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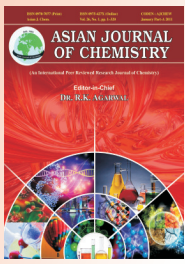

Degradation Properties of Melamine Wastewater with *Streptomyces lincolnensis*

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This study focused on three experiments about utilization of ammonia-nitrogen from melamine wastewater to produce lincomycin by *Streptomyces lincolnensis* screened from melamine wastewater. All three experiments based on mixing the melamine wastewater and the cultural medium in volume ratio 1:10 in shaking flasks. The results showed that during the first experiment fermentation process, there was no differences in biosynthetic activity of the mycelium between the screened and the control groups. The reductive sugar was rapidly utilized in fermentation anaphase with the concentration of 0.23 mg/L. The starting potency of lincomycin was 2030 IU/mL in the lowest stage and reduced by 70 IU/mL compared to the control. In the second experiment, pH value was 5.96-6.98, which was comparatively lower. The speed of metabolism of amino nitrogen was slow and it was 40 mg/100 mL. The potency of lincomycin was 4550 IU/mL in the lowest stage. The third experiment proved that it was effective to promote mycelia secretion by feeding melamine wastewater medium at mid-late stage of fermentation. The potency of lincomycin was 5040 IU/mL in the end, which was 5.88 % higher than the control group. Therefore, the study of recycling utilization for melamine wastewater in the third experiment is quite necessary and significant.

Keywords: *Streptomyces lincolnensis*, Melamine wastewater, Carbamide, Biodegradation.

INTRODUCTION

Melamine from Jinhai industrial park of Suzhou city is obtained by gasification of urea, polymerization onto silica gel and washing. If plenty of ammonia-nitrogen wastewater with high concentration is directly discharged, may significantly harm the environment. Because of these superstandard wastes can be taken as nutrient sources for growth and reproduction of microorganisms[1,2], if transform NH_4^+ to available nitrogen source for microorganisms, can promote growth and reproduction of cells, these provide important pathways for solving high cost of carbon source and nitrogen source production by antibiotics.

Streptomyces lincolnensis is one of an important kind of streptomyces, some researches degraded benzpyrole and diuron wastewater with streptomyces[3,4]. *Streptomyces lincolnensis* can produce lincomycin with ammonium sulfate, lincomycin belongs to aminoglycoside antibiotics, due to broad spectrum and high efficiency of lincomycin, it has better market prospect. NH_4^+ can adjust biosynthesis of lincomycin[5,6], sulfate with higher concentration can stimulate growth and reproduction of microorganisms, can also promote biosynthesis of many antibiotics[7]. Production of lincomycin needs large amount of ammonium sulfate as the nitrogen source for cell growth[8,9].

Production of lincomycin by using ammonium salt in melamine wastewater is an attempt of waste recovery, can not only reduce emission load of melamine producing wastewater, can also promote production of lincomycin, thus can reduce input quantity of ammonium sulfate during fermentation process, so decrease the cost. Currently, there is no report about the related study, is a reciprocal behaviour for energy saving and environment protection. Due to big individual difference of the stains, screening of suitable melamine wastewater and optimization of fermentation condition can adequately utilize useful nutrient sources in wastes, can improve production of antibiotics by the stains. This is a key technique for microbial fermentation. This study provides exploration of feasibility for cyclic utilization, energy saving and environment protection, has practical significance of turn waste into wealth, so as to provide new pathways for treatment of melamine wastewater.

EXPERIMENTAL

Seed culture medium (g/L): Corn starch 1.9, glucose 2.5, soybean cake powder 2.3, corn steep liquor 2.6, $(NH_4)_2SO_4$ 0.2, NH_4NO_3 0.14, NaCl 0.07, $NaNO_3$ 0.086, KH_2PO_4 0.005, $CaCO_3$ 0.68. (Corn steep liquor was purchased from Kangxin Co., Ltd. of North China Pharmaceutical group, its batch No: 20100728).

Fermentation medium (g/L): Corn starch 1.5, starch inverted sugar 3.5, soybean cake powder 2.5, corn steep liquor 1.1, $(NH_4)_2SO_4$ 0.2, NH_4NO_3 0.14, NaCl 0.52, $NaNO_3$ 0.6, KH_2PO_4 0.025, $CaCO_3$ 0.2.

