Study on optimization of proportion between fermented liquid and traditional cultural medium of bioflocculant production and its flocculant performance considering the aerobic fermentation of rice straw as substrate

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Abstract: High cost of traditional culture medium of flocculant is the key element to limit the bioflocculant production. It's therefore much crucial to seek the economic production materials. In this research, part of the traditional culture medium of bioflocculant is replaced by the fermented liquid of rice straw to conduct the discussion on fermentation matching, optimization of fermentation condition and ability of flocculant production. The optimal proportion of aerobic saccharification liquid and traditional cultural medium of flocculant production is 1: 3. The flocculant rates of the economic culture medium of flocculant production are the highest, 65.49% and 71.24%, which are combined by 67d and 109d fermented saccharification liquid and the traditional cultural medium of flocculant production. The growth of flocculant production bacterium is in better situation for composite culture medium of flocculant production. The amount of bioflocculant is 40kg from per ton. The fermentation cost of flocculant saves by 25% comparing with the traditional culture medium. The simple aerobic fermentation technique opens up a new road for low-cost culture medium of flocculant production.

Keywords: Rice straw, aerobic fermentation, fermented saccharification liquid, bioflocculant, performance of flocculant production.

INTRODUCTION

Bioflocculant is a kind of metabolin with flocculant and coagulation produced by microorganism and its possible components are polysaccharide, protein and cellulose, which is obtained through the fermentation, extraction and refinery of the fungi, bacterium and other microorganism (Kurane *et al*., 1994). Bioflocculant is the conomic, advanced and effective agent of water treatment with non-secondary pollution (Salehizadeh *et al*., 2000; Shuangshi *et al*., 2006). It can flocculates a variety of particulate substance quickly, in particular showing unique effect in removing the organic matter including decolorization of wastewater (Krishna *et al*., 2012; Paola *et al*., 2011), sewage (Tingzhi *et al*., 2011), high-salt wastewater (Sanghyun *et al*., 2013) and petrochemical wastewater (Jingbo *et al*., 2010; Wu *et al*., 2007). Large-scale production and industrial application is limited due to the high cost of culture medium of bioflocculant. It's therefore practical to seek the economic production materials and determine the low-cost preparation process. Fujita, *et al* took the volatile aliphatic acid with low molecular weight (LMW) as carbon source to culture the flocculant bacterium to get the bioflocculant (Fujita *et al*., 2000; Kuldeep *et al*., 2014). Shuguang Wang made the wastewater and dairy wastewater using soy sauce to serve as the economic culture medium to further produce microorganism flocculant (Salehizadeh *et al*., 2000); wasterwater of fish meal was used , by Xu Zhou as economic culture medium to culture *Pseudomonas* sp, GX4-1, to obtain the flocculant (Asia *et al*., 2011; Mohammad javad *et al*., 2013). Its performance was also discussed; Considered the Biohydrogen waste as the culture medium and the flocculant rate exceeded 93% with the effective composite bacterium of flocculent production, F2-F6. Lignocellulose is a rich and economic renewable resource on the earth with great development potential, whose efficient use will provide new concept for the low-cost carbon source of bioflocculant (Shuangshi *et al*., 2006). The most suitable carbon source for the bacterium of flocculant production is the amylaceum and straw cellulose is consisted by glucose monomers. Cellulose ruptures in chains after the decomposition of cellulose degrading bacteria and part of the substances with small molecules, such as amylaceum, are released to become the carbon source for the bacterium of flocculant production (Shih *et al*., 2001).

This paper regards the aerobic fermented liquid of rice straw as the partial carbon source and microelement for the culture medium of flocculant production. The ability

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of flocculant production is then discussed through the analysis of saccharides and other components contained in aerobic fermented liquid during different periods. Operation parameters of reactor is regulated to get the best fermented saccharification liquid with the aim to economize the culture medium of flocculant production, offering the effective ways of industrial application.

MATERIALS AND METHODS

Aerobic fermentation reactors of rice straw and its technique parameters

The equipment device of aerobic liquid fermentation is cylindrical reactor (see fig. 1). The air-dried rice straw is cut into 5-8 cm broken sections with scissor. Inoculums are the mixture of marsh liquid and partial fresh cow dung in mixted mesophilic fermented marsh gas tank. The total volume is 2L. Marsh liquid and cow dung are filled into the aerobic pre-treatment reactor to follow the aerobic fermented treatment and monitor the changes of aerobic fermented product.

