Biodegradation of Effluents from Dairy Plant by Bacterial Isolates

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ABSTRACT: *Micro flora of the effluents from a dairy factory in Tehran(Pegah Dairy Processing Plant) were isolated and screened for their ability to reduce the organic matter content and COD of the effluents. 10 bacteria were selected due to reduction in COD content from the 4th to 6th day of incubation at 30 °C and pH =11. Highest COD reduction were obtained by two isolates, BP_3 and BP_4, 70.7 % and 69.5 %,, respectively (The initial COD concentration was 3000 mg/l and reduced to 880 and 920 mg/l).After optimization of the condition for test organisms, big reductions in COD, carbohydrate, fat and protein content of the effluents were observed by BP_3 up to 84.70 %, 98 %, 45.30 % and 53 %, respectively .The mixture of BP_3 and BP_4 did not show the good results as the BP_3 alone .Therefore, BP_3 has been selected as the most efficient microorganism for the system. The overall efficiency of the system will be increased if it is added to anaerobic activated sludge tank.*

KEY WORDS: *Dairy effluent, Biodegradation, Bacterial isolates, COD content, Activated sludge, Optimization.*

INTRODUCTION

Biotreatment leading to bioconversion of the waste materials is probably the most cost-effective technique for managing and utilizing waste. Waste materials associated with the food industry including the wastes generated by the dairy industry are notables [1]. The disposal of large quantities of this wastewater with or without prior treatment and the continuous pile of solid waste from industrial and domestic source rapidly causes deteriorations of the environment. Three effects of the presence of these wastes include contamination of

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drinking water, killing of aquatic life, increased danger in swimming and objectionable physical conditions such as off odors, and accumulation of debris. For example, high concentrations of organic matter have led to the depletion of the dissolved oxygen supply for fish due to the oxidative actions of microorganisms in the surrounding medium. The economy has suffered because many developing countries have resorted to importation of fish to sustain local demands. To save natural bodies of water from possible complete deterioration, stricter and more defined effluent standards should be imposed by the government on different industrial firms. This needs to employ efficient facilities for the various waste materials. The removal of organic matter from the wastewater using chemicals are used in many industrial wastes, and these methods are more expensive than the bioteartment method using micro flora from the same area of the tests. Also the chemical methods may cause further contaminations to the environment. Due to capital draining expense, attention has been drown to the use of microbial culture preparations for waste treatment. The microorganisms of choices should have a strong degradative capacity and high toxic resistance. The process of seeding inoculation of microorganisms for degrading waste materials on streams, rivers and treatment tanks has been rapidly increasing practice in many countries because it is economical and the application is uncomplicated [2]. The objective of this research is to test the ability of some selected bacterial isolates from wastewater to degrade the organic matter of the effluent of the dairy products processing plant after lyophilization.

MATTERIALS AND METHODS

Dairy waste

Wastewater from dairy plant in Tehran (Pegah Dairy Processing Plant) was collected. Table 1 shows the quality and quantity of the wastewater from Pegah firm.

Chemical

All the cultural media used in this research work was supplied by Merck and BDH Companies.

Microorganisms

Some bacterial isolates were obtained from activated sludge of dairy plant according to the method described below:

Table 1: Characteristic of the wastewater from Pegah dairy plant.

Daily Debby	3500 m^3/day
BOD	1500-1800 mg/l
COD	1800-2800 mg/l
Fat	160-200 mg/l
Suspended solid	200-250 mg/l
pH	9 - 12

Enrichment cultural media

In this stage 10 g of the activated sludge was dissolved in 90 ml of sterile physiological serum and it was added to an enrichment cultural medium such as nutrient broth. 1 ml of the suspended solution was inoculated to 250 ml of sterile nutrient broth and was shaked for 24 - 48 hrs at 30 °C.

Specific culture media

In nutrient broth a good growth of bacteria were observed after 24 hrs. Milk broth was used as specific cultural media, which contained: peptone=50 g, yeast extract=3 g, milk solid or fresh milk=10 ml. After preparation and sterilization of this medium, 1 ml of enrichment culture was added to it and shaked for 24-48 hrs at 30 °C. If a good growth of microorganisms occurred, this procedure would be repeated.

