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Isolation of Beneficial Microbes for the Biodegradation of Animal and Plant Oil in Fast Foods Wastewater Effluents

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Abstract

This study on the isolation and screening of aerobic microbes was developed for the treatment of fast foods wastewater effluents to reduce the oil and grease processed under aerobic conditions. The microorganisms were isolated from different fast foods wastewater effluents having both high oil and grease content, a big problem in the environment. From an initial 130 selected isolates, 54 were screened, then 28; thereafter eleven (11) isolates were found the most promising. The isolated microbes then identified as bacteria were screened and applied to the effluent and simulated wastewater for bioprocess treatment. These microbes were inoculated in nutrient broth for 24 hours at room temperature in an environmental shaker. These cultures serve as inoculum for treating contaminated fast foods wastewater in the laboratory for twenty four (24) hours. Analytical results showed that the highest reduction of oil and grease had 93.75% for the isolate $JB_{8/8}$ (uncultured Basidiomycota) in wastewater effluents and isolate $JB_{4/6}$ (Bacillus cereus) was 98.25 % in simulated wastewater.

Keywords: Beneficial microbes, bioremediation, effluents, inoculum, isolate, pollutants.

Introduction

Biological treatment involves the use of microorganisms for degrading household and industrial wastewater wherein water is decontaminated before being released in the environment. It is used in many industries such as food processing, dairies, breweries, paper, plastics and petrochemicals for the removal of organic or biodegradable waste. There are two types of pollutants namely, biological and chemical[1] consisting mainly of nitrogen, phosphorus, heavy metals, detergents, pesticides and hydrocarbons. Waste faecal borne human diseases are mainly caused by waterborne microbes, which harbour viruses, bacteria and protozoa that thrive in drinking and receiving bodies[2].

Fast foods have a big problem in their wastewater, which is animal and plant oil. Grease problems had always been associated with the pumping station (pump failure, float switches, rancid odor, blockage, etc.) which resulted in high maintenance costs for the restaurant, as the pump required cleaning every six months; and a primary cause of sanitary sewer overflows[3].

This study is important since it is not only beneficial to our industry but also generally address the present condition of wastewater management in most companies. Microbial treatment of wastewater effluent is a new rising technology in the Philippines however, many of the industrial companies are not aware of this. Conventional methods of treating wastewater are often complex and costly[4]. Through this study, we introduced another option for fast foods and other industries in treating their wastewater effluents using more effective and efficient microbial strain. The process makes use of newly isolated microbes[5] which can degrade the animal and plant oil.

The search for new technologies involving the removal of high oil and grease from wastewater has directed attention to aerobic treatment: for low initial and operational costs, low sludge[6] and valuable gas production. The newly isolated bacterial strain has the capacity to reduce the organic wastes, oil and grease of wastewater effluents of fast foods chain. The new bioremediation process is expected to provide a technology option that will allow fast foods operators and other related industries able to comply with the stringent wastewater effluents standard for the oil and grease as required by the Department of Environment and Natural Resources (DENR)[7]. The developed process contributes more benefits to all fast foods and other restaurants for increased production and employment. It would be nice to live in a clean, safe and healthy environment.

Impurities found in wastewater are removed through chemical and biological processes. Wastewater organics could provide the energy for micro-biological metabolism and by-products needed for cellular biosynthesis[2]. However, chemical process uses certain chemicals that are harmful to human health[7].

The parameters considered for analyzing wastewater are the five day biochemical oxygen demand (BOD_5) and the total suspended solids (TSS) as a measure for the amount of waste particles suspended in the wastewater sample. For studies involving commercial establishments, we consider the fats, oils and grease (FOG) levels in the waste stream. For FOG based

determination, waste strength is measured in milligrams per liter (mg/L) with values for weak, medium and strong are: 50, 100, and 150 mg/L, respectively. In this study, the Oil and Grease value of 33.33 mg/L for isolate $JB_{8/8}$ is below the range measured in the public sewer industries. The average strength of residential wastewater is approximately 20 mg/L [8].

The chemical composition of fats and oils are free fatty acids and esterified triglycerides. At room temperature fats are solid, while oils are liquid. Animal and vegetable based FOGs are edible; while petroleum or coal based FOGs (mineral based) are inedible, toxic and chemically resistant. Today's commercial production of FOG in a full-service restaurant could reach up to two tons per year and was mostly taken from vegetable oils.