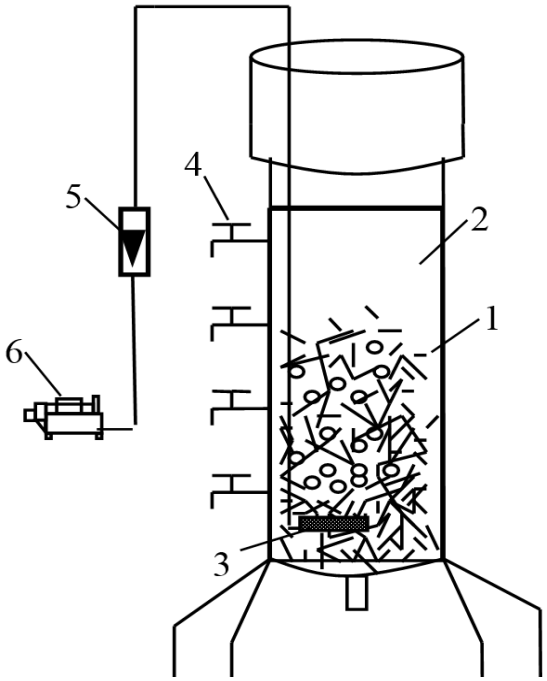

Fig. 1: Illustration of aerobic liquid fermented reactor
1-rice straw; 2-fermented liquid; 3-aeration head; 4-water outlet; 5-Flowmeter; 6-aeration pump

Optimization of culture medium of bioflocculant

Reference for Fermentation condition and methods of flocculant production strains F2-F6 of bioflocculant (Yanbin *et al.*, 2008).

Methods of analysis and test

TOC is measured using TOC-VCPN determinator; TN is measured using TNM-1 total nitrogen determinator. Reducing sugar is measured using 3, 5 - dinitrosalicylic acid (DNS).

RESULTS

Variations of TOC and TN of aerobic fermented liquid in reactor with periods

Aerobic liquid fermentation process needs twice add of marsh liquid. Fig. 2 shows the variations of TOC and TN of aerobic fermented liquid in reactor during different periods.

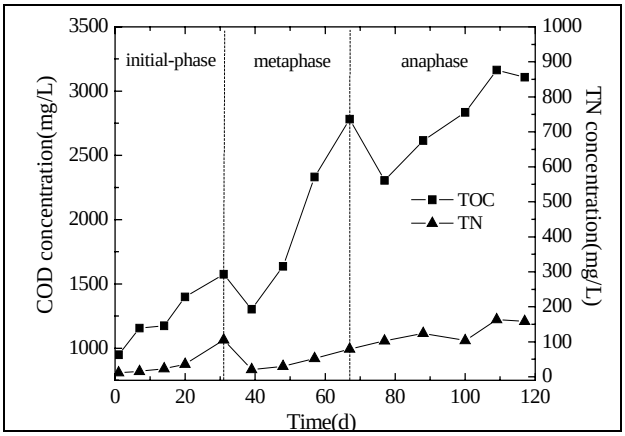

Fig. 2: Variations of TOC and TN of aerobic fermented liquid in reactor during different periods

TOC increases gradually in the early period (0-day31) of aerobic liquid fermented process. Slight reduction is shown during the medium period (day32-67) after adding the marsh liquid, following by the rapid increase; during the late period (day 67-117), the addition of marsh liquid makes TOC decrease a little and then rise up slowly.

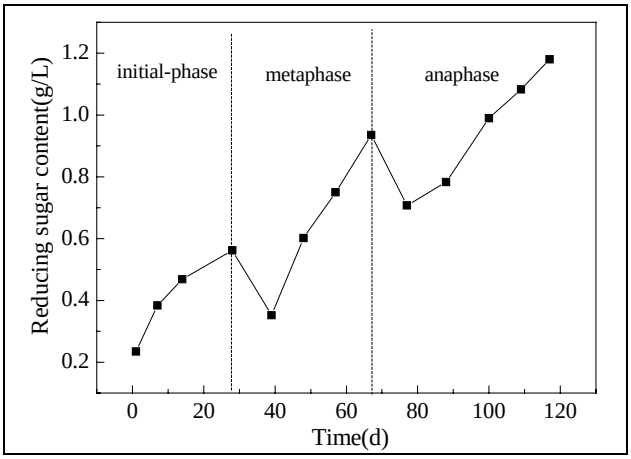

Fig. 3: Changes of total reducing sugars content of aerobic fermented liquid in reactor during

Cellulose of soluble substance and hemi cellulose degrades and the content of organic carbon increases. TOC also rises in its amount. After the first addition of marsh liquid, the sharp increase of TOC indicates that the reactor has a high treatment effect on the rice straw during the first phase. The steady rise of TOC is seen after the

second addition. Aerobic liquid fermented reactor then begins to operate steadily.