Transport to solid culture media

After complete growth of the microorganisms, they were transferred to the sterilized solid cultures such as milk agar and plate count agar, and they were incubated at 30 °C.

Screening

After the wide growth of the bacteria, they were inoculated into the new sterilized media and were incubated at 30 °C for 24 to 48 hrs. Each bacteria grew as a pure colony and was transferred to the prepared slant, for quality and quantity tests [3] and [4].

Product Analysis

The protein, carbohydrate and fat content of the effluents were determined using the methods "Official Methods of Analysis AOAC"[5]. The optical density was measured by spectrophotometer (Spectronic 21 D) as the measurement of the cell growth.

Table 2: Characteristic of the bacterial isolates.

Bacterial name	Microscopic Characteristic	Growth on culture media	Gram	Specified culture media
BM_1	Very small cocci	Good	Negative	Milk agar
BM_2	Very small Coco bacillus	Good	Negative	Milk agar
BP_1	Big bacillus	Fair	Positive	Plate count agar
BM_3	Bacillus smaller than BP_1	Good	Positive	Milk agar
BP_2	Long bacillus	Good	Positive	Plate count agar
BM_4	Medium Coco bacillus	Good	Negative	Milk agar
BP_3	Some sort of Bacillus with curve	Good	Positive	Plate count agar
BM_5	Rather big Coco bacillus	Good	Negative	Milk agar
BM_6	Small Coco bacillus	Fair	Positive	Milk agar
BP_4	Cocci bigger than BM_1	Good	Negative	Plate count agar

RESULTS AND DISCUSSION

10 bacterial isolates were obtained and after Gram method and microscopic observation table 2 was obtained which shows the characteristic specification of the selected bacteria. These selected microorganisms were examined for their ability to reduce the chemical oxygen demand and the other chemical tests. The initial concentration of the wastewater was 3000 mg/l and each bacterium was inoculated to the waste at 30 °C in the shaker incubator at 150 rpm. The pH of the system was adjusted to 11. The COD measurement was carried out during 30 days.

Fig. 1 depicts the reduction of the chemical oxygen demand during 30 days. After 3 days, BP_3 and BP_4 showed reduction up to 52.2 and 51.7 %, respectively. In the tenth day of incubation BP_3 and BP_4 showed reduction up to 71.7 and 69.7 % reduction, respectively. The ability of the other bacterial isolates to reduce the COD concentrations were not suitable for other quantity tests.

From Fig. 1, it is clear that in the 2 to 3 days of incubation, rapid reduction of the COD up to 50 % was due to decomposition of the carbohydrate content of the waste which is a simple biodegradable material. The second biodegradable materials are protein and fat which are degraded after 8-10 days of incubation. The COD reduction was up to 65-70 % and after this time there were no changes in COD content [5]. The highest reduction of COD was after 10 days by these two microorganisms, and COD concentration remained unchanged up to 30 days. Therefore BP_3 and BP_4 were selected as the most effective microorganisms for further experimental performance in order to optimize the efficiency of the test organisms.

In Fig. 2, BP_3 and BP_4, and the mixture of them were examined for their capacity to reduce the COD content of the effluent. The mixture showed only 71.2 % removal of the COD, but BP_3 reduced the COD concentration up to 84.70 %. Figs. 3, 4 and 6 show percentage reduction of carbohydrate, fat and protein content by BP_3 and BP_4. The highest reduction obtained by BP_3 in all cases was 98 %, 45.30 % and 53 %, respectively.

Therefore, BP_3 has been chosen as the most efficient bacterium in the system and the cell growth of this bacteria is shown in Fig. 7. Cell growth increased within 3 hours and maximum growth was obtained after 48 hours (Log phase).

The stationary phase started after 54 h and in 102 h the death phase started. From Fig. 7 the COD reduction increased up to 5 days (120 h) and this is continued until the 30th day of incubation. This is due to retention time of the bacteria in the system and the number of the survivor and dead cells are not involved for the COD reduction.