Material and Methods

Culture Media: All reagents were of analytical grade. Sodium Chloride (NaCl), Calcium Chloride Dihydrate ($CaCl_2{\cdot}2H_2O$), Potassium biphosphate (KH_2PO_4), Dipotassium Phosphate (K_2HPO_4), Ammonium Sulphate $(NH_4)_2SO_4$ and Magnesium Sulphate ($MgSO_4{\cdot}7H_2O$) were obtained from Univar. EDTA and glucose were obtained from Merck. Soybean oil was purchased from the Supermarket. Tween 20 and Tween 80 were bought from a local laboratory supplier. Hydrochloric acid and nitric acid were from Univar. Petroleum Ether was from RCILABSCAN Limited. Nutrient agar, peptone and nutrient broth were from Difco.

Wastewater Effluents: Contaminated wastewater from fast food chains used in the screening of microorganisms was prepared with synthetic water using reagent grade chemicals dissolved in distilled water. Actual wastewater effluents from fast food chains were also used to validate the results. Samples A and B were taken from a particular Fast Foods; A is from an out-of-town site, while B was from Metro Manila site.

Isolation of Microorganisms: One (1) ml each of wastewater from different fast food chains was suspended in 10 ml nutrient broth. Serial dilutions of up to 10^{-5} were prepared from each suspension. A 0.1 ml from each suspension was then spread plated over a nutrient agar dish. The plates were then incubated at room temperature and 24- and 48-hour colonies were picked and streaked on nutrient agar slants. The different isolates were then stored in the refrigerator for screening. Out of the total one hundred and thirty (130) isolates, fifty four (54) were selected visually for subsequent screening.

Screening of Microorganisms: The 54 strains were cultured in media plates and those with a bigger zone of inhibition were further screened; 28 isolates were picked and subsequently 11 promising isolates were selected based on their biodegradability of fat and oil. Instead of distilled water, synthetic water (consisting of 1% Tween 20 and 80, 0.1% peptone, 0.05% NaCl, 0.001% $CaCl_2.2H_2O$; 0.2% Agar) was used as diluent. Then, a liter of simulated wastewater (containing 0.112% K_2HPO_4, 0.0048% KH_2PO_4, 0.05% NaCl, 0.001% $MgSO_4.7H_2O$, 0.02% $(NH_4)_2SO_4$, 0.001% EDTA and 1% Soybean) was cultured with 10% inoculum of a particular selected organisms. After one day of incubation, the 11 isolates were selected as the beneficial microbes for the biodegradation of animal and plant oil in fast foods wastewater effluents.

Inoculum Propagation: Inoculum propagation and screening for biodegradability of animal and plant oil in wastewater effluents were generally of the same media concentration. Nutrient agar slants were streaked with chosen test organism and incubated for 24 hours at room temperature. A 24-hr culture of the bacterial strain was propagated in nutrient broth medium with shaking at room temperature for 24 hours. A 10% volume of inoculum was used directly for screening. The selected microorganism, which was later identified as a bacterial strain, was grown in a one (1) liter bioreactor for treatment using actual wastewater effluents and simulated wastewater. The treatment was stopped after one day and analyzed for the percent reduction of oil and grease. Initial wastewater (raw) sample was collected for analysis and characterized.

Analytical Methods: Wastewater effluents and simulated wastewater were analyzed for oil and grease, pH, Chemical Oxygen Demand (COD), Biological Oxygen Demand (BOD), Total Solid (TS) and Total Suspended Solid (TSS) after 24 hours treatment based on Standard Method of Analysis for Wastewater[9,12]. The BOD5 (Biological Oxygen Demand values read after a period of 5 days) was based on the DIN EN 1899-1 and DIN EN 1899-210, EPA approved, using OxiTop® (BOD) Instrumentation. While, the Chemical Oxygen Demand, ST COD value was determined using a Lovibond® COD VARIO sealed tube test (ISO 15705:2002)10, and samples read in the spectrophotometer. Optical densities of the cells were measured using spectrophotometer at 660 nm.

Results and Discussion

The effluents generated from the wastewater facility of the fast food were generally dark in color (figure-5, untreated/control) while microbial treated samples had considerable reduction in color and quantity (figure-5 and figure-6). The presence of large debris of particles was observed in water, oil and grease; and sediments. It had a very foul odor and the pH ranged from 7.0 to 8.0 (table-1, sample-A).

