

Research Article

Research Progress and Development Trend of Mycelial Pellets

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Abstract: Mycelial pellets are generated by the fermentation of microorganisms as a particle, it has good biological activity, rapid subsidence performance and simple solid-liquid separation technology, so that it can better be used in fermentation production, wastewater treatment and as biological carrier. The researches of using mycelial pellets to ferment production, treat some kinds of wastewater as biological adsorbent and enhance efficiency of treatment wastewater as bio-carrier, were discussed in detail. Development trend of mycelial pellets was presented at end of the article. It was also pointed out that the mycelial pellets as bio-carrier or biological adsorbent will be considered as a wide range of application prospects in wastewater treatment.

Keywords: Bio-carrier, biological adsorbent, fermentation production, mycelial pellet

INTRODUCTION

Researchers are trying to use microorganisms in wastewater treatment process and they found some of microorganisms could form into particle materials in which are provided with the defense of stream shear force, as well as have favorable biological activity and settling velocity. Therefore, the word 'microorganism particle' is proposed firstly. The so-called microbial particles means that microorganisms assemble with each other together in appropriate environmental conditions, accordingly form into microorganism aggregations with big volume, high density, strong environmental adaptability (Liu *et al.*, 2005a).

Microbial particles can be broadly divided into three categories: anaerobic granular sludge, aerobic granular sludge and mycelial pellets. The difference is mycelial pellets contains only a single filamentous fungus, but granular sludge contains a variety kinds of filamentous fungi and bacteria (Liu *et al.*, 2005a). From the morphology of the fermentation, the mycelial pellets is a special form of fermentation of filamentous fungi. Filamentous fungi in submerged fermentation conditions have two main forms: dispersed mycelium and mycelial pellets. Reduce the culture medium viscosity appropriately and change the hydraulic condition to form proper directional shearing force, which can change scattered mycelium into hypha ball in the fermentation process (Wucherpfennig *et al.*, 2010). Compare two granular sludge, advantages of mycelial pellets are listed as follows (Duan, 2012):

- **Good settling properties:** Avoid system instability caused by floating sludge and bacteria lost.
- **Broad growth conditions and better water environment adaptability:** Be able to effectively deal with complex waste water environment, have wider using range and smaller limitations.
- Have the characteristics of easy to cultivate, rapid propagation speed, safety and non-toxic, so mycelium ball has excellent economy effects and is a kind of typical environment-friendly materials.
- Because of the internal morphology and structure, it is easier to adsorpt and storage pollutants.

METHODOLOGY

The formation mechanism of the mycelial pellets: About mycelial pellets formation theory, most studies paid attention to speculation and simple experimental verification stage. Spore germination was the first step for the formation of hyphae ball and it was widely accepted that there were two different forms of spore germination: aggregation and non-aggregation type (Nielsen, 1996; Nielsen *et al.*, 1995). The first is at the beginning of the culture process, the number of spores gathered, then the spores germinated, the aggregates of spores grew into mycelial pellets; Another form is that the single spore directly grew into mycelial pellets. Both ways of spore germination exist in submerged culture process of filamentous fungi.

In the culture process of mycelial pellets, Grimm *et al.* (2004) gave a detailed description. He found that

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there were two distinct particle aggregations during the formation of mycelial pellets and the phenomenon is the concentration of particles in solution significantly reduced twice (Grimm, 2005). The first aggregation occurred at the beginning of culture. After inoculation, the spores began to gather until a steady state and then began to gather spore germination. Spore germination broke aggregates-a stable dispersion state, hyphae grow growing rapidly branched mycelium surface increases, the adhesion of the spore's germinated hyphae triggered the second aggregation of particles in the solution (Grimm, 2005). The second aggregation of particles is mainly affected by pH and the energy input caused by stirring, aeration (Amanullah *et al.*, 2001). Then, the germination of spores and hyphae particle velocity exponentially growing mycelium until it reaches the critical radius of mycelial pellets (usually between 50-200 μm due to different density of mycelial hyphae ball (Hille *et al.*, 2006; Wittier *et al.*, 1986). At this time, it appears insufficient nutrients center phenomenon due to mycelial mass transfer limitations. If there is still adequate nutrition in external media, mycelial pellets continue to grow. When the radius of sphere mycelium exceeded its critical radius, a certain area inside mycelial ball will go into the growth stage of substrate limitation caused by mass transfer, then in the core of the mycelium appeared autolysis and gradually began to hollow (Barry *et al.*, 2009; White *et al.*, 2002). Therefore, regardless of the radius of mycelial pellets, growth activity only lied in the outer sphere, which thickness is determined by the mass transfer conditions and the mycelium medium density (Bizukojc and Ledakowicz, 2010). The whole process of forming mycelial pellets provide basic information for the foundation of researching the formation process and the forming mechanism of the mycelial pellet (Zhu, 2007).