The variation trend of TN is similar to TOC when the aerobic liquid fermented reactor operates under the room temperature. After the addition of marsh liquid, materials is decomposed to release NH_4^+-N gradually, thereby causing the continuous rise of TN in fermented liquid.

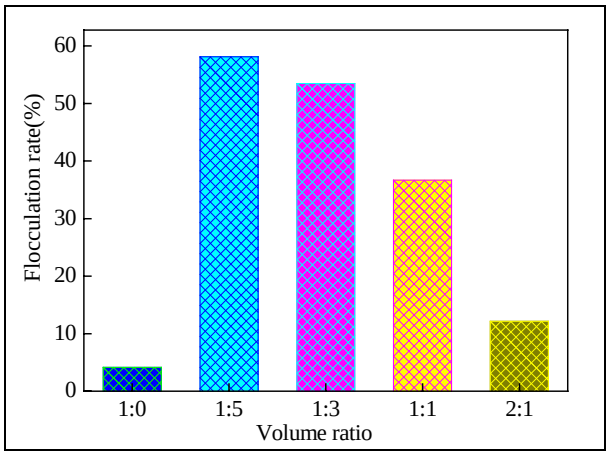

Fig. 4: Comparison of flocculant production of aerobic fermented liquid and traditional culture medium of flocculant production under different matching

Changes of total reducing sugars content of aerobic fermented liquid in reactor during different periods

Twice addition of marsh liquid is conducted during the process of aerobic liquid fermentation and fig. 3 displays the variation of total reducing sugars content of aerobic fermented liquid in reactor during different periods.

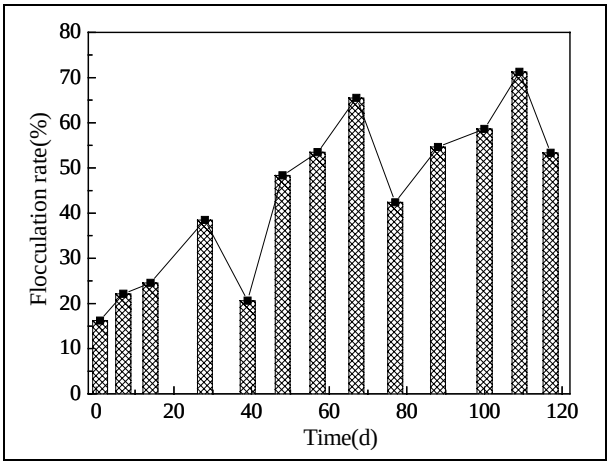

Fig. 5: Flocculation of aerobic composite culture medium of flocculant production

The content of total reducing sugars increases as the time goes by. The content of total reducing sugars decreases after the twice addition of marsh liquid, however, following by the slow increase in the following fermentation process. Bacteria of flocculant production make better use of monosaccharide and disaccharide sugars and the utilization for others is lower.

Optimization of matching between fermented liquid and traditional culture medium of flocculant production

The composite bacteria, which is formed by bacterium of flocculant production F2 and F6 (1:1 in volume), is applied as the bacterium of flocculant production in this experiment. The flocculant result of each matching is tested after 24h. Sterilization of aerobic fermentation liquid serves as the alternative culture medium demonstrates no flocculation. It's therefore considered to mix the aerobic fermented liquid and traditional culture medium of flocculant production in different proportion of volume to constitute the composite culture medium, which will replace the traditional culture medium progressively. The higher total reducing sugars is, the easier the bacteria of flocculant production are used. Aerobic fermented liquid 117d is selected for the optimized experiment of proportion selection. From fig. 4, the highest rate of flocculant is 58.12% when the proportion of aerobic fermented liquid and traditional culture medium of flocculant production is 1:5. The rate is 53.32% when it's 1:3. The rate reduces to 36.67% when it's 1:1. The rate is 12.14% when it's 2:1, almost no flocculant.