Based on the preliminary analysis of wastewater from two different fast food chains, results showed that both samples A and B (table-1) had a high COD content that was not compliant to the standard of the Department of Environment and Natural Resources (DENR)[11]. Due to problems in the collection of samples, we limited our study to one sample (Sample B, from Metro Manila) only. After treatment with the newly isolated microbes the pH ranged from 6.31 to 7.62 and the COD reduction ranged from 60.52% to 92.92% (figure-1 and table-2).

Table-1
Preliminary Analysis of Wastewater Samples

Parameters	Sample A	Sample B
pH	8-9	7.56
COD (mg/L)	15,000	1,360
BOD (mg/L)	1,500	560
TS (mg/L)	5,157	392
TSS (mg/L)	2,443	68.3
VSS (mg/L)	2,253	42.3

The results of the experiments showed that eleven (11) isolates (table-4) were considered the most promising due to their capability to reduce oil and grease of wastewater from the fast food chains. The reported value for Oil and Grease, 33.33 mg/L for isolate $JB_{8/8}$ (table-3) showed a significant decrease, which is below the range measured in the public sewer industries. The isolate $JB_{8/8}$ had 93.75% reduction using actual wastewater effluents and isolate $JB_{4/6}$ has 98.25% reduction (table-3 and figure-4) in simulated wastewater.

Conclusion

This new bioremediation process is expected to provide a technology option that will allow fast foods operators able to comply with the stringent wastewater effluent standard for oil and grease required by the DENR. Data showed clear reduction especially with the isolates, and thus their use in the biological treatment can contribute more benefits to all fast food chains and restaurants; it can increase production and needless to say employment. Another factor to their success has been their ability to target population of low socio-economic background. Of the 11 most promising isolates defined as bacterial strains; only six (6) were analyzed for the 16S rRNA sequence at the Philippine Genome Center, UP Diliman resulted to molecular identification as isolates $TW_{2/1}$ (*Enterobacter cloacae*), $JB_{8/8}$ (*uncultured Basidiomycota*), T_2B_7 (*Bacillus thuringiensis),* $JB_{4/6}$ (*Bacillus cereus*), $JB_{4/8}$ (*Bacillus Cereus*), $T_2B_{7/9}$ (*Bacillus cereus*).

Table-2
Characterization Profiles of the Selected Promising Isolates

Isolates		Optical Density (OD)	pH	COD (mg/L)	% COD Reduction
				(Control) 46,600	
1	T2W2	1.61	6.78	650 (6800)	90.44
	1				
2	JB4	1.482	6.78	9400	79.82
	6				
3	JB4	1.033	6.75	21900	53
	8				
4	JB5	1.113	6.52	8400	80.9
	4				
5	JB8	1.119	6.52	7000	84.97
	8				
6	JB6	1.117	6.31	12400	73.4
	5				
7	T2B7	1.31	6.68	3300	92.92
8	JW6	1.368	7.31	14200	69.53
	9				
9	T2B7	1.293	6.42	4500	90.34
	9				
10	W5	1.429	7.24	7200	84.58
	0				
11	W6	1.319	7.62	18400	60.52
	1				

