VNU Journal of Science, Natural Sciences and Technology 24 (2008) 56-62

Bioremediation of vegetable oil and grease from polluted wastewater in dairy factory

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Received 31 March 2008

Abstract. Vegetable oil and grease polluted wastewater caused many problems in wastewater plan such as physical blockages in sewer, pump, screens and filter distributor arms. Its occurrence in effluent increase COD significantly even its amount is not high. In this paper, bioremediation was investigated to remove vegetable oil and grease. Four strains were positive with lipid degrading test. Cell morphology and optimal conditions such as pH, temperature anf lipid concentration was found. The lipid-degrading activity of A12, B13 and D15 was highest at 30^{o}C and pH 7 while that of D14 is at 34^{o}C and pH 7.5 . The result also showed that the highest lipase production of strains A12, B13 and D14 were observed at 0.1 %w/v of lipid, but for strain E15 concentration of lipid was 0.15 %w/v. Addition of antifoam and surfactant was studied. The optimal concentration of antifoam (CF-18) and surfactant (PVA) was found as 0.4%w/v and 0.2 %w/v respectively. 10% w/v of straw in the best ration of support particle, at which the cell density of bioremediatior was 2x10^{8} cfu/g. The best temperature for storage was 5-7^{o}C, even so at the conditioned temperature (20-25^{o}C) the bioremediation was good until 20 weeks. At optimal moisture 10% and under low temperature (5-6^{o}C) the bioremediator was still as good as its initial after 20 weeks. Application of bioremediator in 25 m^{3}/day wastewater treatment plan indicated that COD removal yield increased s6-7% compared with normal case (no bioremediation).

Keywords: Bioremediation, fat-oil and grease (FOG), wastewater treatment, FOG removal.

1. Introduction

Vegetable and animal oils and greases are handled in specific types of industries such as oil extraction from plant grains including olive oil and palm oil mills, butter, dairy, slaughter, detergent and soap manufacturing where fat or grease are used [1-5]. Heavy oil and grease-polluted effluents caused many problems in wastewater plants such as physical blockages in sewers, pump, screens and filter distributor arms. Lighter oil can accumulate in the wet wells of pumping stations, fouling electrodes or floating systems. Flammable oils may also cause an explosion hazard and during the treatment process fats can be absorbed to activated sludge flocs. The occurrence of vegetable oil and grease in effluents increase COD significantly even its amount is not high. All mentioned problems reduce treatment efficiency and make control system fail to operate. The main aim of this study was to isolate microorganisms that can degrade

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vegetable oil and grease, find out the optimal conditions to produce maximum biomass and produce a bioremediation for vegetable oil and grease biodegradation.

2. Materials and Methods

Medium cultures

Screening medium was Tween 20 (Tw20), which contains per liter distilled water: 10 gram peptone, 5 gram NaCl, 0.1 gram $CaCl_2.2H_2O$, and 20 gram of agar. The medium was supplemented with 1% Tween 20, and the pH was adjusted to 7.5. Tween 20 was separately autoclaved at 121°C for 20 min, and then added before use to the sterilized medium components. Tw20 medium was mainly used to screen and selectively isolate bacterial strains that can degrade Tw20 indicating their ability for lipid biodegradation, where the fatty acids produced as a result of Tw20 degradation react with $CaCl_2$ then forming a precipitate appearing as a zones around lipid degradable colonies. The LMM medium contained 1.12 gram K_2HPO_4, 0.48 gram KH_2PO_4, 5 gram NaCl, 0.1 g $MgSO_4.7H_2O$, 2.0 gram $(NH_4)_2SO_4$ and 0.001 g EDTA per liter of distilled water. Each ingredient was separately autoclaved at 121°C for 20 min before mixing, and then the medium was supplemented with 1% soybean oil as the natural substrate present in the raw effluent of the investigated company. LMM was used in the present study to investigate the ability of the Tw20-screened strains for degradation of the natural pollutant (soybean oil) when supplied as the only carbon and energy source in order to select the strains that can be effectively used in remediation of that polluted effluent [6-10].