APPLICATION OF MYCELIAL PELLETS

The application of mycelial pellets in fermentation production: In the fermentation production, mycelial fungus cultivated in mycelial pellets form has the following advantages. Firstly, it can reduce solution viscosity. Thus, it can properly reduce stirring intensity and avoid increasing operating cost caused by higher viscosity, retention and hard to mixture. Secondly, as mycelial pellets growth and mass transfer character implied, the change of dissolved oxygen concentration is easy to control mycelial pellets fermentation progress. Thirdly, the operative difficulty and cost like product separation considerably reduce (Oncu *et al.*, 2007). Nielsen *et al.* (1995) used *Penicillium chrysogenum* to form little and compact mycelial pellets to product penicillin, which get higher productive rate. Using *Trichoderma* C-30 mycelial pellets to produce cellulase (Domingues *et al.*, 2000), using *Aspergillus niger* mycelial pellets to produce

glucose oxidase (El-Enshasy *et al.*, 1999) and the Production of Endo-p-1, 4-glucanase of mycelium of *Aspergillus terreus* Under Different Submerged Culture Conditions (Wang, 2011) has been reported many times in the articles.

Jiang *et al.* (2009) implemented lactic acid semi-continuous fermentation by *Rhizopus oryzae* mycelial pellets. When stably fermenting 20 times, lactic acid quality remained unchanged. *Phanerochaete chrysosporium* mycelial pellets could grow and synthesize manganese oxidase under nitrogen deficiency and Mn^{2+} (Tobon *et al.*, 2003).

Tripathi *et al.* (1999) controls fermentation conditions to use *Aspergillus niger* mycelial pellets to produce calcium gluconate and output is 149 g/L. Van Wezel *et al.* (2006) controlled fermentation conditions to form *Streptomyces* mycelial pellets in the viscous fermentation broth. It can make streptomycin yield enhance twice than simple microbe fermentation conditions. Du *et al.* (1998) studied the effect of *Rhizopus oryzae* different fermentation form on producing L-lactic acid. *Rhizopus oryzae* was fermented with 76 g/L glucose as substrate. Then lactic acid ultimate concentration was 66 g/L. Compared with mycelium state, fermentative productive rate enhanced from 80 to 86%. Liu and Wu (2012) investigated the effects of surfactant additives and medium pH on mycelial morphology and Exopolysaccharide (EPS) production in liquid culture of a valuable medicinal fungus *Cordyceps sinensis* Cs-HK1. Wang *et al.* (2013) investigated the immobilizing fermentation characteristics and bioflocculant production of two bioflocculant producing bacteria, *Agrobacterium tumefaciens* F2 and *Bacillus sphaericus* F6 using mycelial pellets as biomass carrier and the conclusion was using mycelial pellets as a biomass carrier for the semi-continuous fermentation of bioflocculant is feasible and promising for future industrial production applications.

The application of mycelial pellets in wastewater treatment: It was noticed that mycelial pellet was characterized by specific structure, non-toxicity, huge surface area formed by hypha twining and surface specific adhesion. Researchers began to study the application of mycelial pellets in the water environment protection field. Absorption of mycelial pellets was mainly utilized to remove chroma in dyeing wastewater and heavy metal ion in electroplating waste water (Zhang, 2011).

Except for applying to dye wastewater treatment, mycelial pellets also suitable for adsorbing refractory organic substance in water. Wu and Yu (2006) utilized *Phanerochaete chrysosporium* mycelial pellets to adsorb 2, 4-dichlorophenol in water. And investigated the effect of water solution pH, 2, 4-dichlorophenol initial concentration, temperature and granular size on mycelial pellets adsorption 2, 4-dichlorophenol.

