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ORIGINAL ARTICLE

Isolation and Degradation Optimization of Filamentous Fungi Capable of Degrading Hydrothermal Treated Sludge Leachate

Zhenyu Wang*, Yuan Chen, Lingxiao Xu, Yubin Zhang

College of Ecology, Lishui University, Lishui 323000

Abstract: Discussion on filamentous fungus separation and optimization of degradation conditions of sludge filtrate from silk-degrading hydrothermal treatment is of great significance to the efficient and safe treatment of sludge filtrate from biological enhanced hydrothermal treatment. In this study, filamentous fungi that can efficiently degrade hydrothermal treatment sludge filtrate were isolated and screened from surplus sludge, and their treatment conditions were optimized. The results show that filamentous fungus Mucor sp. LS-1 has a good degradation effect on hydrothermal treatment sludge filtrate, and the optimal inoculation concentration of sludge filtrate is 50% (V/V) original filtrate (~7000 mg/L), and the optimal inoculation concentration of fungus is 15% (V/V). Mucor sp. LS-1 Treatment of sludge filtrate under the optimum conditions can make filtrate COD decrease 64.65%, obviously improving the effluent quality of sludge filtrate. During the treatment of sludge filtrate by Mucor sp. LS-1, the degradation of COD in the filtrate is mainly related to the adsorption and biodegradation of filamentous fungi. Therefore, using Mucor sp. LS-1 to degrade hydrothermal treatment sludge filtrate is a new high-concentration sludge filtrate degradation technology with great potential.

Keywords: Environmental Engineering; Hydrothermal Treatment Sludge Filtrate; Filamentous Fungi; Filtrate COD

Introduction

By the end of 2017, December and December, our cities and counties have built a total of 5027 sewage treatment plants with sewage treatment capacity as high as 188 million cubic meters per day, producing 80% sludge with annual water content of 50 million tons and over 10,000 tons. It is expected that the municipal sludge output will reach 60 million tons, 90 million tons, 10,000 tons and 1,2 tons by 2020. There are a lot of organic matter, pathogenic bacteria, parasites, heavy metals and other substances in the sludge. If not disposed of safely, it will have a serious impact on the environment[2]. Therefore, how to reduce the volume of municipal sludge and eliminate the harm, 40 and finally realize recycling are major problems in sludge treatment.

Hydrothermal treatment technology is an effective sludge pretreatment technology. Sludge hydrothermal treatment technology refers to heating sludge to 130 C and above in a closed container and maintaining it at saturated vapor pressure for 30~90 min, which causes microbial flocs in sludge to decompose, cells to rupture, macromolecules to release and further decompose into micromolecules to release intracellular bound water, resulting in easier separation of water from sludge particles, thus greatly improving sludge dewatering performance[3-7]During the hydrothermal treatment of sludge, the release of intracellular harmful substances due to microbial cell rupture, as well as the dissolution and hydrolysis of solid organic matters, will significantly increase the content of dissolved organic matters in the sludge filtrate, and at the same time produce a large amount of toxic substances, such as phenol, furfural and derivatives thereof[8-11]. The dewatered filtrate produced after hydrothermal treatment of municipal sludge has high

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organic concentration and complex composition, which makes it difficult to treat'[12, 13].

Filamentous fungus treatment is a biological conditioning method, because it has obvious advantages in detoxification of organic wastewater and degradation of organic matter[14-21] Hydrothermal treatment sludge filtrate belongs to high-concentration organic wastewater, and research on degradation of hydrothermal treatment sludge filtrate by filamentous fungi is rare. Filamentous fungi have the following advantages in treating organic wastewater: (1) to form hyphal spheres and adsorb and capture small particles, heavy metals and water-soluble organic substances; (2) Degradation of Organic Matter in Wastewater; (3) biosolids separated from wastewater after fungal treatment contain a large amount of organic matter and nutrients, which can be used for composting; (4) fungi also have the effects of inhibiting pathogenic bacteria and reducing odor,[22, 23] Therefore, this study attempts to isolate filamentous fungi from sludge, which can efficiently degrade hydrothermal treatment sludge filtrate, and study the optimal treatment conditions, so as to provide a reference for efficient and safe treatment of hydrothermal treatment sludge filtrate.

1. Materials and methods

1.1 Materials to be tested

The tested sludge is taken from the sludge concentration tank of a sewage treatment plant in Lishui, and its basic physical and chemical properties are as follows: pH 7.42, solid content ,2.7%, organic matter, 45.6%. The sludge is placed at 121℃ for hydrothermal treatment for 30 min. After cooling to room temperature, the hydrothermal treatment sludge is filtered with Whatman #1. The filtrate is the hydrothermal treatment sludge filtrate, and its basic properties are as follows: pH 6.43, chemical oxygen demand (COD)-14000 mg/L. Hydrothermal treatment sludge filtrate shall be stored in 4 and C refrigerators for later use.