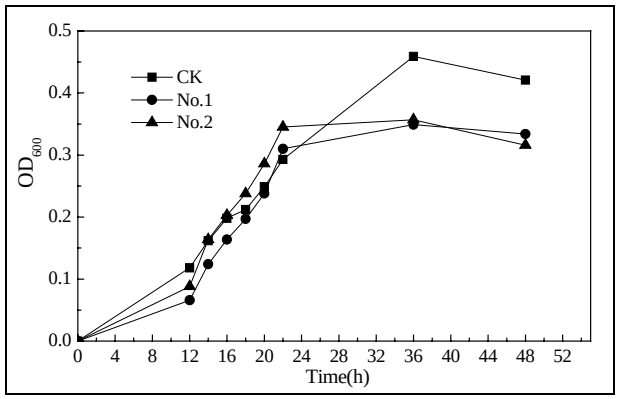

Fig. 6: Growth situations of flocculant production bacteria in the aerobic optimized composite culture medium of flocculant production

The best flocculant occurs when the volume proportion between aerobic fermented liquid and traditional culture medium of flocculant production is 1: 5. Slight decrease relates to the proportion of 1: 3 with limited degree. The ratio of 1: 3 significantly lowers down the cost of culture medium from the economic view. Thereby the ratio of 1: 3 is the optimized based on the maximized savings of the cost.

Comparison of flocculant production ability of fermented liquid under the optimized matching during different aerobic fermented periods

The mixture under the ratio of 1: 3 in volume is selected as the composite culture medium of flocculant production.

24h fermentation follows the sterilization and the final step is to measure the rate of flocculation rate.

According to fig. 5, the flocculation rate of composite culture medium of flocculant production under the optimized matching during the different periods. And the highest rate relates to 67d and 109d, 65.49% and 71.24% in respective. It is indicated that cow dung is hard to be used by bacteria of flocculation production due to the higher amount of nitrogen and low C/N. Suitable C/N is the prerequisite for the growth of bacteria of flocculant production because C/N in 67d and 109d aerobic fermented liquid all ranges from 30 to 35. Moreover, although C/N of aerobic liquid fermentation is suitable for the growth of flocculant production at 20d, other factors required are absent, leading to poor flocculation.

The composite culture medium of flocculation production is thus determined as the optimal aerobic culture medium of flocculant production under the optimized ratio of 1:3 between the aerobic fermented liquid and traditional culture medium at 67d and 109d.

Growth situation of flocculant production bacterium in the optimized aerobic composite culture medium of flocculant production

The growth situation of flocculant production bacteria is observed in the optimized aerobic composite culture medium of flocculant production. The conditions include fermented temperature 30□, the initial pH, 7.0 and the shaker speed 140r/min. The bacteria of flocculant production are accessed into the aerobic optimized composite culture medium of flocculation production at the 8% of inoculums concentration for 36h culture. 1ml fermented liquid is extracted every 2h to determine the turbidity of bacteria ($OD6_{00}$) of the fermented liquid using turbidity of bacteria technique and then draw the growth curve of flocculant production bacteria. The aerobic optimized composite culture medium of flocculant production, which is combined by 67d aerobic fermented liquid and traditional culture medium of flocculant production, is set as No1. The culture medium combined by 109d is set as No2. The traditional culture medium is the blank.

According to fig. 6, the bacteria of flocculant production are in better growth situation for No1 and No2 culture medium. From 1-12h, bacteria of flocculant production begin to grow in No1 and 2. The obvious speed is seen during 12-20h. and 22h is the peak. For traditional culture medium, the bacteria keep a steady growth in the first 24h, following by the same situation between 24h-36h. And it peaks at 36h. It's indicated a course of adaptation for both No1 and 2culture medium. Swift growth occurs after 12h and almost stops after 20h. Peak of flocculant production appears earlier. From estimation, 40kg bioflocculant is produced per t of fermented liquid of rice straw, decreasing by 25% of flocculant production cost.

DISCUSSION

The research indicates that it's possible to produce flocculant with the economic culture medium using aerobic fermented liquid of rice straw. The fermentation of flocculant production is suggested based on the test of reducing sugar during different periods, approximate 0.8mg/L. the optimal matching of aerobic saccharification liquid and traditional culture medium of flocculant production is 1:3. The rate of flocculant of economic culture medium, which is combined by 67d and 109d saccharification fermented liquid and traditional culture medium of flocculant production is the highest, 65.49% and 71.24% in respective. 40kg bioflocculant is generated from pert fermented liquid of rice straw. The cost for fermentation of flocculant is saved by 25% of the traditional culture medium, realizing the economy of substrate for culture medium of flocculant production. The natural methods is applied by aerobic fermentation of rice straw to acclimatize the degradation bacteria of cellulose, which simplifies the fermentation process along with the almost natural fermentation ways in reactor. No special control of operation condition is required, opening up a new road for cost minimization of culture medium of flocculant production (Shuguang *et al*.2006).

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