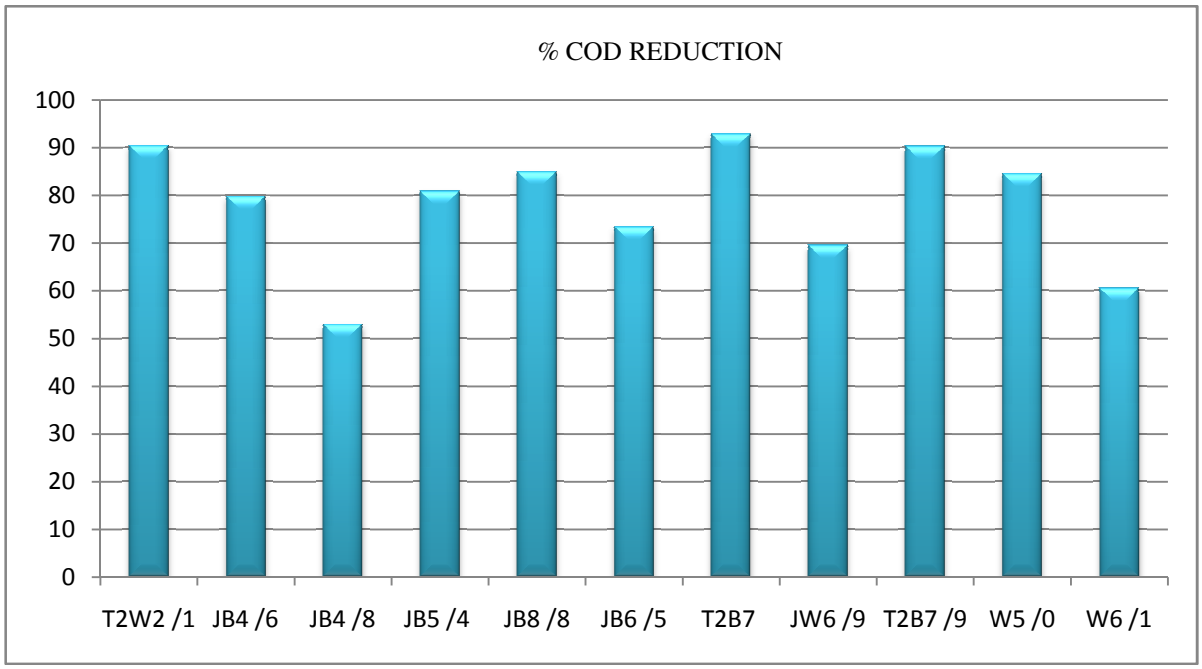

Figure-1
Percentage (%) COD Reduction of the Isolated Microbes with High Potential for Oil and Grease Degradation

Table-3
Oil and Grease Analysis of Wastewater treatment using Isolates and % Oil and Grease Reduction Actual and Simulated Wastewater Effluent

Isolates		**Oil and Grease (mg/L)**	**% Oil and Grease Reduction**	**Oil and Grease (mg/L)**	**% Oil and Grease Reduction**
		Wastewater Effluent		**Simulated Wastewater**	
		(Control) 534 mg/l		(Control) 5,006.30 mg/l	
1	T2W2 1	49.07	90.81	2,116.80	57.72
2	JB4 6	228.2	55.2	87.4	98.25
3	JB4 8	334.93	62.3	770	84.62
4	JB5 4	390.73	26.83	1,630.00	67.43
5	JB8 8	33.33	93.75	1,791.90	64.21
6	JB6 5	198.3	62.87	2,026.40	59.37
7	T2B7	166.54	68.81	1,198.16	76.07
8	JW6 9	214.91	59.75	1,122.40	77.58
9	T2B7 9	104	80.52	2,034.30	59.37
10	W5 0	328.85	38.42	1,469.80	70.64
11	W6 1	652	Nil	3,412	31.85