1.2 Medium

Martin's solid medium is prepared by potassium dihydrogen phosphate, k, 2, hpo, 4, 1.0 g, magnesium sulfate heptahydrate, mgso4, 7h, 2, 0.5 g, peptone, 5.0 g, glucose, 20.0 g, and agar, 15 g to 20 g. Dissolve all the ingredients and add distilled water to make up the volume to 1000 mL ,115 ,30 min[24].

The preparation method of potato-glucose broth ,PDB, 200 g potato is peeled, cut into small pieces, put into 1000 mL distilled water and boiled, 20 min and then filtered with 8 layers of gauze, and 20 g glucose is added to the obtained filtrate. After it is fully dissolved, the liquid is fixed to 1000 mL, then it is filled into conical bottles while hot, and then it is sterilized in 115, 30 min[25].

1.3 Isolation, screening and molecular biological identification of filamentous fungi

Isolation and purification of filamentous fungi: microbial isolation with concentrated sludge. 150 mL of raw sludge was placed in a 250 mL conical flask and incubated in a shaking table at 28℃ for 12 h at 180 r/min to allow rapid growth and enrichment of microorganisms in the sludge. In a sterile environment, the enriched sludge is gradient diluted with sterile water, respectively diluted to 10 ,3 ,10 ,4 and 10 ,5 ,then 100 μL is taken from the solutions of various dilution degrees respectively to a Martin solid medium (fungus selective medium) plate, placed in a 28 C constant temperature incubator for inverted culture to obtain several fungi, and then separated and purified respectively.

Filamentous fungi screening for degrading hydrothermal treatment sludge filtrate: 135 mL of hydrothermal treatment sludge filtrate is added into a 250 mL triangular flask, then 15 mL of PDB-free filamentous fungi inoculum is inoculated, and the triangular flask is placed in a shaking table at 28 degree C. and 180 r/min for constant temperature culture for 3 days. Each processing setting 3 parallel, while setting blank processing. Sample 10 mL per day, filter with Whatman #1 filter paper, and measure the COD of the filtrate. 1 and 1 fungal strains capable of efficiently degrading hydrothermal treatment sludge filtrate were screened out.

Molecular biological identification: Genome DNA extraction and PCR amplification 18S rDNA-Its sequence are carried out on the screened target filamentous fungi. The primers are fungi ITS (Internal Transcribed Spacer) sequence to amplify universal primers Its1 and Its4. Then, the products of PCR and PCR are entrusted to relevant companies for bidirectional sequencing. The obtained nucleotide sequence results are submitted to NCBI database for comparison, and typical strain series are found for homology comparison. Mega 6.0 software is used to construct phylogenetic tree to

determine the classification status of the strain.

2. Results and discussions

2.1 Isolation and screening of objective filamentous fungi

The original sludge was screened for microorganisms through Martin's medium, and after separation and purification, 11 strains of fungi with different forms were obtained. Inoculate fungus inoculum into the sludge filtrate of hydrothermal treatment to study the degradation of the sludge filtrate by fungi, so as to screen out the target fungi capable of efficiently degrading the sludge filtrate of hydrothermal treatment.

By comparing the degradation effects of fungi on hydrothermal treatment sludge filtrate, finally, the 4# strains used in this experiment were selected. Through sequencing and homology comparison, the fungus was identified as Mucor, with strain numbers of MUCOR and SP. LS-1 .The evolutionary tree is shown in Figure 1. The mycelia of this bacterium on Martin's medium are dark gray, and the mycelia are longer. After PDB, medium culture, 2 days,the mycelia are dispersed evenly and do not intertwine into mycelial balls.

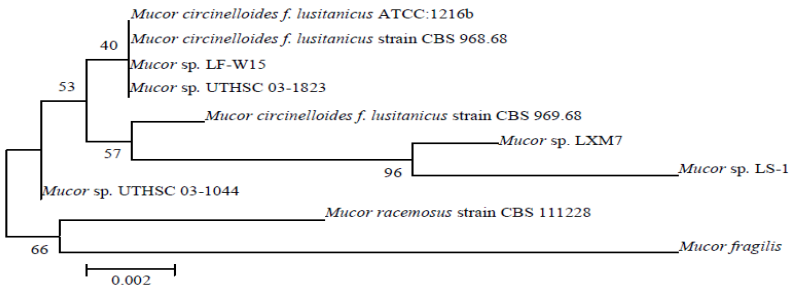

Figure 1. The development tree of Mucor sp. LS-1 and ITS related strains.

