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著作権は学会に帰属する
Fungal Decolorization of Microbially Treated Night Soils

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Abstract

A fungal strain LM-12 was selected for the decolorization of microbially treated night soils {filtrate(iv) in Fig. 1} and it was identified as Penicillium janthinellum. Strain LM-12 decolorized 50% of the color of the medium with 5.0 g/l glucose and 0.6 g/l ammonium sulfate at 30°C for 24 hr. Over 50% decolorization was not attained in any culture conditions tested, but the increase of the mycelial amount enhanced the decolorization rate. So by using repeated replacement culture, the decolorization period was reduced to 2.0 hr. The amount of nutrient addition could be reduced in further replacement culture.

Key words: decolorization of night soils, night soil decolorization, Penicillium janthinellum, repeated replacement culture

INTRODUCTION

Recently night soil treatment in villages is carried out in a compact night soil treatment plant. Figure 1 shows a plant in Kamiukena, Matsuyama, and it is composed of following steps: (i) removal of solids such as sands, metals, and plastics by sedimentation and screening; (ii) treatment by aerobic microbes to remove BOD, COD, nitrogenous compounds, and SS (suspended solid); (iii) microbial denitrification; (iv) membrane filtration to remove microorganisms and SS; (v) addition of chemicals and alkali to promote aggregation of phosphates and colored materials; (vi) membrane filtration to remove aggregates; (vii) decolorization with activated charcoal; (viii) disinfection with hypochlorite addition.

By the operation of this plant some color remains and so the effluent from this system is not discharged without adsorption on active charcoal.

In this study we tried to decolorize the microbially treated night soil {filtrate (iv) in Fig. 1} for the cancellation or reduction of the use of activated charcoal. We found a fungal strain to decolorize it to half and optimized the reaction period to 2 h by the repeated replacement culture.

MATERIALS AND METHODS

Solution for decolorization The filtrate in step (vi) in Fig. 1 is used for the decolorization to save the amount of active charcoal. The solution after step (viii) can be used, but color of the solution is very faint (less than A<sub>∞</sub> 0.2), which is not easy to distinguish the color removal by naked eyes in screening of microorganisms. Also after decolorization the solution is expected to return to step (iii) in Fig. 1. The solution at step (ii) or (iii) can be used, but the cleared solution is better because most of microorganisms is removed and we can keep it long during experiments.

The filtrate in step (iv) is obtained from Environmental Sanitation Center, Kami-
RESULTS AND DISCUSSION

Absorption spectrum of the culture filtrate  The absorption spectrum (320 to 700 nm) of the medium gave a monotonous increasing curve toward shorter wave length (Fig. 2A), even before and after the microbial decolorization, which meant the absorption was composed of many colored materials. The absorption of the medium before and after the decolorization at 400 nm was not affected much by pH 2 to 8 (Fig. 2B), though a little increased in alkaline side. In the experiments we used the pH range between 2 and 6, where the absorption did not depend on pH, and so the color is measured without adjusting pH of the supernatant of medium.

Fig. 2  A Absorption spectra of the medium supernatant containing microbially treated night soil before and after the microbial decolorization. B Effect of pH on the absorption at 400 nm before and after the decolorization of the medium.
Screening of microorganisms for the decolorization One loop from 300 soil samples was inoculated into a test tube containing 5 ml of the medium, and it was incubated on a reciprocal shaker at 30°C. After one week, the decolorization was judged by naked eyes, and one loop of which was seeded into the fresh medium. It was cultivated for 5 days, and 25 decolorized samples were seeded into 100-ml conical flasks containing 20 ml of fresh medium together with 15 stock cultures in our laboratory. They were incubated for 3 days on a rotary shaker and those absorbance at 400 nm of the supernatant were measured.

A culture to show the highest decolorization, strain LM12, was selected, which had been isolated as a lignin decomposer before. It is used in this study.

The decolorizing activity was not located extracellularly, because the culture filtrate supplemented with the fresh medium was not decolorized at all.

The photo of strain LM12 is shown in Fig. 3. Taxonomical identification of strain LM-12 was carried out by NCIMB Japan (Shimizu, Japan) and identified as Penicillium janthinellum.(1, 2).

The fundamental properties are as follows: hypha has cephal; conidiophore blanches irregularly; double-ring penicilli are composed of blanches, meturoe and fialides; ascus or screlotium is not formed; colony is whitly cotton fiber-like until conidiospore formation and turns to green, then dark green; the reverse side of agar plate is dark orange; spores is mainly globe, rough in surface, and about 3 μm in diameter; size of colony is about 6 cm in diameter on a potato-dextrose agar plate after 10-day growth. It was quite rare that P. janthinellum was isolated as a decolorizing fungus, because white rot fungi such as Phanerochaete chrysosporium (3), Geotrichum candidum (4), and Coriolus hirsutus (5) were usually found, as decolorizers of azo dyes, molasses, and melanoidine, respectively. It was maintained on malt-extract agar medium.

Optimal culture condition The good decolorization was observed at 30-37°C in 2-day culture as shown in Fig. 4, and 30°C was used in further experiment.

The best decolorization was observed at initial pH of 7.0 as shown in Fig. 5, but in further experiments the initial pH of 6.0 was selected because glucose in the medium gave colored material at pH 7.0 after sterilization.

The decolorizations were high at 2.5~10.0 g/l glucose concentrations in the medium, the fastest at 5.0 g/l glucose (Fig. 6), and this concentration was used in further experiments. The strain could not decolorize the medium without the addition of glucose.

The decolorizations were high at 0.6~2.4 g/l ammonium sulfate concentration (Fig. 7), the fastest at 0.6 g/l ammonium sulfate, and this concentration was used in further experiments.
experiments. Strain LM-12 could not de-
colorize without the addition of ammonium
sulfate.