Heavy metal in industrial wastewater is a kind of substance to severely pollute the environment and harm human health. Since heavy metal has toxicity for microorganism, the treatment method was usually physical chemistry method for treating heavy metal wastewater. But using mycelial pellets to absorb heavy metal will offer new ideas for treatment of heavy metal wastewater. In the experiment of mycelial pellets absorbing Cr (VI). Wang (2007) studied the effect of environment factors on absorbing Cr (VI). In the optimum condition, mycelial pellets adsorption rate was 98.8%. Adsorption kinetic curve described mycelial pellets's adsorption process to heavy metal. It could provide guidance to study mycelial pellets adsorption characteristic and engineering application. Liu *et al.* (2005b) inspected adsorption condition to Pb^{2+} of mycelial pellets of the strain HM6 and analyzed the change of surface groups of mycelial pellets after adsorbing Pb^{2+} . Then speculated the major mechanism of mycelial pellets adsorbing Pb^{2+} , ensured that mainly adsorption function groups were hydroxyl and amide (Liu and Chen, 2008). Also there are the researcher presenting that the Cr (VI) remover mainly is reduction and controlled by diffusion. The reaction of adsorbing heavy metal was of first order (Wang *et al.*, 2010). Changing mycelial pellets surface nature can enhance adsorbing capacity of heavy ion (Jin *et al.*, 2006). In conclusion, the applied research of removal heavy metal and dye decoloration has been intensive. But the application research of mycelial pellets as biological carrier is still very weak.

The application of mycelial pellets as biological carrier in wastewater treatment: Recently, some researchers started to apply mycelial pellets as biological carrier to realize immobilization of every functional bacteria, which can be used in water treatment and industrial fermentation fields to reach expectant achievement. The mycelial fungus plays the function of skeleton structure which formed mycelial pellets in the activated sludge or granule sludge. Thus, mycelial pellets has great advantage from the biocompatibility and combining capacity of functional bacteria. In addition, mycelial pellets has many advantages like well sedimentation performance, easy solid-liquid separation, wide growth condition, easily grew, and so on. Mycelial pellet with these characteristic is suitable as biological carrier (Zhang *et al.*, 2011a; Zhao *et al.*, 2007; Gao, 2006; Shan *et al.*, 2008).

Zhang *et al.* (2010) studied the effects of aniline biodegradation for mycelial pellets made from a combination of *Aspergillus niger* Y3 and the bacteria JH-9. The results showed that the best inoculation ratio was 1 mL fungal spore suspension of Y3 ($A_{620} = 0.25$) with 3 mL bacterial suspension of JH-9 (bacteria concentration: 1.2×10^9 cfu/mL). Aniline concentrations

of less than 500 mg/L did not cause inhibition for the combined mycelial pellet. The effluent of the batch reactor was stable. Aniline was completely eliminated and the Chemical Oxygen Demand (COD) was reduced 60%. The experiment results (Zhang *et al.*, 2011b) showed the stable degradations of aniline and COD were observed in both reactors. In the SBR with combined mycelial pellet, the biological removal efficiency was about 0.9 mg aniline/ (L·day). It was much higher than that in the activated sludge reactor. Tourmaline was added to this system in order to improve the aniline removal performance using combined mycelial pellet. It was suggested that tourmaline could enhance the number and activity of the aniline-degrading bacteria immobilized on the mycelial pellet. Therefore, the performance of mycelial pellet as a biomass carrier could be improved by tourmaline (Zhang *et al.*, 2011b).

CONCLUSION

Using biomass as the carrier materials can not only realize the function of the immobilized bacteria, some of them can also be used as nutrients of microbe. More importantly, on the foundation of not changing the water treatment process conditions, the compatibility with microorganisms is effectively improve, and the engineering cost is reduced. However, due to the structure characteristics of these biomass carrier, determines that the performance of mass transfer, adsorption performance is not good, which do not lead to its widely application in the world. Mycelial pellets are generated by the fermentation of microorganisms as a particle, it has good biological activity, rapid subsidence performance and simple solid-liquid separation technology, so that it can better be used in fermentation production, wastewater treatment and biological carrier.

Mycelial pellets as biological carrier has certain advantage and feasibility, which establishes theoretical basis of the application of strengthening wastewater treatment. However, preparation of mycelial pellets carrier has not formed systematic method. And the stability of the carrier is low. These problems had becoming the most urgent problems to be solved in bio-augment wastewater treatment for mycelial pellets as biological carrier.

ACKNOWLEDGMENT

This research was supported by 'Twelfth Five-Year Plan' National Key Technology R&D Program of China (No. 2012BAD14B06-04), the National Natural Science Foundation of China (No. 51478140 and 51408200), Science and Technology Research Project of Heilongjiang Education Department (No. 12541701), Promising Youngsters Training Program of

Heilongjiang University of Science and Technology (No. Q20120201) and State Key Laboratory of Urban Water Resource and Environment (Harbin Institute of Technology) (2015DX06).

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