Separation and screening of strains: The wastewater was sampled from melamine workshop of Suzhou Zhongyuan chemical Co., Ltd in Aug, 2012, detection results showed that: the sample contained 256 mg/L of NH_2-N, the pH was 9.5. Concentrated 20 mL of the wastewater into 10 mL and added it into peptone isolation medium of *Streptomyces lincolnensis* for sterilization, under aseptic condition, poured into a petri dish for solidification while hot. After cooling, drew aseptic washed lincomycin mycelial solution with a straw, inoculated the stains in the medium in the petri dish with streak plate method, cultured for 7 days at 30 °C, selected big, plump and undefiled bacterial colonies, kept it in a 4 °C freezer for standby application.

Preparation of the stains: Under sterile operation condition, selected mycelia in the screened bacterial colonies, transferred to the bevel of a eggplant type flask with streak plate method, screened 8-10 plump and undefiled bacterial colonies by a inoculating shovel, then transferred into a sterile seed medium (liquid amount 30 mL/250 mL), at 30 °C, cultured for 3 days on a 150 rpm shaking bed, kept in a 4 °C freezer for standby application.

Design of fermentation test: Inoculated the stains in a shake flask for fermentation (50 mL/500 mL), the inoculums size was 10 % of the shake flask, cultured for 168 h on a 150 rpm shaking bed at 30 °C, sampled a bottle of the fermentation broth for detecting all metabolism parameters of fermentation. The experiment are performed for 3 times, test results are three times' average values while the experiment was designed as 3 groups.

(1) 5 mL of the wastewater was added into the mother medium for seed culture, 5 mL of the wastewater was added into the fermentation broth for fermentation cultivation.

(2) Directly inoculated the seeds into the fermentation medium, add 5 mL of the wastewater into the medium for fermentation cultivation.

(3) When the seeds were directly added into the fermentation medium, cultured for 48 h, took down from the shaking bed and took into buffer preparation room of sterile room, disinfected ektexine of the shaking flask with 75 % alcohol wipes, on the ultra-clean working table of sterile room, unfastened multilayer gauze, added 5 mL of the wastewater (sterilized for 0.5 h at 0.1 Mpa and 120 °C) with sterilized sucker, tied up the bottleneck again for fermentation cultivation.

(4) For control group cultured the seeds in a normal seed medium, inoculated in a normal fermentation medium for fermentation cultivation.

Data handling

Biomass detection: Took 10 mL of the fermentation broth into a centrifuge tube and centrifuged at the rate of 3500 rpm, poured out the supernatant liquor and readed the precipitation number; content test of reducing sugar (RS), Fehling titration[10]; content test of amino nitrogen (NH_2-N), formaldehyde oxidation method[11], lincomycin titer detection, WZZ-2A automatic polarimeter (Shanghai physical optics instrument plant), reading of the polarimeter × 7000[12], treated and detected by Wanbei pharmaceutical group.

RESULTS AND DISCUSSION

Effect of different adding time of melamine wastewater on mycelial concentration: Three tests in Fig. 1 showed that adding time and adding quantity of melamine wastewater to mother flask and fermentation medium affected mycelial growth in a certain degree, in test 1, after addition of melamine wastewater, mycelia grew adaptively, when transferred to the fermentation medium, mycelia was well adapted to the medium, the concentration of the medium was 20 %, so the fermentation process was similar to that of the control group. In test 2, mycelia in mother flask grew well, but after transferred to the fermentation broth with melamine wastewater, mycelia was not adapted to the growing environment, the concentration of the medium was 16 %; when fermentation time was within 96 h, the minimum mycelial concentration was still 32 %, the concentration was still low until entry into the flask. Test 3 showed that: after normal growth of the mycelia, they were inoculated to the fermentation medium for fermental cultivation, after 48 h, added melamine wastewater, mycelia were well adapted to the fermentation environment, the maximum concentration was 25 %, in the earlier stage of the fermentation process within 96 h, mycelial concentration of test 3 was 54 %, far above that of the control group 40 %.

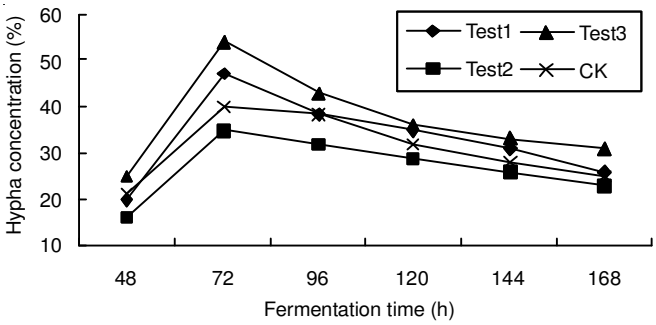

Fig. 1. Effect of fermentation time on mycelial concentration

Effect of different adding time of melamine wastewater on pH of fermentation broth of lincomycin: Variation of pH of the fermentation broth was a combined effect of all kinds of metabolic activities of *Streptomyces lincolnensis*, variation of pH can not only reflect nutriture of *Streptomyces lincolnensis*, can also indirectly reflect its physiological activity, lincomycin producing ability, so pH is a key parameter for fermentation process of lincomycin[8,9].

