Study on Degradation Kinetics of Co-metabolic Biodegradation of Linear Alkylbenzene Sulfonate Strains

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ABSTRACT. The degradation kinetics of co-metabolic biodegradation of two linear alkylbenzene sulfonate (LAS) strains was researched. Two bacterial strains which could degrade linear alkylbenzene sulfonate by co-metabolism mechanism, named as L-2 and L-15 were determined to Klebsiella sp.and Enterobacter sp.The optimal degradation conditions of L-2 and L-15 were investigated by the orthogonal experiment.The results suggested that the degradation rate of LAS (50mg/L) by L-2 was up to 94.2% when glucose was chosen as growth substrate under the conditions as : 30˚C, pH 7.5, glucose concentration 500mg/L,while the degradation rate of LAS (50mg/L) by L-15 was up to 92.2% under the conditions as: 30˚C, pH 7.5, glucose concentration 1000mg/L.The degradation reactions were consistent with characteristics of first-order kinetics when the concentration of LAS ranged from 25mg/L to 100mg/L. Certain theoretical basis of the treatment of LAS wastewater was provided by this study.

Introduction

Linear alkylbenzene sulfonate (LAS) is an anionic surfactant which is the main ingredient of synthetic detergent,has been widely used for domestic and industrial purposes[1-2].However,LAS may has a negative impact on the environment during its life-cycle.There remains 20%~50% LAS after a long period of degradation,through it is biodegradated easily under aerobic conditions[3]. The LAS in wastewater can affect the activity of the enzyme, dissolve the biological membrane of microorganisms and invertebrates,damage the structure of the protein and endanger the health of animals and plants[4-7]. Therefore, the method of removing LAS in wastewater high efficiently has become a hot research topic. The methods of treating LAS mainly include physical separation, catalytic oxidation and biological treatment at present. The biological treatment method, which has the advantages of large scale, simple equipment, low cost and wide application, is the most studied method.

Two bacterial strains which could degrade linear alkylbenzene sulfonate by co-metabolism mechanism, named as L-2 and L-15 were isolated from sewage treatment plant and determined to Klebsiella sp.and Enterobacter sp.before this experiment.Degradations of linear alkylbenzene sulfonate by L-2 and L-15 were optimized by varying the culture temperature,pH,the volume of culture fluid and the glucose concentration. The degradation kinetics was analyzed while LAS of different concentrations were degraded by L-2 and L-15.

Materials and methods

Medium

The component of the Medium (g/L): $MgSO_4 \cdot 7H_2O$ 0.14,Ka_2HPO_4 0.43 ,KaCl 0.06,$FeSO_4 \cdot 7H_2O$ 0.005,NH_4Cl 0.75,LAS 0.05.

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Experimental instrument

Full temperature oscillator(HZQ-FX,Harbin Donglian Electronic Technology Co. Ltd.), Autoclave (HVE-50, Hirayama, Japan), Upright fluorescence microscope (BX51, Olympus, Japan), Microcentrifuge(MIKRO 200R, Hettich, Germany), Fluorescent spectrophotometer (F-7000, Hitachi, Japan), Electronic balance (BS-124S,Sartorius,Germany)

Methods

LAS was determined using a fluorescent spectrophotometer.The medium was centrifuged at 8000r/min by 10 minutes after it was put into the centrifuge tube.Then,1.0 ml supernatant was removed into 25 ml colorimetric tube. Taking 5 ml of $NH_3 \cdot H_2O$-NH_4Cl buffer solution (pH=10.6) into the 25 ml colorimetric tube mentioned above.The fluorescence intensity F was measured at the excitation wavelength of 230 nm, the emission wavelength of 290 nm.The concentration of LAS was obtained from the standard curve according to the fluorescence intensity F measured.

In this experiment,the two bacteria strains were inoculated in the culture medium. Degradations were optimized by varying the culture temperature,pH,the volume of culture fluid and the glucose concentration. LAS concentrations were detected, and the degradation rate of LAS was calculated after the medium in 250ml conical flask was cultured for 5 days in full temperature oscillator.Under the optimal degradation conditions, the degradation rate of LAS by L-2 and L-15 was fitted by Monod equation.