The time course of the decolorization under
the optimal condition was shown in Fig. 8.
The growth became active after 12 h and
continued to grow until 24 h. The pH began
to decrease sharply after 12 h and reached to
pH 3.0 at 16 h. The decolorization also
started at 12 h, and after 24 h 50% of
decolorization was attained.

Decolorization degree After the incu-
bation for 24 h, 5.0 g/l glucose and 0.6 g/l
ammonium sulfate were added to the culture
and it was further incubated for more 24 h.
The cell mass increased, but the
decolorization did not proceed any more (data
were not shown).

The culture filtrate was recovered after 24
h, to which glucose and ammonium sulfate at
5.0 and 0.6 g/l, respectively, were added,
LM12 was inoculated, and it was incubated
for another 24 h. Strain LM-12 could grow
as well as before, but the decolorization did
not proceed any more (data were not shown).

To check the effect of amount of mycelia on
the decolorization, the mycelia were
harvested by filtration from one, two, or four
flasks of 24-h culture, and they were
suspended to fresh media to give different
amounts of mycelia (1-4 folds), which were
incubated for more 24 h, but the

![Fig. 5](image-url)  
**Fig. 5** Effect of the initial pH on the decolorization of
the medium by strain LM12 for 2 days.

![Fig. 6](image-url)  
**Fig. 6** Time courses of the decolorization of
the medium by the addition of different
concentration of glucose by strain LM12.

![Fig. 7](image-url)  
**Fig. 7** Time courses of the decolorization of
the medium by the addition of different
concentration of ammonium sulfate by strain
LM12.

![Fig. 8](image-url)  
**Fig. 8** The decolorization of the medium under the
optimal culture condition.
Symbols: △, color; ○, pH; ●, dry cell weight.
Fungal Decolorization of Microbially Treated Night Soils

Decolorization were about 50%, however, the decolorization periods were reduced to 3.5 h by the replacement culture (1 fold) from 24 h in the previous batch experiments. And also the increases of mycelial amount to 2 and 4 folds reduced the decolorization period to 2.0, and 1.5 h, respectively (Fig. 9). This meant that some part of colored components in the filtrate were resistant to further decolorization by strain LM-12. However, the maximum decolorization rates were 0.09, 0.15, and 0.26 ΔA_{400}/h, with 1, 2, and 4 folds of mycelial amount, respectively, and so decolorization periods were also reduced in the same order. These results meant that the higher amount of mycelia promoted the decolorization rate very much and we should use the higher mycelial amount in the decolorization.

**Decolorization by replacement culture**

So to obtain higher mycelial amount, the strain was precultured for 48 h with intermittent additions of nutrients (5.0 glucose and 0.6 g/l ammonium sulfate) at 24 and 36 h, respectively. The mycelia were harvested by filtration from the preculture, suspended in the fresh medium, and incubated for 2 h. The decolorization attained to a steady level, then the mycelia were harvested, used for the further rounds of replacement cultures at 2 h-interval as shown in Fig. 10. This experimental results showed that the replacement culture worked well for the reduction of decolorization period.

In the next experiment 24-h culture was used as a preculture without obtaining higher mycelial amount in the first round of replacement culture. So the culture was replaced when the decolorization attained to a steady level as shown in Fig. 11. The decolorization time in the first culture was 4.5 h, that in the second was 3.0 h, those in the third and fourth was 2.5 h, and those after the fifth were 2.0 h. This 2-h decolorization was attained for at least following 10 replacement cultures without using 48-h preculture. This reaction system would be very convenient for the scale-up, because one replacement culture takes only 2 h and replacement culture could be operated by natural sedimentation of mycelia, removal of supernatant, and addition of the fresh medium without filtration, and mixing of mycelia in the next decolorization process.

Though a constant amount of 5 g/l glucose and 0.6 g/l ammonium sulfate addition into the replacement culture was employed, but such much amounts of nutrient additions may not always be necessary and the amount of nutrient may be reduced in the replacement culture. So the replacement culture was carried out with addition of

![Fig. 9 Effect of cell mass in the replacement culture on the decolorization. The different amounts of mycelia were prepared by harvesting mycelia from different numbers of flasks precultured for 24 h. Symbols (relative cell mass): ○, 1 fold; ■, 2 folds; △, 4 folds.](image)

![Fig. 10 The replacement culture by using higher amounts of mycelia. The higher amounts of mycelia were prepared from the precultures, which was incubated for 48 h with twice additions of the nutrients.](image)
different amounts of glucose and ammonium sulfate. The standard nutrient concentration is composed of 5.0 g/l glucose and 0.6 g/l ammonium sulfate. The first replacement cultures with addition of 10 and 20% of the standard nutrient did not give sufficient decolorization, and that with 40% nutrient addition gave a little less decolorization than that with the standard nutrient addition as shown in Fig. 12. But the second replacement culture with 40% nutrient addition gave the same decolorization as that with the standard nutrient addition. So it may be possible to reduce the addition of nutrient more in prolonged replacement cultures.

CONCLUSION

We selected *P. janthinellum* LM12 as a decolorizer of microbially treated night soil (filtrate in step (iv) in Fig. 1) with addition of 5 g/l glucose and 0.6 g/l ammonium sulfate, which decolorized 50% of the filtrate within 2-day culture. We optimized culture conditions for the decolorization, where the reaction period was reduced to 2 h by replacement culture but more than 50% of the decolorization was not attained. In a prolonged replacement culture the amount of glucose and ammonium sulfate addition is suggested to be reduced.

REFERENCES


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