2.2 Effect of hydrothermal treatment sludge filtrate concentration on degradation effect of filamentous fungi

High concentration sludge filtrate has inhibitory effect on fungal activity, and the presence of a large number of toxic substances has toxic effect on fungi, affecting the degradation efficiency of fungi on sludge filtrate. Clarifying the optimal inoculation concentration of sludge filtrate plays an important role in improving fungal activity and improving the treatment efficiency of sludge filtrate. The degradation effect of Mucor sp. LS-1 on sludge filtrate with different dilution ratios is shown in figure 2. MUCOR and SP. LS-1 have degradation effects on sludge filtrate with different dilution ratios, and the larger the dilution ratio, the more obvious the degradation efficiency. COD of the filtrate decreased obviously in the early stage, which may be that Mucor sp. LS-1 has strong adsorption performance. After Mucor sp. LS-1 treated 100%, 75%, 50% and 25% filtrate respectively for one day, COD degradation rates were 24.72%, 28.27%, 39.45% and 45.97% respectively, while COD degradation rates were 40.22%, 44.66%, 57.31% and 60.83% respectively after 3 days of treatment. It can be seen from this that the degradation rate of fungi to 75% filtrate and COD only increased by 3.55~4.44% . It may be that there are high concentrations of harmful substances in the filtrate, which inhibit the growth of MUCOR and SP. LS-1 ,thus affecting its degradation effect on sludge filtrate. However, the degradation rates of Mucor sp. LS-1. LS-1, 50%, filtrate ,COD and 14.73~17.09% ,significantly higher than that of 75% and filtrate treatment. This is mainly because the concentration of harmful substances in sludge filtrate decreases, weakening its inhibitory effect on filamentous fungi. although the degradation rates of Mucor sp. LS-1. ls-1, right , 25%, filtrate, COD and cod are still improved, the improvement effect is not obvious. At the same time, the sludge filtrate is diluted to 25%. The overall treatment efficiency of the filtrate decreases and the treatment cost increases, which is not conducive to the efficient and economic treatment of the filtrate. Therefore, in order to improve the treatment activity of MUCOR and SP. LS-1 and the treatment efficiency of sludge filtrate, it is appropriate to select 50% (V/V) sludge filtrate, i.e. hydrothermal treatment sludge filtrate COD with a concentration of about 7000 mg/L as the treatment concentration of MUCOR and SP. LS-1.

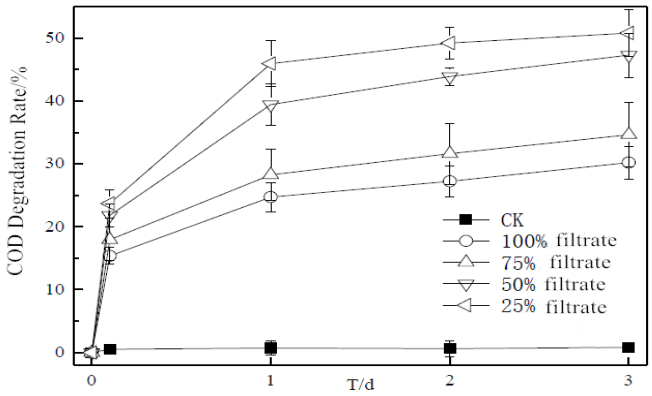

Figure 2. The degradation of COD in hydrothermal treated sludge leachate with different dilution levels by filamentous fugal Mucor sp. LE-1.

2.3 Effect of inoculation amount of filamentous fungi on degradation effect of sludge filtrate from hydrothermal treatment

Inoculation amount of filamentous fungi has an important influence on the degradation of sludge filtrate, which is mainly manifested in the fact that the degradation efficiency of sludge filtrate is not significant when the inoculation amount of filamentous fungi is too low, while the treatment cost increases when the inoculation amount of filamentous fungi is too high [14] <rt1>0> 3 shows the degradation effect of Mucor sp. LS-1 on 50%(V/V) sludge filtrate with different inoculation amounts. As shown in the figure, the more fungi are inoculated, the more obvious the degradation efficiency of sludge filtrate is. When the inoculation amount is gradually increased from 5% to 20% , MUCOR , SP. LS-1 and adsorption removal rate for sludge filtrate are respectively 7.15%, 21.83%, 35.14%, 40.62%, MUCOR ,SP. LS-1 have strong adsorption performance for sludge filtrate. The adsorption efficiency of filamentous fungi on sludge filtrate will directly affect its biodegradation efficiency[27]. As the reaction progresses, filamentous fungi biodegrade the sludge filtrate. When the sludge filtrate was treated with filamentous fungi with different inoculation amounts 3 days later, the degradation rates of filtrate COD were 21.62%, 47.31%, 64.65%, 66.86%, The degradation efficiency of filamentous fungi on sludge filtrate gradually increases with the increase of inoculation amount. When the inoculation amount exceeds 15% ,the degradation effect of sludge filtrate is not obvious. There are some substances in sludge filtrate, which are not easily degraded by filamentous fungi. In addition, too much inoculation will reduce the overall treatment efficiency of sludge filtrate, increase the treatment cost, and lead to difficulty in dehydration in the later stage. The experimental results are similar to those of Zheng Xiang and other researchers[28]Mycelia itself is the reason for improving sludge dewatering performance. The larger the amount of mycelium added, the better the sludge dewatering improvement effect and the higher the relative cost. The mycelium can wrap the sludge after being added to the sludge, reduce the size of fine flocs in the sludge and increase the dehydration performance of the sludge. Therefore, for Mucor sp. LS-1. LS-1 and treating hydrothermal treatment sludge filtrate, 15% inoculation amount can effectively degrade high-concentration sludge filtrate and improve effluent quality.

