Available online www.jocpr.com

Journal of Chemical and Pharmaceutical Research, 2015, 7(3):993-996

Research Article

ISSN : 0975-7384
CODEN(USA) : JCPRC5

Biodegradation of phenanthrene by *Pseudomonas aeruginosa* with treatment of rhamnolipid biosurfactant

ShaojunZhang[1,2], Wanqing Wu[1*] and Qinggong Zheng[1]

[1]*Marine Engineering College, Dalian Maritime University, 1 Linghai Road, Dalian, China*
[2]*Maritime College, Shandong Jiaotong University, 5 Jiaoxiao Road, Jinan, China*

ABSTRACT

In order to investigate the role of rhamnolipid on the degradation of polycyclic aromatic hydrocarbons (PAHs), biosurfactant rhamnolipid produced by Pseudomonas aeruginosa was invested on the degradation of phenanthrene. The result showed that the solubility of phenanthrene have linear relationship with rhamnolipid when below or above the critical micelle concentration (CMC). The concentration of rhamnolipid showed stronger solubility when below CMC than above CMC. Then, Pseudomonas aeruginosa strains treated by rhamnolipid were applied in degradation. The results showed that the growth of bacterium was inhibited when treated with 75 mM (1 CMC) rhamnolipid. However, the bacterial treated with 750 mM (10 CMC) rhamnolipid accelerated the degradation. There was no degradation phenomenon occured without treated with the rhamnolipid. This result showed that the cells could not degradate phenanthrene directly. We can deduce from the experiment that rhamnolipid improve the degradation efficiency by increasing the solubility of carbohydrates. This conclusion is significant for the evaluation of rhamnolipid surfactant in the remediation of phenanthrene contaminated sites.
Keyword: rhamnolipid; phenanthrene; biodegradation

INTRODUCTION

Application of biosurfactant in remediation of hydrocarbon contaminated soil or water is one of the most effective technologies in the world. However, the low bioavailability of hydrocarbons is the bottleneck to restrictthe biodegradation[1].Adding the biosurfactant is an effective method to improve the bioavailability.Biosurfactantcan increase the solubility of hydrophobic organic compounds in the aqueous phase, so as to increase the mass transfer rate and bioavailability[2,3]. The synthetic surfactant itself is toxic and difficult to degradate, so itis limited inthe application.Biological surface active agent is non-toxic and easy for degradation, which make it has a wide application in the treatment of petroleum hydrocarbon polluted sites[4].

Rhamnolipid is a famous biosurfactants produced by *Pseudomonas aeruginosa (P.aeruginosa)*, its synthesis pathway, molecular structure and aggregation properties caused more and more attention[5,6]. However, its mechanismin petroleum hydrocarbon degradationremains for further research.The physical and chemical character ofrhamnolipid can form hydrocarbon copolymer for increasing hydrocarbon solubility.This copolymer can improve the mass transfer of hydrocarbonfrom water to the organic phase, or they can be swallowed as a whole by microbial cells[7,8].Rhamnolipid can enhance the cell surface hydrophobicity, so as to improve the direct contact of microbial cells and hydrocarbons, or enhance the permeability of cell membrane, for the convenient transportation of hydrocarbons into the cell.In order togrope for the function ofrhamnolipid in the hydrocarbon degrading process, the researchersusually using an excess of hydrocarbon, which leading to the emergence of various forms of carbon source of emulsion or a large number of hydrocarbons. As there are complex forms of carbon source co-exist, it is difficult to distinguish the role of rhamnolipid on the microbial biodegradation of hydrocarbons.Phenanthrene is widely used in dyes, pesticides and other production process that it is acancerigenicpollutant in soil and river.Thisexperiment intends toresearch the effects of rhamnolipidproduced by*P.aeruginosaATCC27853*on phenanthrene degradation[9,[10]].In order

to research the biological function of rhamnolipid, some cells were treated with/without rhamolipid. The experiment is designed to explore the rhamnolipid plays a decisive role in hydrocarbon degradation.

