Highly Efficient Degradation of PAHs by the Strains NPA-5 and K-4

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Abstract: The naphthalene and isoquinoline were the recalcitrant pollution of PAHs(polycyclic aromatic hydrocarbons) in the coking wastewater processing system, whose contents had a direct impact on effluent water quality. This study was aim at reducing the recalcitrant pollution of naphthalene and isoquinoline more effectively, the biological activated technique was adopted. The highly efficient degradation strains NPA-5 and K-4 were isolated from the long-term polluted soil in the coking plant and the activated sludge in the coking wastewater treatment plant. The High Efficiency Strains (HES) degradation experiment showed that two strains possessed idiographic capability to degradate naphthalene and isoquinoline. The average removal rate of COD by NPA-5 static Biological Activated Carbon (BAC) was 13.4% higher than the method of Granular Activated Carbon (GAC) and 9.2% higher than that of Activated Sludge Biomass(ASB) in treating coking wastewater. After reduplicative treatments for 12 times, the removal rate of COD by BAC was 54.88% higher than GAC and 16.36% higher than ASB.

Keywords: High Efficiency Strains; PAHs; Coking wastewater; Biological Activated Carbon

1 Introduction

Coking wastewater is a kind of difficult to treat industrial and noxious wastewater[1-2]. After treated by AAO technology, the coking wastewater still contained approximately 118 sorts of organics under the detection by GC-MS. Among these of organics, the content of quinolones was the highest, total quality percent was 14.3%; the naphthalenes' total quality percent was 5.16%. They both total quality percent were 19.46%.

The PAHs is low water soluble in the nature, difficult biodegradable, potential carcinogenic and easy adsorbed by environmental granule characteristics, so they are identified as main environmental contaminants[3-4]. The biological method using specific degrading bacteria is more feasible and cost-effective than physical and chemical treatment of industrial wastewater[5].

Although the biodegradation of naphthalenes in the effluent treatment system have been investigated prosperously at home and abroad, the qulnolones' biodegradation effect was undesirable because their nitrogenous heterocyclic structures were not catabolized by most microorganism enzymes[6].

This paper was focus on the descrease the content of naphthalenes and qulnolones in the coking wastewater. Naphthalene and isoquinoline biodegradation strains were isolated and screened from the long-term polluted soil in the coking plant and the activated sludge in the coking wastewater treatment plant, which provided strains to biodegradate the PAHs in the coking wastewater more effectively.

2 Materials and methods

2.1 Main instrument and chemicals

Spectrophotometer U-3010 was procured from Hitachi Ltd.(Japan). Spectrophotometer 722-200 was procured from Shandong gaomi caihong analytical instrument Co., Ltd.(China). GC Agilent 6890 was procured from Agilent technology Ltd.(USA). Biochemical incubator Spx-250B-Z was procured from Shanghai boxun industry & commerce Co. Ltd. medical equipment factory (China). Constant temperature shaker HZ200LB was procured from Wuhan ruihua instrument and equipment Co., Ltd.(China).

Isoquinoline was analytical pure grade and purchased from Anshan beida synthetic chemical factory(China). Naphthaline was analytical pure grade and purchased from Shanhai traditional Chinese medicine Co., Ltd.(China).

2.2 Media and culture condition

All cultures were maintained in liquid mineral salts medium (MSM, 1L) containing $NaNO_3$ 1.0g, NaCl 0.5g, K_2HPO_4 0.5g, $MgSO_4$ 0.25g and $FeSO_4$ 0.01g. The final pH of MSM was 7.0 and sterilized at 121.3℃for 20 min, then added the naphthalene and isoquinline as unique carbon source respectively when solid medium added 1.5~2.0% agar.

During the experiments, the deionized water was required and the other chemical reagents were all analytical pure grade. The cultures were maintained at 25℃ on a constant temperature shaker (150 rpm).

2.3 Isolation and screening of strains

2.3.1 Enrichment, isolation and purification

There were activated sludge pretreatment and soil solution different pretreatment and enrichment as below.

(1) Activated sludge pretreatment and enrichment

100 ml activated sludge from coking plant was loaded into 250 ml erlenmeyer flask with several glass beads, then 2 drops of 0.02% sodium pyrophosphate was added for deflocculation. The treated sludge was placed in the constant temperature shaker under 30℃ and shaked for 30 min with 150 r/min. The sludge was centrifuged under 5000r/min for 10 min. The pretreated activated sludge mixed with uniformly sediment liquid was inoculated into the 100 ml enrichment medium[7] in the constant temperature shaker culturing for 24h.

