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Technical Communication

Biosurfactant production and use in oil tank clean-up

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A proprietary bacterial strain (Pet 1006) produced biosurfactants when grown on both glucose and an immiscible hydrocarbon as carbon sources. Pilot-plant-scale (1500 l) production gave, on repeated batch runs, 2 tonnes of culture broth containing active biosurfactant. The product was used as a substitute for chemical surfactants in a clean-up demonstration test carried out by Cargo Fleet Chemical Company Ltd. (UK) on an oil storage tank belonging to Kuwait Oil Company, Kuwait. The clean-up was successful in removing the sludge from the tank bottom, and it also allowed the recovery of more than 90% of the hydrocarbon trapped in the sludge. The recovered hydrocarbon had excellent properties and could be sold after being blended with fresh crude.

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In view of the annexation of Kuwait by Iraq in August 1990, this paper has been accepted without return to the author for attention to minor details and for approval of certain editorial changes that have been made. The Editor-in-Chief therefore assumes full responsibility for any errors or omissions.

The problem of cleaning sludges and waste oil deposits from storage and holding tanks is a major cost in crude oil production. Sludges and heavy oil deposits accumulate in the tanks because they are not lifted by conventional pumps. Tanks must be cleaned periodically, usually by manual means. The procedure is hazardous, time-consuming, labour intensive and expensive. To resolve these problems, numerous studies have been carried out to determine the suitability of surface-active compounds for cleaning, and it has been recognized that sludges can be cleaned up, and a significant percentage of the oil can be recovered from the sludge layer when treated with biosurfactants (Jobson *et al.* 1972; Cundell & Traxter 1973; Mulkilns-Philips & Stewart 1974; Walker & Colwell 1974; Singer *et al.* 1983; Hitzmann 1983).

Biosurfactants are complex biopolymers produced by microorganisms, and have better characteristics than many synthetic surfactants for oil industry application. In tank cleaning, their mode of action involves dispersion of heavy oil components by reducing the sludge's viscosity by forming either macro- or microemulsions of either oil-in-water or water-in-oil. The less viscous emulsion is easily pumped and the oil fraction can be easily separated after breaking the emulsification.

Biosurfactants are mainly produced by hydrocarbon-degrading microorganisms to increase their interfacial area for contact to give improved uptake of such substrates (Hisatsuka *et al.* 1971; Rapp *et al.* 1979; Kappeli & Finnerty 1980; Ito & Inoue 1982; Prince & Morton 1989; Robert *et al.* 1989). However, they have also been reported to be produced from soluble carbon sources, such as carbohydrates (Cooper *et al.* 1981; Guerra-Santos *et al.* 1984; 1986; Person & Molin 1987).

Although many investigators have sought to characterize biosurfactants and requirements for their production, few assessments of their performance in oil storage tank cleaning have been reported. The purpose of this study was to produce biosurfactant with a bacterial culture, Pet 1006, and to assess the biosurfactant's suitability for cleaning a large oil storage tank in order to evaluate the effectiveness of such a cleaning procedure and to estimate the amount of oil that can be recovered from the sludge.

Table 1. Chemical composition of modified BSM media.

Component	Modified BSM*
Macroelements (mM)	
$(NH_4)_2SO_4$	1.00
KH_2PO_4	10.00
K_2HPO_4	10.00
$(NH_4)_6Mo_7O_{24}.4H_2O$	0.01
$MgCl_2$	0.80
$CaCl_2.2H_2O$	0.18
Na_2SO_4	5.00
Microelements (uM)	
$FeSO_4$	2.00
$NaMoO_4$	8.00
$MnCl_2$	5.00
$ZnSO_4.7H_2O$	1.00
$CuSo_4.7H_2O$	1.00
$CoCl_2.6H_2O$	1.00
Carbon source (% w/v)	
Glucose	2.0
Immiscible hydrocarbon (% v/v)	2.0†

* 6.5% (v/v) NH_4OH was used to adjust the pH and to act as a nitrogen source.
† Added after the glucose was consumed.

Materials and Methods

Microorganism

A bacterial isolate, Pet 1006, a proprietary strain from Petrogen Inc. (USA), was used. It was maintained on nutrient and plate count agar and was preserved as frozen glycerol culture (2:1 mixture of freshly grown cells and glycerol solution) at −70°C.