Three mediums TYGA, NGS and IAM were used in this study to find out which nutrient conditions the isolates could produce the maximum biomass [11,12]. The TYGA medium contained 5 gram trypton, 2.5 gram yeast extract and 1 gram of glucose per liter of distilled water. The medium has been sterile at 121°C for 15 minutes after adjusting to pH 7 separately with glucose. The NGS medium contains per liter of distilled water: nutrient broth 4 gram, potato starch 5 gram, glucose 8 gram and 0.5 gram of yeast extract. Glucose was sterile separately with other components in all above conditions. The IAM medium contained 0.15 gram $NaCOOCH_3$, 0.15 gram glucose, 5 milliliter of vitamin solution, 10 milliliter solution A and 1 milliliter of phosphate solution. Solution A was prepared by mixing of 10 gram of $(NH_4)_2 SO_4$, 5 gram KCl, 5 gram $MgSO_4 .7H_2O$, 2 gram $CaCl_2. 2H_2O$, 2 gram $CaCO_3$ and 0.05 gram of $FeCl_3 .6H_2O$ with 1 liter of distilled water. The phosphate solution contains per liter of distilled water: 124 gram Na_2HPO_4, 15.4 gram NaH_2PO_4. The IAM medium was adjusted to pH 7,2 then sterile at 121°C for 15 minutes, vitamin solution was added to medium when the temperature of medium was about 40°C after sterile [13,14].

Isolation of lipid-degrading bacterial

Samples were taken at the sewers of dairy enterprises, slaughters, food processing enterprises and milk collections. After collection, all samples were isolated in the lab where it was serially diluted up to 10^{-8} and spread on solid sterile surfaces of Tw20 agar plates as triplicate. The plates then were incubated at 30°C for 24 hours. Only bacterial strains that have ability to degrade lipid were grown [13,15].

Lipase assay

Lipase assay was implemented by using modified method of Nguyen Trong Can (1998) [2]. The substrate was soybean oil instead of olive oil.

COD measurement

COD was measured as specification TCVN 6491-1999.

Total lipid analysis

Total lipid analysis was calculated by using Roese-Gottllied (JAS-SOP 35) [15].

3. Results and Discussions

Isolation of Micro-organisms

35 strains were isolated in screening medium Tw 20. After testing the lipid-degrading ability of screened strains there were 4 strains as A12, B13, D14 and E15 were positive. The precipitation of free fatty acids with calcium give a white zone around colony has been used for detection of lipid degrading microorganisms and producing lipase.

Optimization of temperature

The results shown that at fixed conditions as pH 7 the optimal temperature of isolated strains was 30^{o}C for A12, B13 and E15 when D14 produced highest lipid-degrading capacity at 34^{o}C. At the optimal temperature lipase activities were 1.79, 1.43, 0.94 and 1.5 for A12, B13, E15 and D14 respectively.

Optimization of pH

At optimal temperature condition of strains and after 24 hour of incubation, the results indicated that the activity of lipid degradation of strain D14 was at pH 7.5 while the others were at pH 7. The lipase activity of D14 was 0.97, and that of A12, B13 and E15 were 1.66, 1.43 and 1.51 respectively.

Optimization of FOG concentration

Five FOG concentrations were tested to find out the optimal substrate concentration for lipolytic activity. This range of concentration was a common range of FOG concentration in wastewater of food processing companies. The results confirmed that the at 0.1% by weight of soybean oil the highest degradative activity of A12, B13 and D14 was observed as 1.79, 1.43 and 0.91 respectively, while E15 produced the highest activity at 0.15%.

Extra-cellular lipase producing by microoganisms was controlled by many factors such as initial pH, growth temperature, and type and substrate concentration. In this paper the optimal temperature and pH were investigated as shown by other reports (Stams A.G *etc* 1997 and Jaeger, K.E *etc* 1994) [1,8]. The results also indicated that the lower concentration of soybean oil (play the role of carbon source) the lower lipolytic activities were observed, this may due to the lack of substrate for microorganism growth. At higher optimal concentration of soybean oil lipid activity of strains was reduced; this can be explained by the inhibition of oxygen dissolve at high concentration of soybean oil.

Combination of strains

In facts, the wastewater treatment plan worked on varied conditions as pH, substrate concentration and operation temperature. To overcome this situation the combination of isolated strains was investigated. The fixed condition as pH 7, 30^{o}C and 0.1% of soybean oil was used. Ratios by weight of A12, B13, D14 and E15 such as 1:1:1:1, 1:2:1:2, 2:1:2:1, 2:2:1:1, 1:1:2:2 respectively were tested. The results shown that lipid-degrading activity of mixed strains was highest at ration 1:1:1:1 while the lowest activity was observed at ratio 2:1:2:1(figure 1). The explanation of this phenomenon may be the inhibition or promotion of mixed strains in fixed condition.