EXPERIMENTAL SECTION

2.1. Microorganisms and MSM

The rhamnolipidbiosurfactant was produced by *P. aeruginosaATCC27853*in mineral salt medium (MSM) with 20 g/L ofglucose (carbon source). 2-bromoacetophenoneand triethylaminewere purchased from SigmaAldrichU.S.A., and phenanthreneof analytical grade was obtained from Kermal Chemicals(Tianjin, China). Other reagents were of analytical grade andused as received. *P. aeruginosaATCC27853*was used both as theproducer of rhamnolipid and the degrader of phenanthrene, and maintainedat 4 ˚C. It was transferred every month and activated for 24 hat 37 ˚C before use.The fraction of MSM is as follows: $NaNO_3$(0.20%), KH_2PO_4(0.15%), $Na_2HPO_4 \cdot 12H_2O$ (0.15%), $MgSO_4$ (0.01%), $FeSO_4 \cdot 7H_2O$ (0.001%) in pH 6.5

2.2. Solubilization ofphenanthreneby rhamnolipid

Phenanthrene(10 mL) wastaken outinto a 50-mL Erlenmeyer flask, then 5mL of MSMsolution ofrhamnolipid was added to the flask, and incubatedat 30 ˚C for 12 h. The solution was centrifugedat 400 rpm for 5 min to separate the phenanthrene droplets from MSM.Extraction approximately 2 mL of saturated rhamnolipid solubilized phenanthrene solutionusing a needle.In order to investigate the effect of pH on the enhancedsolubilization of crude oil, thepH of rhamnolipid MSMsolutionwas adjusted using 10% HClor NaOH.Angas chromatography/mass spectrometry (GC/MS) system (GC/MS-QP 2010 Plus, Shimadzu,Japan)equipped with a DB-5MS capillary column (30 m×0.25 mm×0.25 μm) was used for the determination of phenanthrene concentration in the solution.. The injection mode wassplitless with volume of 1 μL. The carrier gas was helium, and the column flow was set at 26.2 cm/sec. MS ionization was EI mode, and electron impact mode was 70 eV. Ion source temperature was 200˚C. GC/MS interface temperature was 280˚C, and SIM mode was set for acquisition. Thecolumn temperature programwas set at 90˚C for 1 min, and then raised by25˚C/min to 180˚C, held for 1min, raised by 5˚C per min to320˚C and held for 1 min.

2.3. Degradation experiment

MSM solution300 mL containing 750 mMrhamnolipid and 0.5 mL phenanthrenewere added intoa 500mLErlenmeyerflask, and shakedon a gyratory shaker at 100 rpm for 12 h. The solution was then transfered into a separatory funnel for24 h, andcollected the bottom solutionto obtain saturated rhamnolipidsolubilizedphenanthrenesolution.Enriched the *P. aeruginosa*from slants in 50 mLof MSM solution and cultured at37 ˚C for 24 h.The supernatant was removedafter the sample was separated after centrifugation, then added 1 mL of NaOH(0.1mol/L) into the centrifuge tube.Dinitrosalicylic acid(DNS) methodwas used for determination of glucose.

RESULTS AND DISCUSSION

3.1. Solubilization ofphenanthreneby rhamnolipid

Figure 1 showed therhamnolipidthat solubizaed in the MSM containingphenanthrenesolution. TheCMCofrhamnolipidin MSM is analyzed on 75 mM. As therhamnolipid concentrations below or above CMC, it was showed a linearly relationship between rhamnolipid concentration and phenanthrene solubility.In MSM solution, the amount of solubilized phenanthrene is also in linearrelationshipbetween therhamnolipid below and above the CMC.

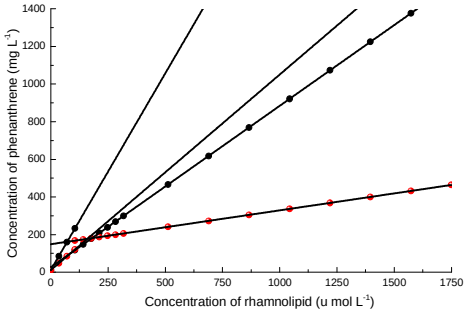

Fig. 1.Solubilization of phenanthrenein the MSM soulutioncontaingrhamnolipid

Figure 2 showedin MSMtherhamnolipidenhanced solubility of phenanthrene in the range of pH6-7.5. The solubility reached the highest at 255 mg/L.As pH of MSM increased to 8.0, the solubilityrapidly dropped to lower than 100 mg/L.This result showedthat the strong activity ofrhamnolipidto solubilizephenanthrenewhen the solution was weakly acidic (pH rangingfrom 6.0 to 6.8). So pH 6.5was selected in the following experiments.