Media

The composition of the medium used for propagating Pet 1006 is listed in Table 1.

Cultivation Conditions and Production

Laboratory studies were carried out in a 7 l Chemap (Männedorf, Switzerland) fermenter operating at pH 6.8, temperature 34–37°C, oxygen saturation level 20% and working volume 4 l controlled by a weight-control system. The fermenter was equipped with a foam detector and control using a silicon-based chemical antifoam agent. Large pilot-plant production was carried out in a 1500 l nominal volume (900 l working volume) stainless steel Chemap automated fermenter equipped with an EFFIGAS turbine system for gas dispersion. The pilot fermenter had a mechanical foam breaker (FUNDA FOAM) and a control system for temperature, pH, pressure, dissolved oxygen and weight. Samples were collected periodically during fermentation runs, and analyses for dry weight, emulsification capacity, surface and interfacial tension, nitrogen and glucose were carried out as required.

Biosurfactant Detection

Three biosurfactant detection methods were used:

1. Xylene or oil emulsification assay. This test was carried out by adding 5.0 ml 20 mM Tris buffer, pH 8.0, to a scrupulously clean 16 × 150 mm glass test tube to which 35 µl sample and 35 µl xylene were added just above the air/water interface. Using a clean 21-cm-long pasteur pipette attached to a regulated air supply and flow meter, the liquid was aerated for 45 s at 2.0 l min^{-1} with the pipette tip placed at the tube bottom. The mixture was allowed to sit at room temperature for 20 min without further agitation before the absorbance at 660 nm was read in a colorimeter. Low-viscosity crude oil may be substituted for the xylene. High absorbance indicates high-level dispersion of the oil or xylene in the buffer and, hence, the presence of surface-active compounds; lower absorbance indicates low-level surface active compounds.

2. Surface and interfacial tension measurements. Both were carried out using a Kruess K 10 T ring tensiometer (Kruess, Optische-Mechanische Werkstatten, Hamburg, Germany).

3. Thin-layer chromatography (TLC). Culture broth samples were spotted on silica gel plates run in a solvent containing chloroform/methanol/acetic acid (65:15:2; by vol.) and developed using 2% anthrone solution in concentrated sulphuric acid. The plates were dried at 110°C for 10 min to determine qualitatively the presence of biosurfactant spots characteristic of the Pet 1006 culture.

Oil Storage Tank Cleaning

The tank cleaning involved four steps.

1. Surveying. Personnel from Cargo Fleet Chemical Company Ltd. surveyed the proposed tank for cleaning (tank No. 12 at Kuwait Oil Company's South Tank

Farm, Ahmadi, Kuwait) and carried out bottom dipping to determine the sludge topography and sludge volume. The sludge quality and the optimum biosurfactant requirements to lift the sludge were also determined.

2. *Mobilizing.* All necessary equipment (pumps, hoses and circulation boxes to provide circulation and biosurfactant mixing in the tank) were installed. Pipe connections were arranged and pipes were connected to supply fresh water and fresh crude oil for the cleaning operation.

3. *Desludging.* A blend of the produced biosurfactant, brackish water and fresh crude oil were introduced into the tank and circulated until all sludge was lifted from the bottom of the tank as a result of its emulsification. The circulation was then stopped, and the emulsion was allowed to separate into oil and water layers using an emulsion breaker. The oil layer was pumped out and transferred to a storage tank and the water layer was removed for disposal.

4. *Cosmetic.* Any inorganic deposits (sand, gravel and non-hydrocarbon materials) were removed manually before tank inspection.

Results

Laboratory Fermenter Experiment

In this experiment the modified BSM-58 medium (see Table 1) was used. Glucose (2% w/v) and the hydrocarbon component (2% v/v added after glucose consumption) were used as carbon sources. The results (Table 2) showed rapid growth and glucose consumption. The glucose was consumed within 14 to 16 h (Figure 1), after which the growth rate, as measured by biomass production, decreased dramatically. The use of glucose as an initial carbon source led to high biomass production of 7 g l^{-1} dry weight. The emulsification assay (Figure 2) showed increased biosurfactant production, especially after the initial 14 h, when glucose was available. Since glucose is a readily available substrate, it was utilized without the need for biosurfactant production; only when it was consumed and the immiscible hydrocarbon was the only available carbon source did biosurfactant production increase to facilitate its dispersion in water for easier uptake by the microorganisms.

