. Mater.*, 261(2013): 470-490.
- [7] Powell, S.M., M. Paul, P.M. Harvey, S. J. Stark, I. Snipe, and J.M., Biodegradation of petroleum products in experimental plots in Antarctic marine sediments is location dependent. *Mar. Pollut. Bull.* 54(2007): 434-440.
- [8] Machin-Ramirez, C., A. I. Okoh, D. K. Morales, M. Mayolo-Deloisa, R. Quintero, M. R. Trejo-Hernandez, Slurry-phase biodegradation of weathered oily sludge waste. *Chemosphere*, 70(2008):737–744.
- [9] Chang Hong, Nie maiqian, Ge Bizhou, Liu Chao, Yang Qin, Zhou Lihui, Fan Xiaoyu, Sun Chao, Wei Rongrong, Jiang Xin, Effect of rhamnolipid on oil degradation by *Pseudomonas aeruginosa* strain NY3. *Chinese Journal of Environmental Engineering*, 7(2013): 771-776.
- [10] Maiqian, Nie, Xihou, Yin, Chunyan, Ren, Yang, Wang, Feng, Xu, Qirong, Shen, Novel rhamnolipid biosurfactants produced by a polycyclic aromatic hydrocarbon-degrading bacterium *Pseudomonas aeruginosa* strain NY3. *Biotechnol. Adv.* 28(2010): 635–643.
- [11] Swaranjit Singh Cameotra, Pooja Singh, Bioremediation of oil sludge using crude biosurfactants. *Int. Biodeter. Biodegr.* 62 (2008): 274–280.
- [12] Yimin Zhang and Raina M. Miller, Effect of Rhamnolipid (Biosurfactant) Structure on Solubilization and Biodegradation of n-Alkanes. *Appl. Environ. Microb.* 61(1995): 2247–2251.
- [13] P. Bharali, J.P. Saikia, A. Ray, B.K. Konwar, Rhamnolipid (RL) from *Pseudomonas aeruginosa* OBP1: A novel chemotaxis and antibacterial agent. *Colloid. Surface. B.* 103(2013): 502-509.
- [14] N. Grossowicz, Peyuta Hayat, Y. S. J., Pyocyanine Biosynthesis by *Pseudomonas aeruginosa*. *J. gen. Microbial.*, 16(1957), 576-583.