(2) Soil solution pretreatment and enrichment

5g soil sample was loaded into 50ml sterilized sodium pyrophosphate. The sodium pyrophosphate bacterium suspension was prepared, then cultured for 24h[8]. 1ml sodium pyrophosphate bacterium suspension was inoculated into 100ml enrichment medium, and cultured in constant temperature shaker for 24h.

(3) Isolation and purification

1ml enriched bacterium suspension was loaded into 100ml MSM with 200mg/L naphthalene or isoquinoline, cultured in the constant temperature shaker for 3d. After a number of domestications, the mass concentration of naphthalene or isoquinoline step-wise increases from 200mg/L to 1000mg/L, then the different pure strains would obtain via gradually dilution on the solid medium. The strains were purificated by solid plate streaking to obtain single colonies, and then the better degradation rate strains were stored on solid slant medium in the refrigerator at 4℃.

2.3.2 Screening of high efficiency strains (HES)

The purified single strains were enriched in LB medium[7]. 1 ml bacterium suspension was inoculated into 100ml MSM with naphthalene or isoquinoline as unique carbon source, and cultured under in the constant temperature shaker for 3d. During this period, the changes of MSM were observed and recorded. The best bacterial growthism and degradation rate strains were selected as next experimental HES.

2.4 Degradation experiment of HES

The mass concentration of substrates affected HES degradation rate and bacterial growthism. HES on solid slant medium were inoculated by incubation loop and enriched via LB medium, and 1ml enriched bacterium suspension inoculated into 100ml MSM with mass concentration gradient of naphthalene and isoquinoline in the constant temperature shaker cultured for 3d, and then the naphthalene and isoquinoline degradation rate were calculated by the strains, the baterium growthisims in MSM were also recorded.

2.5 NPA-5 BAC static degradation experiment

2.5.1 Static NPA-5 BAC preparation

The cylinder activated carbon with opengrain was selected as static biological activated carbon carrier. The biological materials came from the screened and domesticated HES and the activated sludge in the coking wastewater treatment plant. The static NPA-5 BAC preparation specific method was as below.

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A certain intermediate cultured mount of HES solution(or the activated sludge) and worked cylinder activated carbon were added into 1000ml bunsen beaker, agitated by magnetic stirring apparatus at 25℃ with the speed 150r/min for 30min, then standed till the bacterium solution concentration was not changed to ensure activated carbon and microorganisms adequately mixed.

The NPA-5 biological activated carbon (BAC), activated sludge biomass (ASB) and granular activated carbon (GAC) prapared materials were as below.

Table 1. BAC, ABS and GAC prapared material

Activated Carbon Type	Biological Source Type	Activated Carbon
BAC	High efficiency strain (HES) NPA-5	Cylinder carbon
ASB	Activated Sludge	Cylinder carbon
GAC	None	Cylinder carbon

2.5.2 NPA-5 BAC static degradation experiment

(1) Experimental water sample quality

Experimental water sample was from a coking plant secondary sedimentation tank effluent, pH=8.0, COD =200mg/L, chromaticity =900 degree.

(2) Processing time affect on COD removal rate

4g/L prepared BAC were added into 250 ml erlenmeyer flask with 100ml coking wastewater, and processed at 30℃ for a certain time. Then 20ml water sample was extracted, the COD average removal rate was detected after filtrate and centrifuge which was compared with GAC and ASB. The total processing time was 48h.

(2)Processing times affect on COD removal rate

Processing method as (1), the BAC repeatedly treated coking wastewater which was compared with GAC and ASB.

2.6 Analysis methods

2.6.1 Determination of substrate concentration

(1)Determination of naphthalene concentration

The mass concentration of naphthalene was determined by GC under column temperature 150℃, vaporizer temperature 200℃, detecting device temperature 220℃, carrier gas nitrogen flow rate 1ml/min, hydrogen flow rate 100ml/min, split ratio 1:200, sample size 1μl. According to standard curve, the naphthalene mass concentration in the MSM was extracted by normal hexane that was centrifuged at 12000r/min for 10min and determined by GC.

(2)Determination of isoquinoline concentration

In this study, the isoquinoline degradation rate was determined the MSM UV photometric that was quantity to the effect of the strains on degradation isoquinoline [9].