An analysis of a sterilized sample of the final product showed effective reduction of surface and interfacial tension at dilutions up to 10^{-4} in synthetic deposit

Table 2. Batch fermentation of Pet 1006 in modified BSM medium.

Time (h)	Dry cell weight (g l^{-1})	Glucose concn (g l^{-1})	Xylene emulsification (A_{660})	Kuwait oil emulsification (A_{660})	Surface tension (dynes cm^{-1})	Interfacial tension (dynes cm^{-1})
0.0	0.00	33.00	0.00	0.00	76.0	45.5
1.0	0.38	32.50	0.12	0.19	76.0	45.5
3.0	NA	32.15	0.07	0.18	76.0	44.5
6.0	1.03	24.07	0.16	0.28	74.2	41.5
10.0	1.96	5.71	0.31	0.46	49.0	20.8
14.0	4.41	0.10	0.43	0.50	37.0	10.5
18.1	6.24	0.00	0.56	0.54	38.0	10.2
22.0	6.84	0.00	0.74	0.69	34.5	7.9
26.0	6.50	0.00	0.68	0.42	33.4	9.2
30.0	7.10	0.00	0.60	0.92	33.2	8.1

Hydrocarbon substrate was added at 12 h.
NA—not analysed.

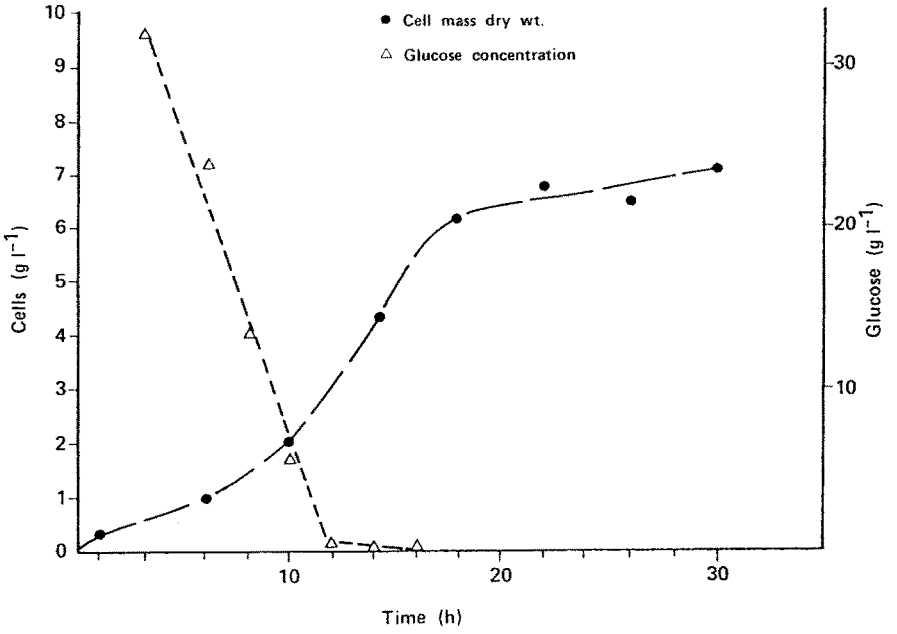

Figure 1. Increase of cell mass as measured by dry weight and decrease of substrate (glucose) during second batch fermentation experiment. ○—Cell mass dry wt; ▲—glucose concentration.

water (SDW) (Figure 3). This proved the presence of a highly active and heat-stable biosurfactant in the Pet 1006 fermentation broth.