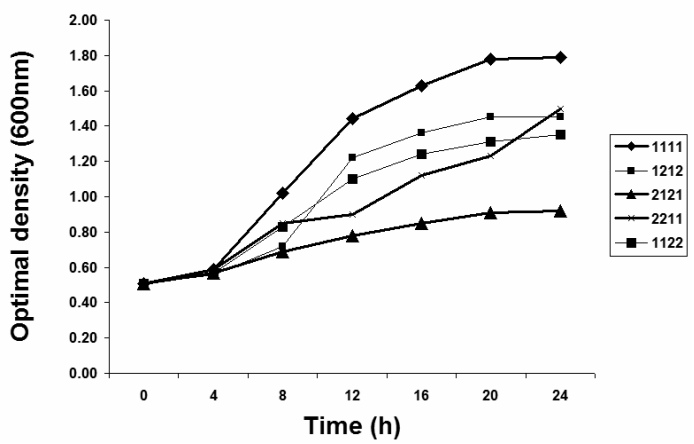

Fig. 1. Lipid degrading activity of mixed ratios.

Addition of antifoam and surfactant

CF-18 is common antifoam using in wastewater treatment system. In this study, the optimal concentration of CF-18 was investigated. The result (Table 1) showed that increasing of CF-18 concentration caused increasing of cell density. The maximum value of cell density was observed at 0.4 %w/v. With the higher concentration of CF-18 the cell density was decreased and it was quite inhibited at CF-18 of 1.5% w/v. This can be explained by the increasing of surface contact between phases at low concentration while at high concentration CF-18 may inhibit cells and reduce the dissolvent of oxygen to water phase. These results are similar to previous reports (Kurita 1999, Bhumibhamon etc 2003, Daigger etc 2003) [6,7,10].

Table 1. Effect of CF-18 to the bioremediator

CF-18 (% w/v)	Blank	0,2	0,4	0,6	0,8	1,0	1,5	2,0
Cell density (10^3 CFU/ml)	151	222	259	254	182	25	2	1

Surfactants in fat polluted wastewater treatment plays an important role, it increases the surface contact of oil-phase and water-phase. In this paper, PEG (polyethylene glycol) and PVA (polyvinyl acetate) were tested. Results (table 2) have shown that at 0.2 %w/v of PEG cell was inhibited and no cell was observed at 0.8%w/v. According to Cao Xuan Thang (2004) [9] this may due to inhibition of PEG on cell and dissolve of oxygen.

Table 2: Effect of surfactants to the bioremediator

Surfactant concentration (%w/v)	Cell density x 10^3 (cfu/ml)
PEG (polyethylene glycol)	
0	131
0.2	101
0.4	9
0.6	3
0.8	0
1	0

PVA (polyvinyl acetate)	
0	148
0.1	158
0.2	159
0.3	180
0.4	187
0.5	191

Results in table 2 also showed that PVA not significantly effect to cell. The cell density was increased even PVA added. The increasing of cell density may due to attachment of cell to the PVA network. Optimal concentration was advised as 0.2 %w/v.

Storage and application of bioremediator

Straw was used for microorganism immobilization. After milling, drying and sterile at 121^{o}C for 15 minutes, 0.4% w/v of CF-18 and 0.2 %w/v of PVA were added to mixed starter. Mixed solution was incubated and dried to 10% of moisture.

Table 3. Effect of concentration of straw on cell density in bioremediator

Concentration of straw (% w/v)	cfu/gram bioremediator
5	6 x 10^6
10	2 x 10^8
20	5 x 10^6
30	4 x 10^5

Table 3 showed the relation between concentration of straw and cell density of bioremediator. The results indicated that 10% of straw was the optimal concentration at which bioremediator has a highest cell density (2x10^8 cfu/g). When then concentration of straw was increased the cell density was decreased. The explanation can be the increasing of surface when the cell concentration was assumed as a fixed factor.

Bioremediator was dried to 8, 10 and 15% of moisture and keep in low temperature (5-7^{o}C), conditioned temperature (20-25^{o}C) and ambient temperature (30-35^{o}C). After 20 weeks of experiments, the results (not shown) indicated that the higher temperature and moisture the shorter time for storage and 10% is optimal moisture. Bioremediator can be good at 10% of moisture and in conditioned temperature. In case of 10% of moisture and in low temperature, the bioremediator showed the same quality as initial.