The structural drawing was shown below, the isoquinoline showed the biggest ultraviolet characteristic absorption peak under λ=318 nm (Fig.1).

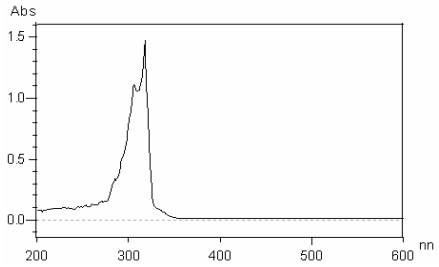

Fig.1. UV absorption peak of isoquinoline

A series of isoquinoline mass concentration solution C were prepared with the acetidin taken as solvent, the absorbance value A was determined under λ=318 nm and the standard curve of C-A was drawn. The MSM was centrifuged under 12000r/min for 10 min and supernatant was extracted by acetidin, the absorbance value was determined under λ=318 nm under the optimal mass concentration.

2.6.2 Biodegradation rate computational formula

The parallel blank experiment remaining substrate mass concentration was C_0 and the substrate mass concentration after biodegradation was C_1. The biodegradation rate η computational formula was as follows.

$$\eta = \frac{C_0 - C_1}{C_0} \times 100\% \quad (1)$$

2.6.3 Bacteri[illegible] nethod

The bacterial growthism could be measured in determining MSM optical density values OD_{600} at λ=600 nm by the 722 spectrophotometer or recorded the changes in the MSM.

3. Results and discussion

3.1 Result of isolating and screening strians

(1) Result of naphthalene degradaton strains

10 strains were acquired in all the isolation after re-duplicative domestication and purification which could grow in the MSM with naphthalene as the main carbon source and named NPA-1～NPA-10. The strains in the MSM with naphthalene mass concentration 400mg/L and their growth are as table 2.

Table 2. The changes in the MSM with different single strains

NO.	1st Day	2nd Day	3rd Day	NO.	1st Day	2nd Day	3rd Day
NPA-1	-	+	+	NPA-6	-	+	+
NPA-2	-	+	+	NPA-7	-	-	+
NPA-3	+	++	++	NPA-8	+	+	++
NPA-4	+	++	++	NPA-9	-	-	+
NPA-5	+	++	++	NPA-10	-	+	+

++: MSM turbidity changed significantly, +:MSM turbidity changed obviously, -: MSM turbidity changed not obviously.

In table 2, the strains NPA-3, NPA-4, NPA-5, NPA-8 grew rapidly in the MSM with naphthalene as the main carbon source. The degradation rates of these strains were as Fig.2.

From table 2 and Fig.2, NPA-5 was the highest efficient degradation and rapid growth strain which degradation rate was 99.2% and was the HES in next experiment.

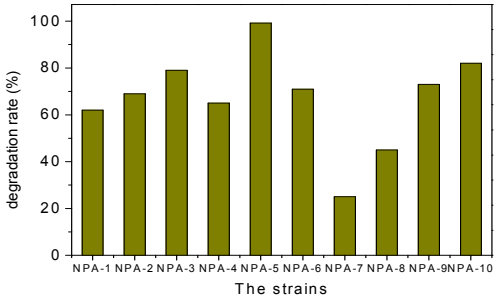

Fig.2. All kinds of strains naphthalene biodegradation rate

(2)Result of isoquinoline degradaton strains

10 strains were acquired in all the isolation after reduplicative domestication and purification which could grow in the MSM with isoquinoline 500mg/L as the main carbon source and nominated K-1～K-9. Fig.3 showed 72h degradation rate and OD_{600} values of the isoquinoline degradation strains. From this figure, we could find K-4 strain was the highest efficient degradation strain. From Fig.3, K-4 was the highest efficient degradation and bacterial growthsim strain which degradation rate 82.3% and was the HES in next experiment strain.