Pilot-plant Production

Culture broth was prepared using the modified BSM-58 medium under the conditions described earlier. Both glucose (2% w/v) and hydrocarbon (2% v/v added after the glucose was consumed) substrates were used, whereas ammonium hydroxide was used to control the pH in the fermenter. Inoculum was prepared by growing Pet 1006 in a laboratory fermenter as described earlier. The results of a typical pilot-plant production run are shown in Table 3. A decrease in the

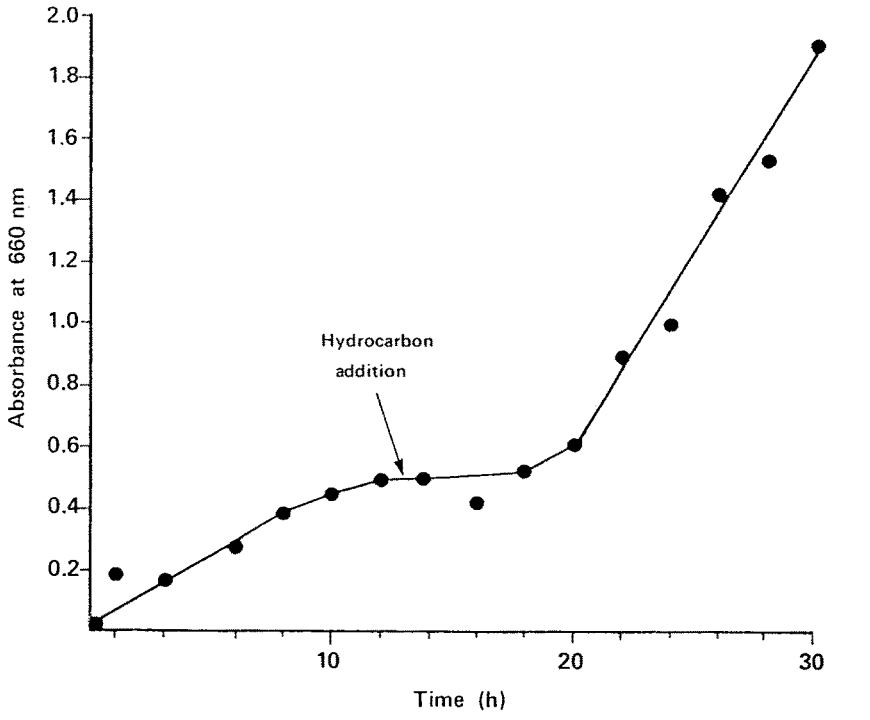

Figure 2. Biosurfactant activity as measured by oil emulsification assay for Kuwaiti crude oil at different intervals.

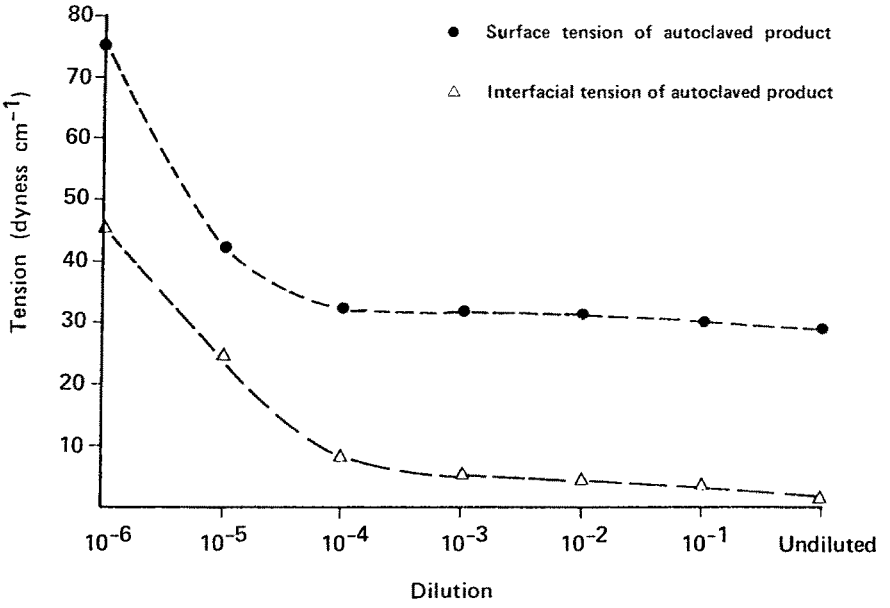

Figure 3. Surface and interfacial tension activity in fermentation broth of second batch fermentation experiment at different dilutions into standard salt water. ○—Surface tension of autoclaved product; ▲—interfacial tension of autoclaved product.

product's surface tension measurements occurred due to biosurfactant production that reached a maximum at 18 to 19 h. Glucose was consumed within 8 h of the pilot run, after which the hydrocarbon was added. The growth rate of Pet 1006 (as measured by cell dry weight) was high when glucose was available but dramatically decreased after glucose consumption and hydrocarbon addition. The hydrocarbon addition forced the existing cells to produce high concentrations of biosurfactants, as detected by the TLC results (Table 3).