The increasing of the concentration of polluted material, caused the lowering down of the efficiency of the system. For BP_3, increasing the concentration of the COD , increased the resistance of this bacteria to the

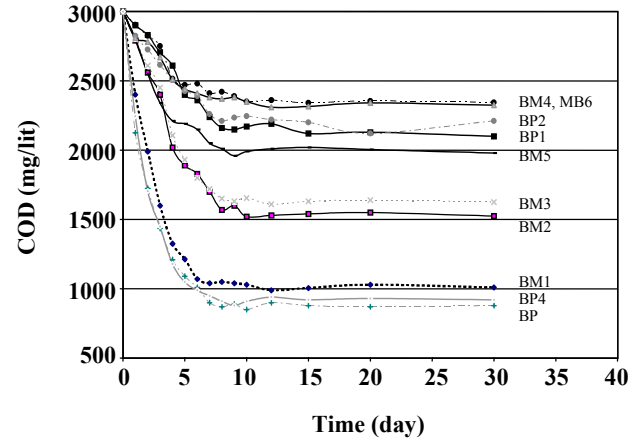

Fig. 1: COD reduction by 10 bacterial isolates pH = 10.5, T=30 °C.

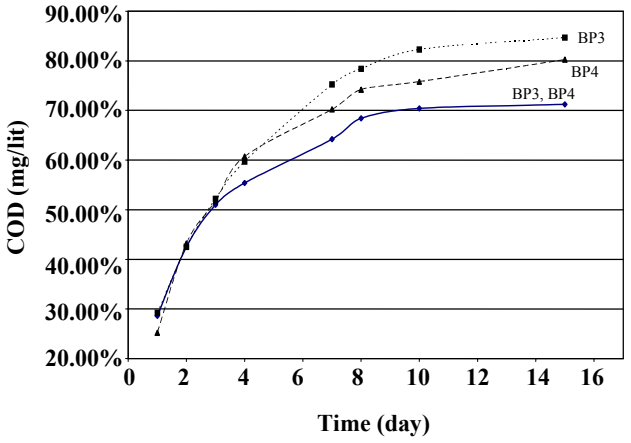

Fig. 2: COD removal by BP_3 , BP_4 and mixture of BP_3 and BP_4.

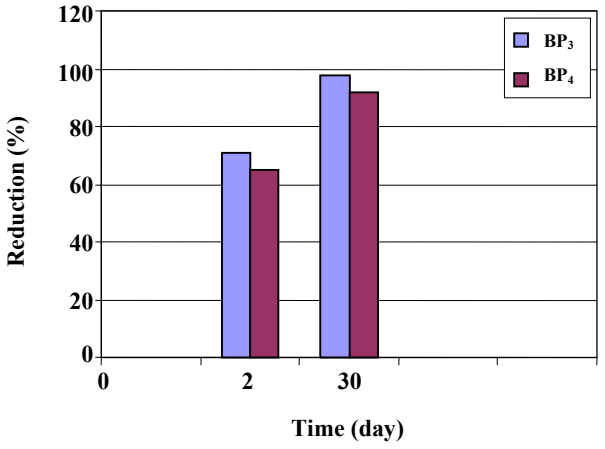

Fig. 3: Reduction of Carbohydrate content of dairy waste by BP_3 and BP.

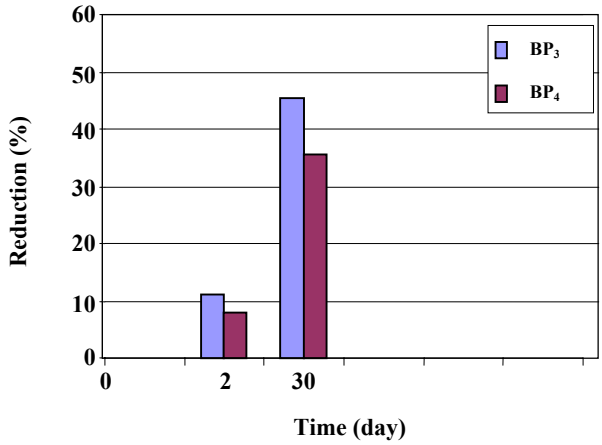

Fig. 4: Reduction of fat content of dairy waste by BP_3 and BP_4.