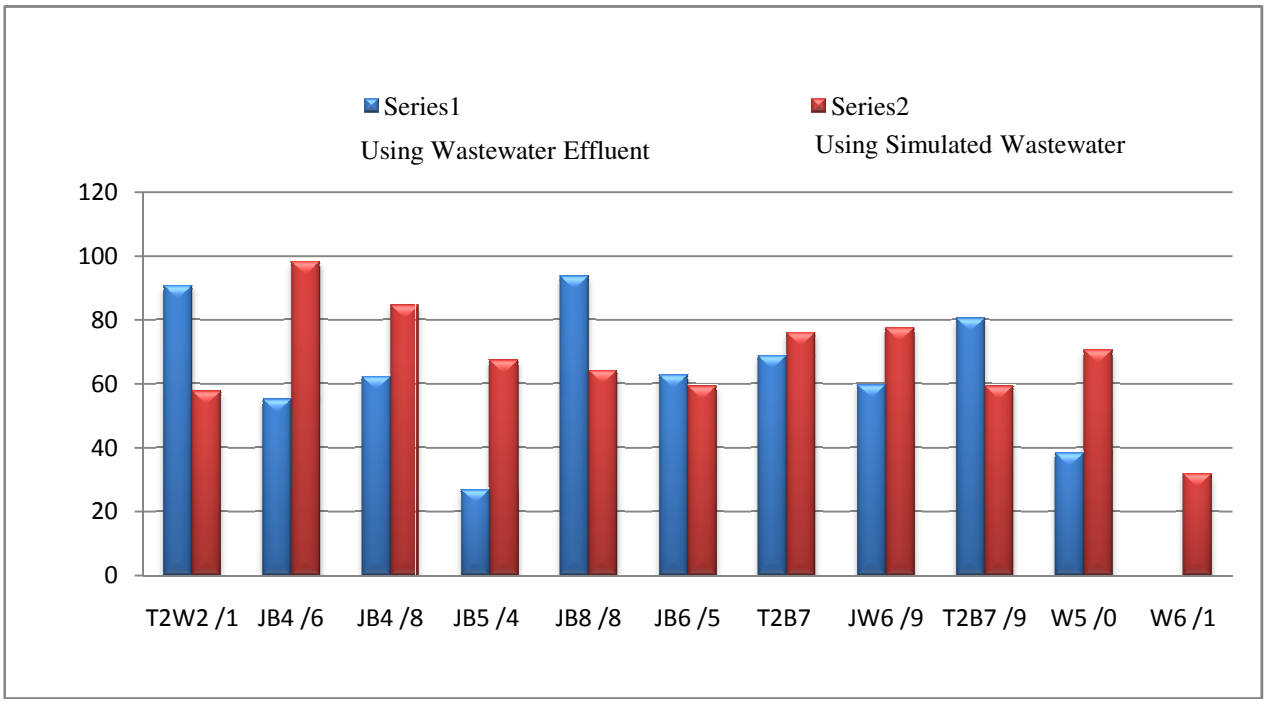

Figure-2
Oil and Grease % Reduction using Simulated Wastewater and Actual Effluent Wastewater the Isolated Microbes with High Potential for Oil and Grease Degradation

Figure-3
Isolation of Microorganisms

Figure-4
The eleven (11) Most Promising Isolates

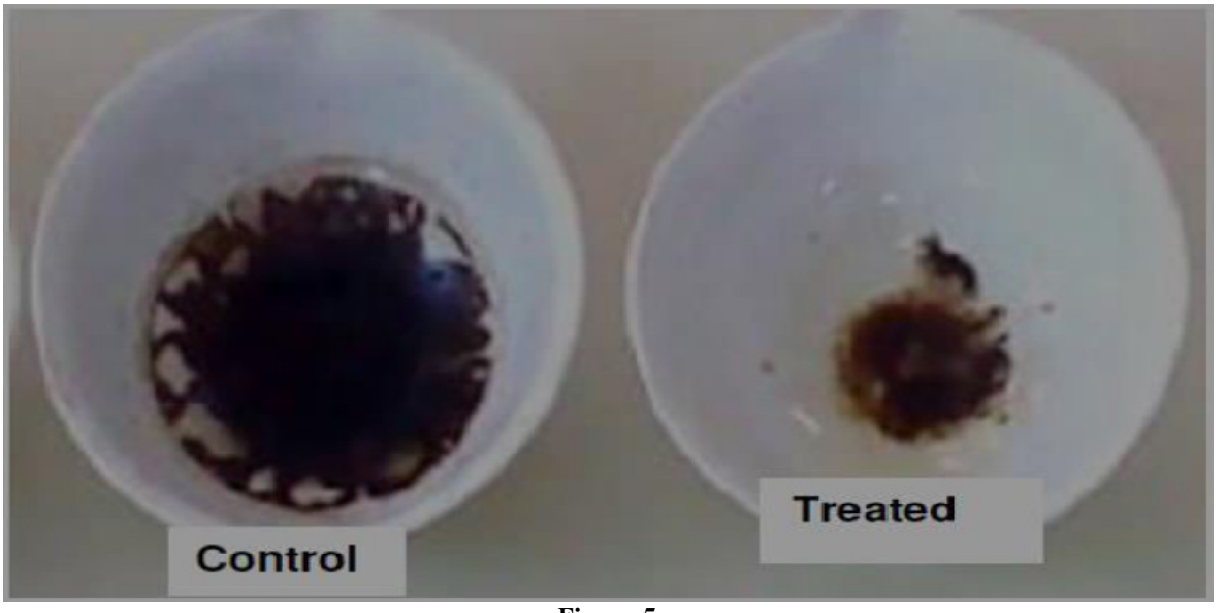

Figure-5
Oil and Grease Analysis Using Wastewater Effluent using Wastewater Effluent from fast food (after oven-drying)

Figure-6
Oil and Grease Analysis using Simulated Wastewater (after oven-drying)

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