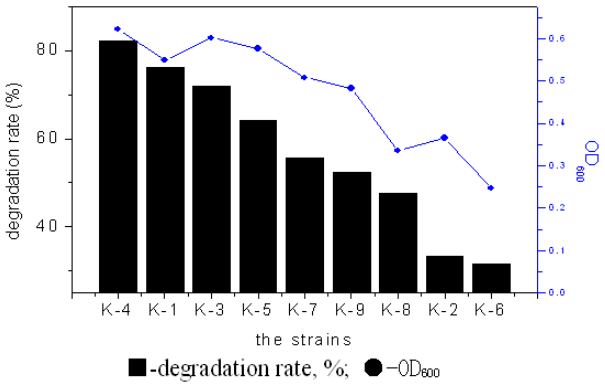

Fig.3. All kinds of strains isoquinoline biodegradation rate and bacterial growthsim

3.2 HES degradation experiment

(1) Naphthalene HES NPA-5 degradation experiment

From the Fig.4, the strain NPA-5 degradation rate was higher than 50% with the naphthalene mass concentration below 1200mg/L. From the biodegradation experiment, the MSM changed from clear to turbid, as time lapsing, which changed from pale yellow to light pink. The naphthalene mass concentration 2000mg/L was limiting degradation mass concentration of the strain NPA-5.

Table 3. The changs in the MSM with different naphthalene concentration

Concentration	1st Day	2nd Day	3rd Day	Concentration	1st Day	2nd Day	3rd Day
200	++	++	++	1000	-	+	++
400	++	++	++	1200	-	++	++
600	+	++	++	1500	-	-	+
800	-	+	++	2000	-	-	-

++: MSM turbidity changed significantly, +:MSM turbidity changed obviously, -: MSM turbidity changed not obviously.

(2) Isoquinoline HES K-4 degradation experiment

From the Fig.5, the strain K-4 degradation rate was higher than 50% with the isoquinoline mass concentration below 1000mg/L. From the experiment, the isoquinoline culture medium changed from clear to turbid, as time lapsing, the floc bacteria group was found in the

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chipped heels. The isoquinoline mass concentration 1500mg/L was the strain K-4 limiting degradation mass concentration.

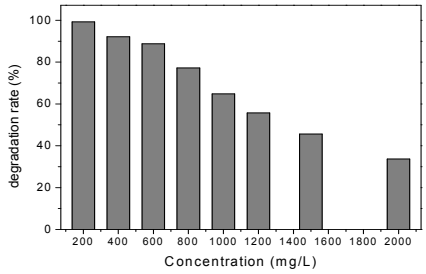

Fig.4. The strain NPA-5 biodegradation rate with different naphthalene concentration

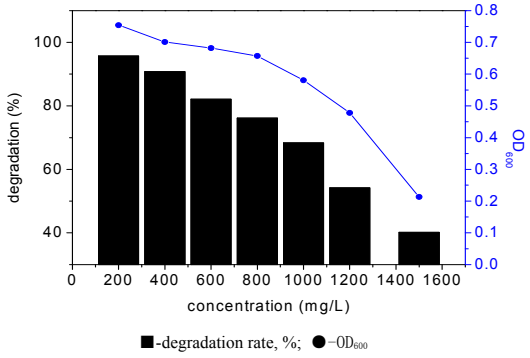

Fig.5. The strain K-4 biodegradation rate with different isoquinoline concentration

From the HES degradation experiment, the strains NPA-5 and K-4 could live in high naphthalene or isoquinoline mass concentration and possessed diographic capability to degradate naphthalene or isoquinoline.

3.3 Static treatment coking wastewater with NPA-5 BAC

(1) Processing time affect on COD removal rate

From the Fig.6, at 4h, the three kinds of treatment treatments were unanimous. The removal rate of COD rapidly increased because of activated carbon adsorption. After 4h, the effect of GAC on removal rate of COD was not increased; the ASB and BAC on removal rate of COD velocity of increase also slowly increased. The bacterium on the activated carbon gradually played a role of biodegrading the refractory organics adsorbed and not adsorbed substance. Comparing to GAC and ASB, the BAC on average removal rate of COD was increased by 13.4% and 9.2%.

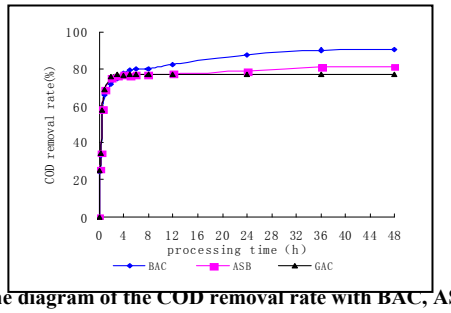

Fig.6. The diagram of the COD removal rate with BAC, ASB and GAC under processing time

(2) Process times affect on COD removal rate

From the Fig.7, the GAC treatment effect declined evidently in pace with transactions increased and the removal rate was less than 20%. The ASB and BAC still had superior removal rate of 40% and 65% respectively. It was reason that the bacterium on activited carbon biodegradated the adsorbed organics and the biological activited carbon was regenerated. Comparing with ASB, the BAC on removal of COD was increased by 25% because the HES exclusive pertinence biodegradation was superior to activated sludge and could biodegradated organics in coking wastewater more effectively. After reduplicative processed for 12 times, the average removal rate of COD by GAC, ASB and BAC were 78.08%, 61.32% and 23.20% respectively. Comparing to GAC and ASB, the BAC on removal of COD was increased by 54.88% and 16.36%.