Table 3. Results of large pilot-plant run for biosurfactant production using Pet 1006.

Time (h)	Dry cell weight (g l^{-1})	pH	Surface tension at 10^{-3} in SDW	Surfactant present*
0.0	0.29	6.78	71.4	—
3.0	0.90	6.55	71.4	—
4.0	1.60	6.40	—	—
5.0	2.50	6.45	70.3	—
6.0	3.35	6.50	71.7	—
8.0	5.18	6.35	70.8	—
10.0	5.80	6.80	44.0	—
12.0	6.39	6.60	48.6	+
14.0	6.58	6.64	44.1	+ +
16.0	6.60	6.51	44.1	+ +
18.0	6.61	6.51	37.9	+ + +
19.0	6.62	6.49	37.6	+ + +
19.5	6.63	6.48	37.1	+ + +
Sterilized product	—	—	33.5	+ + +

Glucose was consumed at 8 h, and hydrocarbon substrate was added 9 h after start of run.
* Surfactant determined by thin-layer chromatography (see Materials and Methods).
SDW—synthetic deposit water.
— No biosurfactant detected.
+ Slight biosurfactant detected.
+ + Much biosurfactant detected.
+ + + Maximum biosurfactant detected.

At the end of the production run, the culture broth was sterilized while in the fermenter and was harvested into 200 l pre-sterilized drums for use in the clean-up operation.

In all, 2 m^3 (= 2 tonnes) of medium were processed in this manner.

Clean-up Operation

The initial survey, carried out by dipping in 17 points at the bottom of the floating roof tank (44 m diameter) showed two main sludge areas about 1 to 1.5 m high. The results of the tank survey, sludge analyses and laboratory tests on the sludge carried out by Petrogen (USA), Cargo Fleet (UK) and Kuwait Consulting and Investment Company (Kuwait) determined the presence of some 750 m^3 sludge, which was potentially easily emulsified. They also predicted a good oil recovery from the proposed clean-up. Accordingly, it was determined that 1 t biosurfactant would be sufficient to effect the necessary emulsification and recover the hydrocarbons trapped in the sludge. Nevertheless, nearly 2 t biosurfactant was produced to allow for any inaccuracies in the sludge sampling or any activity loss of the product due to long storage or unforeseen delay in its application.

Upon the installation of the circulation pumps, hoses, circulation boxes and connections to manholes, 1.5 t biosurfactant was added to the tank in addition to 1:1 crude oil to sludge and 1.25:1 brackish water to total hydrocarbon (sludge + fresh crude). Circulation in the tank was started by powerful suction at the water-oil interface and the reinjection of the water–oil mixture through the tank bottom. It continued for 24 h per day for 5 days, after which sampling showed that most sludge had been processed and resuspended. This meant that the sludge had broken down into small droplets and had mixed with the water, which had removed all inorganic materials and impurities from it and resuspended its hydrocarbon component in the main oil body; this stayed on top of the water layer. The inorganic impurities sank back to the bottom. Although 10 days had been allowed for the circulation, only 5 days were needed to achieve the required results. This was due mainly to the use of 1.5 t as opposed to the calculated 1 t of biosurfactant and to the high summer temperature (40 to 50°C). After an emulsion breaker was mixed in, time was allowed for the water to separate from the crude oil. The two layers were extracted to be reused; the water in future clean-up operations and the oil hydrocarbon layer for blending and resale as crude oil. The remaining inorganic impurities were manually removed through cosmetic cleaning of the tank.