Three curves of the three tests in Fig. 2 showed that before 96 h, compared to the control group, pH increased gently. Organic acids generated from mycelial growth secreted out of the cells, NH_4^+ was fast applied to produce R-NH_3^+, generated H^+, CO_2 in the fermentation broth, partly soluble in the fermentation broth, all of the factors above leaded to low pH of the fermentation broth[13]. Fig. 2 showed that after 120 h, pH of all the tests changed obviously. In this stage, increase rate of pH of test 3 was the fastest, far above that of the control group.

This manifestation was related to the adding time of melamine wastewater in fermentation cultivation, also due to H^+ produced by mycelium in the middle stage was buffered

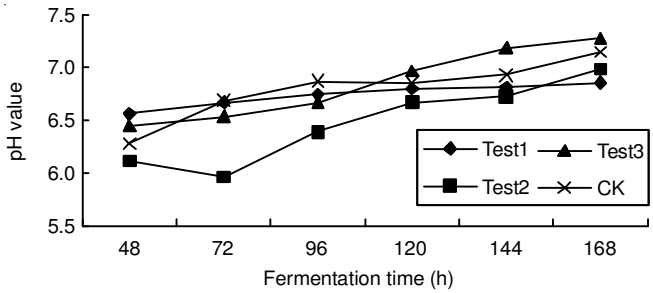

Fig. 2. Effect of fermentation time on pH of the fermentation broth

by physiologically alkaline salts (*e.g.*, sodium nitrate and calcium carbonate[8,13]. pH increased gradually and vigorous mycelial growth was over, consumption and metabolism of sugar and ammonia nitrogen were slow, production of organic acids was less than secreting quantity of antibiotics, pH increased continuously.

Effect of different adding time of melamine wastewater on fermentation of reducing sugar by lincomycin: Fig. 3 showed that compared to the control group, reducing sugar metabolism of *Streptomyces lincolnensis* screened in test 1 was normal, among the 72 h, metabolic exhaustion of reducing sugar in the earlier 24 h was the fastest (0.14 mg/L). The stains in test 2 were not domesticated by melamine wastewater, when inoculated to the fermentation broth with melamine wastewater, in the earlier 48 h, metabolism of reducing sugar was the slowest, consumed 0.04 mg/L, in the later 24 h, mycelia were well adapted to the environment, metabolism of reducing sugar was the fastest, consumed 0.16 mg/L of reducing sugar.

But in the later stage, reducing sugar was almost unconsumed, it was always maintained at 0.40 mg/L or so, mycelial metabolism was abnormal, microscope detection showed that most of the mycelia were dissolved. New mycelia decreased gradually. Compared to the control group, metabolism of reducing sugar at 72 h in test 3 was slower, probably due to addition of melamine wastewater at 48 h affected mycelial growth environment. After 72 h metabolism of reducing sugar was similar to that of the control group. It also indicated that screened and domesticated lincomycin stains which adapted to melamine wastewater had its function, they are adapted to the fermentation environment in test 3, tendency of the whole fermentation process was superior to that of the control group.

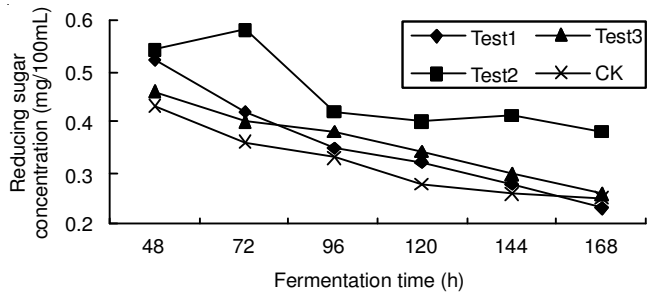

Fig. 3. Effect of fermentation time on concentration of reducing sugar

Effect of different adding time of melamine wastewater on amino nitrogen utilization in lincomycin fermentation process: Amino nitrogen is the most important nitrogen source for microculture, protein synthesis of mycelia originates in it. Content of amino nitrogen in the fermentation broth determines growth and metabolism rate of the mycelia. Fig. 4 showed that in test 1, the stains grew in mother flask with melamine wastewater, then transferred to the fermentation medium containing melamine wastewater, utilization rate of amino nitrogen by mycelia was stable, when entry into the flask, the content of amino nitrogen was 23 mg/100 mL.