Application on wastewater treatment system

The bioremediator was applied to 25 m^3/day wastewater treatment system. Properties of effluent were controlled as 750-850 mg/l of COD, 0.1 - 0.16 %w/v concentration of lipid. pH was 7.0 ± 1 and ambient temperature. Bioremediator was added to system depending on flow rate of effluent and controlled as 0.5 %w/v and re-added after 24 hours of treatment. The results (not showed) affirmed that COD removal increased 6-7 % compared with normal case (no bioremediation).

4. Conclussion

Four strains were positive with lipid degrading test. Cell morphology and optimal conditions such as pH, temperature anf lipid concentration was found. The lipid-degrading activity of A12, B13 and D15 was highest at 30^{o}C and pH 7 while that of D14 is at 34^{o}C and pH 7.5. The result also showed that the highest lipase production of strains A12, B13 and D14 were observed at 0.1 %w/v of lipid, but for strain E15 concentration of lipid was 0.15 %

w/v. Addition of antifoam and surfactant was studied. The optimal concentration of antifoam (CF-18) and surfactant (PVA) was found as 0.4%w/v and 0.2 %w/v respectively. 10% w/v of straw in the best ration of support particle, at which the cell density of bioremediatior was 2 x 10^8 cfu/g. The best temperature for storage was 5-7^{o}C, even so at the conditioned temperature (20-25^{o}C) the bioremediation was good until 20 weeks. At optimal moisture 10% and under low temperature (5-6^{o}C) the bioremediator was still as good as its initial after 20 weeks. Application of bioremediator in 25 m^3/day wastewater treatment plan indicated that COD removal yield increased s6-7% compared with normal case (no bioremediation).

Acknowledgement

This study was supported by Ministry of Industry and Trade, Vietnam.

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Bổ trợ sinh học trong hệ thống xử lý nước thải ô nhiễm dầu mỡ động thực vật tại nhà máy chế biến sữa

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Sự có mặt của dầu mỡ động thực vật gây ra rất nhiều khó khăn trong hệ thống xử lý nước thải như: làm tắc hệ thống ống dẫn, tắc hệ thống bơm, hệ thống chắn rác và hệ thống lọc phân phối. Chỉ với một lượng nhỏ của dầu mỡ động thực vật trong nước thải có thể làm tăng đáng kể COD dòng vào. Trong báo cáo này là các kết quả nghiên cứu bổ trợ sinh học để loại bỏ dầu mỡ động thực vật trong nước thải nhà máy chế biến sữa. Bốn chủng phân lập được cho kết quả dương tính với khả năng phân hủy chất béo cao. Các đặc tính hình thái của vi sinh vật và các điều kiện sinh trưởng tối ưu như pH, nhiệt độ, và nồng độ chất béo đã được nghiên cứu. Các chủng A12, B13 và D15 cho hoạt lực phân hủy chất béo cao nhất tại 30^{o}C và pH 7 trong khi chủng D14 lại đạt giá trị cao nhất tại 34^{o}C và pH 7,5. Kết quả nghiên cứu cho thấy khả năng sinh tổng hợp lipaza của các chủng A12, B13 và D14 cao nhất ở nồng độ 0,1 % chất béo, nhưng chủng E15 lại quan sát thấy nồng độ tối ưu là 0,15 %. Kết quả nghiên cứu bổ sung chất chống tạo bọt và chất hoạt động bề mặt cho thấy 0,4 % chất chống tạo bọt CF-18 và 0,2 % chất hoạt động bề mặt PVA là nồng độ tối ưu. Tỉ lệ 10% chất đệm vỏ trấu nghiền cho mật độ tế bào cao nhất (2x10^{8} cfu/g) của chế phẩm là tỉ lệ tối ưu. Nhiệt độ bảo quản tốt nhất là 5-7 ^{o}C, mặc dù vậy nếu ở nhiệt độ 20 -25^{o}C chế phẩm có thể bảo quản được tốt sau 14 tuần ở độ ẩm 10%. Kết quả cũng cho thấy ở độ ẩm 10% và nhiệt độ bảo quản 5-7 ^{o}C thì sau 20 tuần chế phẩm vẫn có chất lượng tốt như ban đầu. Ứng dụng bổ trợ sinh học vào hệ thống 25 m^{3}/ ngày đêm trong 3 tháng, kết quả là hiệu suất xử lý theo COD tăng 6-7% so với không dùng phương pháp bổ trợ sinh học

Từ khóa: Bổ trợ sinh học, dầu mỡ động thực vật, xử lý nước thải, loại bỏ chất béo.