The clean-up was successful; the oil storage tank was inspected by the owners, and a final acceptance certificate was issued. The amount of sludge in the tank was more than predicted (850 m^3); nearly 91% (774 m^3) was recovered as crude oil, whereas 76 m^3 remained as impurities at the tank bottom and consisted mainly of nonhydrocarbon materials. The crude oil recovered was tested before and after being blended with fresh crude oil at 1:1 ratio and also after storage for two months. The test results (Table 4) showed API volumes ranging between 27.6 and 29.8, similar to the value for Kuwaiti crude, and a total hydrocarbon content of 100%. These results indicate that the recovered oil could be sold as crude at the market value of Kuwaiti crude.

Discussion

Foaming was a major problem during the laboratory fermentation experiment because of the lowered surface tension in the culture broth, which increased the air in the water emulsion. This was controlled by using minimum air flow in the fermenter while operating under high agitation speed to maintain the dissolved oxygen at above 10% saturation level. The use of chemical antifoam for foam control was minimized, since its addition interfered with the surface and interfacial

Table 4. Characteristics of recovered crude oil samples before and after blending with fresh crude and after two months' storage.

Property	Test method	Unit	Sample		
			1	2	3
Specific gravity	IP 160/D1298	g ml	0.883	0.877	0.888
API gravity	IP 200/D1250	API	28.6	29.8	27.6
Viscosity kinematic at 20°C	IP 71/D445	cSt	166.6	54.1	196.6
Water content (A)	IP 74/D95	% vol	ND	ND	ND
Sediment (B)	IP 53	% vol	ND	ND	ND
Water & sediment (BS&W) (A + B)	—	% vol	ND	ND	ND
Asphaltic material	IP 143	% wt	2.09	2.14	1.05
Oil content 100-(A + B)	—	% vol	100	100	100

Analyses by Division of Petroleum, Petrochemicals and Materials, Kuwait Institute for Scientific Research.
ND—none detected.
Sample 1—crude oil extracted using biosurfactant before blending.
Sample 2—crude oil extracted using biosurfactant after blending.
Sample 3—sample 2 after two months' storage.

tension measurements. However, pilot-plant production encountered no such problems, since the fermenter had a mechanical foam breaker that effectively controlled all foam produced.

The pilot-plant-scale production of Pet 1006 was successful, and the biosurfactant product had strong activity, as demonstrated by the surface tension measurements at 10^{-4} dilution in standard salt water. The product was also heat stable and withstood the sterilization procedure with no activity loss; on the contrary, a slight increase of activity was usually detected after sterilization, which may have been due to the release of intracellular biosurfactants and cell-water or capsular fragments having biosurfactant activity (Kosaric *et al.* 1983).

Gutnick & Rosenberg (1977) have reported the production of an extracellular emulsifier named 'Emulsan' from *Acinetabacter calcoaceticus*. This product was also reported to be used in small-scale cleaning of oil-contaminated vessels and in the reduction of crude oil viscosity in pipelines, and to have potential for the management of oil spills (Rosenberg 1986; Desai 1987). Although similar microbial products have been reported by other workers, the use of microbial biosurfactants for cleaning large oil storage tanks is a new application that, according to our knowledge, has not been evaluated anywhere large scale.

The present cleaning operation recovered 91% of the sludge volume in the oil storage tank as saleable crude oil; the recovered 774 m^3 (= 5550 barrels, at US $20/barrel) is worth $110,000. This can turn the currently expensive clean-up operations, costing from $100,000 to $150,000 per tank, into a financially rewarding process. At worst, the value of the recovered hydrocarbons should virtually cover the clean-up costs. The future of biosurfactants for such uses will also be governed by the economic gain between their production and their application, where the fermentation step is expected to be the key operation in the overall process economics (Desai 1987).

The evaluation of biosurfactant use for oil sludge cleaning should consider other advantages:

1. The process uses nontoxic, nonhazardous, biodegradable and environmentally acceptable compounds, as opposed to the environmentally unacceptable chemicals now widely used.

2. The process, when optimized, may significantly reduce the operational time lost for oil storage tank cleaning from that lost with current manual cleaning methods.
3. The process reduces the amount of residual sludge to be disposed of by burning or land farming.

The successful completion of this financially rewarding clean-up operation attracted much interest from Kuwait's oil industry and may open up new applications of biotechnological and industrial biosurfactant fermentation in the Gulf region.

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