Results

The orthogonal experiment

This orthogonal experiment contains the factors of the culture temperature(A),pH(B),the volume of medium (C) and the glucose concentration(D). Table 1 reflects the level of each factor . Orthogonal experiment analysis table 2 and 3 reflect the degradation rate of LAS in the different levels of different factors after cultured for 120 hours.

Table 1.The different factors and levels in orthogonal experiment

Level	Temperature（A）/°C	pH/（B）	Volume of medium (C)/ml	Glucose concentration(D)($g \cdot L^{-1}$)
1	30	6.5	50	100
2	20	8.5	100	500
3	40	7.5	150	1000

Table 2.The degradation analysis of L-2 in the orthogonal experiment

Number	A	B	C	D	L-2 degradation/%
1	1	1	1	1	34.9
2	1	2	2	2	57.6
3	1	3	3	3	69.3
4	2	1	2	3	25.1
5	2	2	3	1	16.1
6	2	3	1	2	68.3
7	3	1	3	2	16.6
8	3	2	1	3	42.9
9	3	3	2	1	11.9
K_1	161.8	76.6	146.1	62.9	
K_2	109.5	116.6	94.6	142.5	
K_3	71.4	149.5	102	137.3	
k_1	53.9	25.5	48.7	21.0	
k_2	36.5	38.9	31.5	47.5	
k_3	23.8	49.8	34	45.8	
Range	30.1	24.4	17.2	26.5	
Order			A>D>B>C		
Optimal level	30	7.5	50	500	

Table 3.The degradation analysis of L-15 in the orthogonal experiment

Number	A	B	C	D	L-2 degradation/%
1	1	1	1	1	34.9
2	1	2	2	2	57.6
3	1	3	3	3	69.3
4	2	1	2	3	25.1
5	2	2	3	1	16.1
6	2	3	1	2	68.3
7	3	1	3	2	16.6
8	3	2	1	3	42.9
9	3	3	2	1	11.9
K_1	199.5	72.8	123.9	57.3	
K_2	85.8	119.5	110.5	136.8	
K_3	58.1	151.1	109	149.3	
k_1	66.5	24.3	41.3	19.1	
k_2	28.6	39.8	36.8	45.6	
k_3	19.4	50.4	36.3	49.7	
Range	47.1	26.1	5	30.6	
Order			A>D>B>C		
Optimal level	30	7.5	50	1000	

For the factor A,the experiments 1,2 and 3 reflect the influence of A_1 on the degradation,while 4,5 and 6 reflect the influence of A_2,7,8 and 9 reflect the influence of A_3.The influence on the degradation rate of A_1 ,A_2, A_3 can be judged by the value of k_{A1}、k_{A2}、k_{A3}. A_1 is the optimal level of A factor since $k_{A1}>k_{A2}>k_{A3}$. According to the same method, B_3,C_1 and D_2 are the optimal level of B,C and D.

Kinetics

The degradation of LAS wastewater with initial concentration of 25,50,100mg/L was studied. The results are shown in Fig.1.

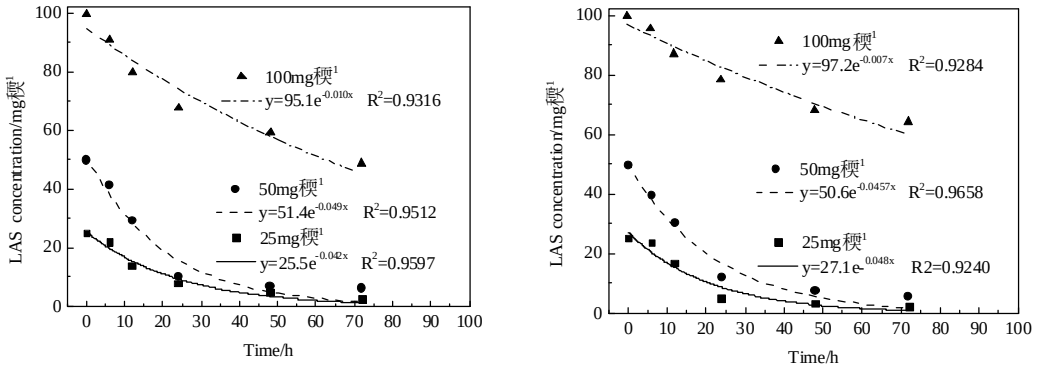

Fig.1 The degradation of different initial LAS concentrations under the optimal condition by L-2 and L-15