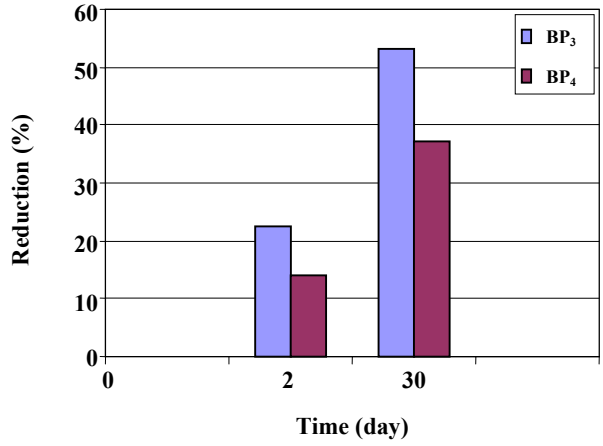

Fig. 5: Reduction of protein content of dairy waste by BP_3 and BP_4.

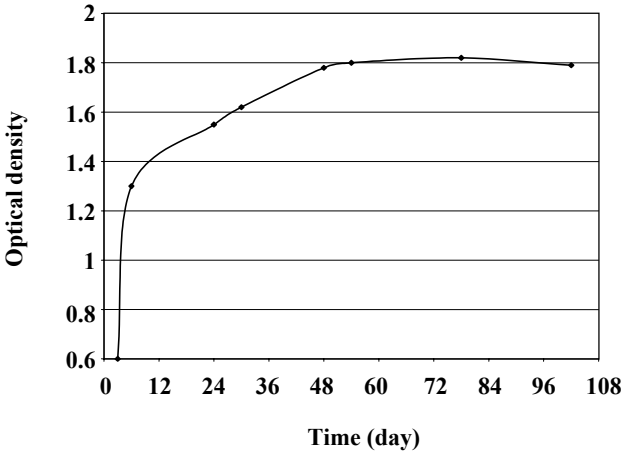

Fig. 6: Cell growth of BP_3.

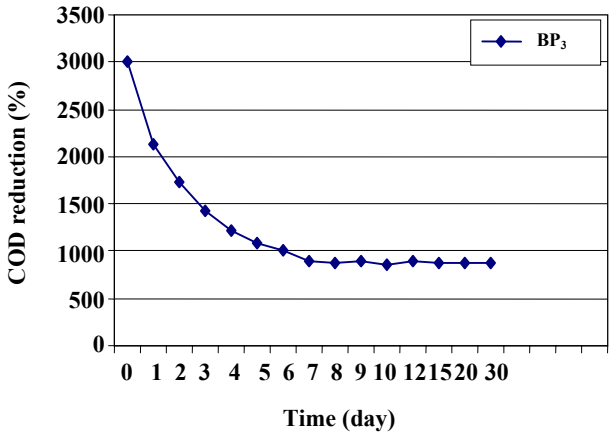

Fig. 7: COD reduction by BP_3.

variation of the concentration of polluted load. Increases in concentration of the COD from 500 to 10000 mg/l decreased the activity of the tests organisms 37 %, 29.6 % and 38.5 % for BM_1 ,BP_3 and BP_4, respectively. The most efficient bacterium BP_3.

In this study, the rate of aeration was not measured, but the shaker incubator provides enough air for better growth of the aerobic microorganism. The COD removal is higher at shaking rate of 150-160 rpm than the other values. The best temperature intervals were found to be 30-35 ^{o}C (data not shown). The effect of temperature variation was lower for BP_3 than BP_4 and BM_1, and it was the most resisted bacteria in the system. pH variation from 7 - 11, reduced the removal of the COD content up to 10 % for BP_3 and BP_4, and 13 % for BM_1. The optimum pH was 11 [8].

CONCLUSIONS

According to the results, bacterial isolate BP_3 is the most effective organisms to reduce the COD concentration more than 84 %. This value was obtained at 32 °C, pH=11 and the shaking rate of 150 rpm. It also decreased the carbohydrate, fat and protein content of the waste by 98 %, 45.30 % and 53 %, respectively. We suggest that the addition of bacterium to the microbial mixture of the activated sludge will increase the overall efficiency of the treatment system. It can also reduce the bulking problems of the activated sludge by preventing the load of the organic matter from becoming too high. Further work is needed in the pilot plant system with these bacteria, to improve the effectiveness of the treatment system.

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