In test 2, when normal cultured seeds were transferred to the fermentation medium with melamine wastewater, after 72 h, content of amino nitrogen in the fermentation broth was 50 mg/100 mL, it was 12 mg/100 mL higher than that of the control group, amino nitrogen metabolism in the later stage was slower than that of the control group, mycelia are not adapted to the medium. In test 3, when normal cultured seeds were transferred to the fermentation medium, after 48 h, concentration of amino nitrogen in normal fermentation broth was 41 mg/100 mL, when melamine wastewater was added, concentration of amino nitrogen was up to 48 mg/100 mL, the content of amino nitrogen was the highest in detections at the same stage, due to mycelial metabolism in the earlier stage of the fermentation process was similar to that of the control group, mycelia had good adaptation, concentration of amino nitrogen in the later stage of the fermentation process was down to 36 mg/100 mL. It was not affected by addition of melamine wastewater.

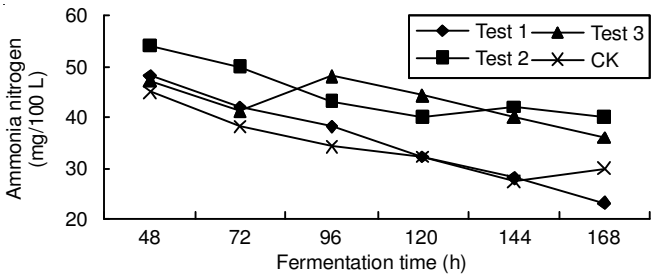

Fig. 4. Effect of fermentation time on content of amino nitrogen

Effect of different adding time of melamine wastewater on biosynthesis of lincomycin: Results of 3 tests were shown in Fig. 5, in test 1, the starting titer was 2100 IU/mL, the final fermentation titer was 4690 IU/mL, slope of the titer curve showed that in the later stage, the fermentation titer at 144 h increased 420 IU/Ml, when the fermentation time was prolonged,titer's improvement had staying power. In test 2, from the 96 h to the 120 h, titer increased 2100 IU/mL sharply, microscopic examination showed that in the later stage of the fermentation process, mycelia aged sharply, production of antibiotics had no staying power, the final fermentation titer was 280 IU/mL less than that of the control group. In test 3, synthesis of lincomycin was stable, the fermentation titer was up to 5040 IU/mL. It was 5.88 % higher than that of the control group. These showed that when the wastewater was added in the later stage of the fermentation process, secretion of lincomycin was promoted, microscopic examination showed that big vacuoles were contained in the mycelia, autolysis was slow, compared to the control group, when the wastewater was added to the medium, ammonium sulfate in the medium

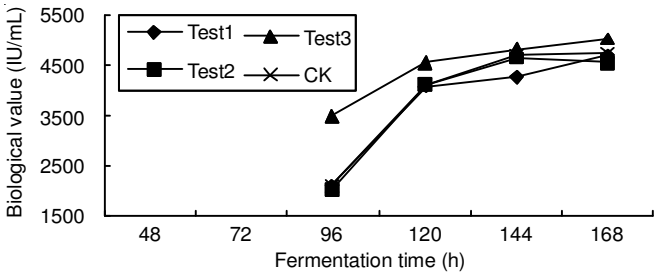

Fig. 5. Effect of fermentation time on the biological titer

simulated metabolism of mycelia, thus production ability of antibiotics by the mycelia was improved.

Conclusions

(1) Melamine wastewater can inhibit primary growth of lincomycin mycelia, the mycelia cannot grow well, pH of the fermentation broth was not stable, metabolism of reducing sugar and amino nitrogen was slow, the minimum starting titer was 2030 IU/mL.

(2) Lincomycin seeds were cultured normally, then transferred to the fermentation medium, when cultured for 48 h, added melamine wastewater for fermentation, results showed that compared to test 1 and test 2 as well as in test 3, metabolism of carbon and nitrogen was faster, pH was stably controlled, the fermentation titer was 5040 IU/mL, it was 350 IU/mL higher than the control group.

(3) Addition of melamine wastewater in mother flask for cultivation and domestication of *Streptomyces lincolnensis* is important, when transferred to the fermentation medium for 48h, addition of melamine wastewater can improve production of lincomycin (increase by 5.88 %). By optimization of technology conditions, amino nitrogen content in the control group and test 3 was detected. The results showed that ammonium sulfate concentration in the medium can be reduced by 20 %, so it is available for cyclic utilization.

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