As shown in the Fig.1, the concentration of LAS decreased exponentially.The concentration of LAS decreased rapidly at the initial stage, and then slowly. The fitting curves accorded with first order reaction kinetics equation.The general reaction process can be expressed as the following:

$$V = V_m C/(k + C) \quad (1)$$

Where:

V=Reaction rate, t^{-1}

V_m=The maximum reaction rate, t^{-1}

C= Concentration of substrate, mg/L

k=The half-saturation constant of reaction rate

When the degradation reaction follows the first order kinetic equation and C<<k, Eq. (1) can be expressed as:

$$V = V_m C/k \quad (2)$$

$k_0=V_m/k$, The relationship between concentration of substrate and time is the following：

$$C = C_0 e^{-k_0 t} \quad (3)$$

Table 4.The degradation kinetic equation of L-2

LAS concentration（mg/L）	Degradation kinetic equation	$k_0(h^{-1})$	R^2
25	$C=25.5e^{-0.042t}$	0.042	0.9597
50	$C=51.4e^{-0.049t}$	0.049	0.9512
100	$C=95.1e^{-0.010t}$	0.010	0.9316

Table 5.The degradation kinetic equation of L-15

LAS concentration（mg/L）	Degradation kinetic equation	$k_0(h^{-1})$	R^2
25	$C=27.1e^{-0.048t}$	0.048	0.9240
50	$C=50.6e^{-0.046t}$	0.046	0.9658
100	$C=97.2e^{-0.007t}$	0.007	0.9284

The degradation kinetic equation of LAS in different sets of experiment is compared in Table 3. and Table 4. Under different initial LAS concentrations conditions, the actual value of LAS degradation rate is in good agreement with the theoretical value. The fitting correlation coefficient squared R2 were all above 0.92. With the increase of the initial concentration of LAS, the constant of reaction rate increased first and then decreased. The growth and reproduction of the bacterias were inhibited, and the metabolic activity slowed down because of the toxicity of LAS when the initial concentration was higher than 50mg/L.Therefore,the degradation rate of LAS declined,and this belongs to the typical inhibition kinetics.

Conclusions

This prelimunary study indicated that under the conditions of 30˚C, pH 7.5, the degradation rate of LAS (50mg/L) by L-2 was up to 94.2% when glucose which concentration was 500mg/L was chosen as growth substrate,and the degradation rate by L-15 was 92.2% when the concentration of glucose was 1000mg/L. The reactions were in accordance with the first order kinetic equation when the initial concentration of LAS was less than 100mg/L.

Acknowledgements

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REFERENCES

[1] Nomura Y, Ikebukuro K, Yokoyama K, *et al*. Application of a linear alkylbenzene sulfonate biosensor to river water monitoring[J]. Biosensors and Bioelectronics, 1998, **13**(9): 1047-1053.

[2] Schleheck D, Lechner M, Schönenberger R, *et al*. Desulfonation and degradation of the disulfodiphenylethercarboxylates from linear alkyldiphenyletherdisulfonate surfactants[J]. Applied and environmental microbiology, 2003, **69**(2): 938-944.

[3] Mungray A K, Kumar P. Fate of linear alkylbenzene sulfonates in the environment: a review[J]. International Biodeterioration & Biodegradation, 2009, **63**(8): 981-987.

[4] Jensen J. Fate and effects of linear alkylbenzene sulphonates (LAS) in the terrestrial environment[J]. Science of the total environment, 1999, **226**(2): 93-111.

[5] Bragadin M, Perin G, Raccanelli S, *et al*. The accumulation in lysosomes of the anionic detergent linear alkylbenzene sulfonate[J]. Environmental toxicology and chemistry, 1996, **15**(10): 1749-1751.

[6] Cserháti T, Forgács E, Oros G. Biological activity and environmental impact of anionic surfactants[J]. Environment international, 2002, **28**(5): 337-348.

[7] Duarte I C S, Oliveira L L, Saavedra N K, *et al*. Treatment of linear alkylbenzene sulfonate in a horizontal anaerobic immobilized biomass reactor[J]. Bioresource technology, 2010, **101**(2): 606-612.