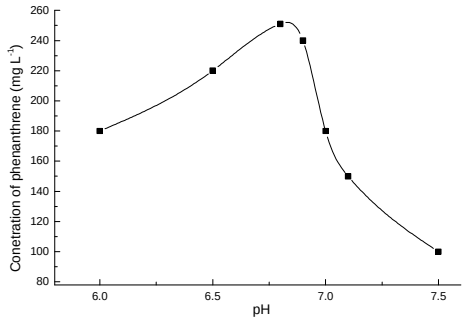

Fig. 2Solubility of phenanthrene at pH6.0-7.5.

3.2. Degradation of phenanthrene

Figure3showed the degradation results ofphenanthrene. At thebeginning, the concentration of bothphenanthreneandrhamnolipiddecreased remarkable for all the*P. aeruginosa*indicating the adsorption of phenanthreneaggregates to*P. aeruginosa*. The adsorption reached equilibrium in 20 min, about half of the aggregates being transferred to the surface of cell.However,in thefollowing 10 days, the concentration of phenanthrenedid not show further decrease.The concentration ofrhamnolipid in the cultures alsoincreased after 72 h of cultivation.

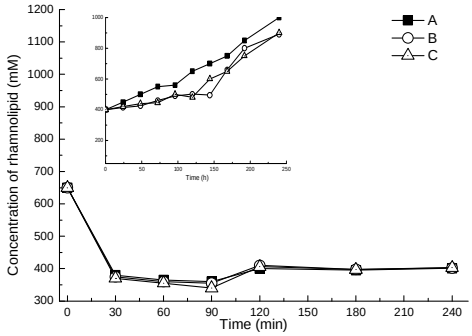

Fig. 3.Effect of rhamnolipid treatment on degradation ofphenanthreneby *P.aeruginosa.*

3.3. Cell surface hydrophobicity

The hydrophobicity ofrhamnolipid effectedthe cell surface was also investigated.CMC solutions 75 mM and750 mM containing rhamnolipidwere tested. Astreated with750 mMrhamnolipidcaused a slightly decrease ofcell adhesion, however when treated with75 mMrhamnolipidreduced the rate to 10.1%. It indicated there was a highly hydrophilic cellsurface.

CONCLUSION

Rhamnolipidtreated cellsshowed a stimulating effect on the degradation ofphenanthrene。Phenanthrene and rhamnolipid formed co-aggregatesinMSM culture. The result supports the hypotheses that facilitatinginterfacial enhancing cell surfacepermeability is an important role of rhamnolipid in enhancedhydrocarbon degradation. The results also indicated the effectivenessof using rhamnolipid to enhance the bioavailability ofhydrocarbons.

Acknowledgments

This research was supported by the funds of Shandong Natural Science Foundation Project(ZR2014JL038);Projects of Medical and Health Technology Development Program (2014WS0281), Shandong, China.

REFERENCES

[1] EllenCristinaSouza,TherezaChristinaVessoni-Penna,etal.*InternationalBiodeterioration&Biodegradation*,**2014**,89 :88-94.
[2] XuhuiMao,RuiJiang,WeiXiaoetal..*JournalofHazardousMaterials*,**2015**,285:419-435.
[3] CeliaF.C.daRosa,DeniseM.G.Freire,HelenC.Ferrazetal. *JournalofEnvironmentalChemical Engineering*,**2015**,3(1): 89-94.
[4] HanenBenAyed,NawelJemil,HanaMaalej,etal. *InternationalBiodeterioration&Biodegradation*,**2015**,99:8-14.
[5] HuaZhong,YangLiu,ZhifengLiuetal. *InternationalBiodeterioration&Biodegradation*,**2014**,94:152-159.
[6] KellK.Andersen,DanielE.Otzen. *FEBSLetters*,**2014**,588(10):1955-1960.
[7] HabaE.,PinazoA.,PonsR.etal. *BiochimicaetBiophysicaActa(BBA)-Biomembranes*,**2014**,1838(3):776-783.

[8] TylerDillardRenfro,WeijieXie,GuangYangetal. *ColloidsandSurfacesB:Biointerfaces*,**2014**,115:317-322.
[9] FengLi,LizhongZhu,DongZhang. *JournalofEnvironmentalSciences*,**2014**,26(11):2298-2306.
[10] PantsyrnayaT.,DelaunayS.,GoergenJ.L.etal. *Chemosphere*,**2013**,92(2):192-195.