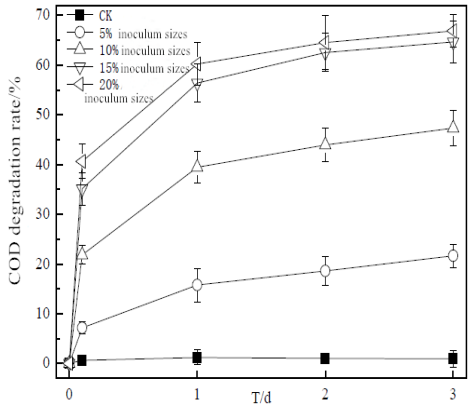

Figure 3. Degradation of COD in hydrothermal treated leachate by Mucor sp. LE-1 with different inoculum sizes.

2.4 Study on degradation of sludge filtrate from hydrothermal treatment by filamentous fungi

In order to further clarify the degradation of sludge filtrate by filamentous fungi, the degradation effects of different activities Mucor sp. LS-1-1 on hydrothermal treatment sludge filtrate were studied. The results are shown in Figure 4. At the early stage of treatment, the adsorption removal rate of activated Mucor sp. LS-1 on sludge filtrate was 35.14%. Due to the biodegradation of the filtrate by filamentous fungi, the removal rate after treatment 1 and days can reach 56.37%. With the extension of treatment time, the treatment effect continues to increase, and the removal rate reaches the maximum after 3 days, 64.65%, filtrate, COD, concentration decreases to 2474.5 mg/L After the filamentous fungi are inactivated, the adsorption removal rate of the filamentous fungi to the sludge filtrate in the early stage is 43.34% ,and the adsorption performance of the filamentous fungi to the sludge filtrate is improved. The reason may be that high temperature treatment increases the active sites on the hypha surface. Due to the inactivation of filamentous fungi, the sludge filtrate adsorbed in the early stage cannot be effectively biodegraded. The sludge filtrate on the hypha is gradually desorbed, and the concentration of filtrate COD gradually increases. After treatment 3 days, the removal rate of filtrate COD decreases to 10.73%. Therefore, the mechanism of filamentous fungi degrading high-concentration sludge filtrate is that filamentous fungi first adsorb high-concentration filtrate through the adsorption of hyphae, and then biodegrade through microbial degradation.

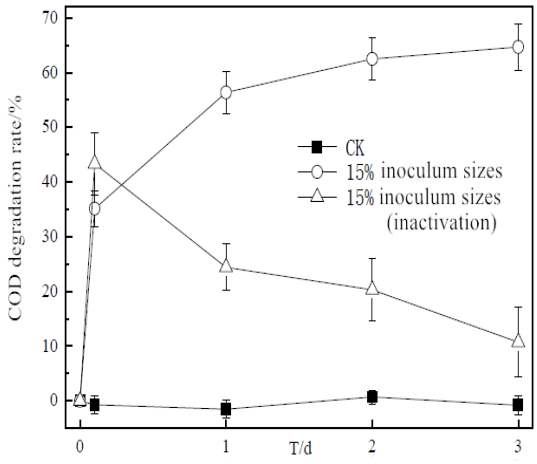

Figure 4. Degredation of COD in hydrothermal treated sludge leachate by Mucor sp. Ls-1 with different activities.

3. Conclusion

In this paper, a filamentous fungus, MUCOR, SP. LS-1, which can efficiently degrade hydrothermal treatment sludge filtrate was isolated from excess sludge. The optimum conditions for the filamentous fungi to degrade hydrothermal treatment sludge filtrate are as follows: the inoculation concentration of sludge filtrate is 50% (V/V) (original filtrate) (~7000 mg/L), and the inoculation concentration of fungi is 15% (V/V). Mucor sp. LS-1-1 Under optimum conditions, sludge filtrate from hydrothermal treatment COD reduction 64.65% and filtrate COD removal are mainly related to the adsorption and biodegradation of filamentous fungi.

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