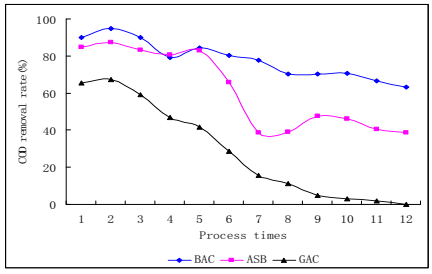

Fig.7. The diagram of the COD average remval rate with BAC, ASB and GAC under processing time

The times of treatment coking wastewater by BAC, ASB and GAC achieved the national industrial water effluent standard were as below (Table 4).

Table 4. Reach the effluent standard with BAC, ASB and GAC

	BAC	ASB	GAC
COD achieved standard times	10	5	3

According to the result of static NPA-5 BAC treating coking wastewater experiment, the HES NPA-5 played the effective role of reducing PAHs in the coking wastewater.

3. Conclusions

After enrichment, isolation and purification, the naphthalene HES NPA-5 and isoquinoline HES K-4 were isolated and screened from the long-term polluted soil in the coking plant and the activated sludge in the coking wastewater treatment plant. The maximal degradation mass concentration of strain NPA-5 was 2000mg/L and the maximal degradation mass concentration of strain K-4 was 1500mg/L. The HES degradation experiment results showed that two strains could live in high naphthalene or isoquinoline mass concentration and possessed idiographic capability to degradate naphthalene or isoquinoline.

According to static treatment coking wastewater with NPA-5 BAC experiment results, the average removal rate of COD by NAP-5 BAC was 13.4% higher than the method of GAC and 9.2% higher than that of ASB. After reduplicative treatments for 12 times, the average removal rate of COD by BAC was 54.88% higher than GAC and 16.36% higher than ASB.

The admixture of the naphthalene and isoquinoline HES were not made BAC to treat the coking wastewater which would be studied in the future experiment.

References

[1] Zhang M, Tay J H, Qian Y, et al. Coke plant wastewater treatment by fixed biofilm system for COD and NH_3-N removal[J]. *Water Res*, 1998, 32(2), 519-527.

[2] Jim M, Lu G. Methods for improving biochemical treatment of coke plant wastewater[J]. Shanghai Environmental Sciences, 2002, 16 (2), 13-14.

[3] Mueller J G, Cerniglia C E, Pritchard P H. Bioremidiation of environments contaminated by polyeyclic aromatic hydrocarbons [M]//Crawford R L, Crawford D L. Bioremediation Principles and Applications. Cambridge, Cambridge University Press, 1996.

[4] Johnsena AR, Wickb L Y, Harmsb H. Principles of microbial PAH-degradation in soil[J]. *Environmental Pollution*, 2005, 133,7l-84.

[5] Yaohui Bai, Qinghua Sun, Cui Zhao, et al. Bioaugmentation treatment for coking wastewater containing pyridine and quinoline in a sequencing batch reactor[J]. Microbiol Biotechnol, 2010, 87, 1943-1951.

[6] Edward J O'Loughlin, Staci R Kehrmeyer, Gerald K Sims. Isolation, characterization, and substrate utilization of a quinoline-degrading bacterium[J]. *International Biodeterioration & Biodegradation,*1996,38(2):107-118.

[7] Sambrook J, Russell D Molecular cloning: a laboratory manual, 3rd edn[M]. NewYork, Cold Spring Harbor Laboratory Press, 2001.

[8] Vazquez G, Cancela MA, Riverol C. Application of danckwerts method in a bubble column: effect of surfactants on mass transfer coefficient and interfacial area[J]. *Chemical Engineering Journal*, 2000, 78:13-19.

[9] Fang Miaomiao, Zhang Xiuxia, Wang Xin, et al. Screening of strain Q5 and its degradation ability for quinoline from oil contaminated soil[J]. ACTA Petrole Sinica(Petroleum Processing Section), 2007, 23(5),111-116.

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