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EFFECT OF XANTHAN ON THE GROWTH OF SULPHATE-REDUCING BACTERIA IN MARINE SEDIMENTS

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ABSTRACT - Chemical additives are used extensively in the off-shore oil industry. For secondary oil recovery, the biopolymer xanthan has been recommended as a vicosifying agent but its use may provoke microbial problems. The effect of adding xanthan to marine sediments on the growth and metabolic activity of sulphate-reducing bacteria (SRB) was studied. In all instances, its addition resulted in an increase in SRB numbers of up to 250-fold. This was accompanied by a marked increase in the electron transport activity of the sediments when these were incubated under either aerobic or anaerobic conditions. The products formed as a result of the biodegradation of the polymer by indigenous heterotrophic bacteria in these sediments appear to have acted as suitable substrates for SRB growth.

Key words: xanthan, secondary oil recovery, sulphate-reducing bacteria, ETS activity.

RÉSUMÉ - Les additifs chimiques sont utilisés de façon considérable dans l'industrie pétrolière off shore. Pour la récupération assistée du pétrole, le biopolymère xanthane a été recommandé comme agent de viscosité mais son utilisation peut provoquer des problèmes microbiens. L'effet de l'addition du xanthane à des sédiments marins est étudié sur la croissance et l'activité métabolique de bactéries sulfato-réductrices (SRB). Dans tous les cas, cette addition entraîne une augmentation du nombre de bactéries sulfato-réductrices qui peut atteindre un facteur de 250. Ceci s'accompagne d'un développement important de l'activité de transport des électrons dans le sédiment, l'incubation ayant lieu en milieu aérobie ou anaérobie. Le produit formé lors de la biodégradation du polymère par les bactéries hétérotrophes du sédiment semble être utilisé comme substrat disponible favorisant le développement des bactéries sulfato-réductrices.

Mots clés: xanthane, récupération assistée, bactéries sulfato-réductrices, activité de transport des électrons.

INTRODUCTION

Sulphate-reducing bacteria (SRB) create many problems for the oil industry. Their activities are perhaps most difficult to control in the sulphate-rich environment of the North Sea. The group respire sulphate to sulphide during the anaerobic oxidation of a somewhat restricted range of organic substrates such as simple organic acids and alcohols. Some strains can, however, utilize higher fatty acids (up to C-14) and aromatic compounds as carbon sources and electron donors (Pfennig *et al.*, 1981). SRB appear incapable of growth on high molecular weight compounds such as the biopolymer cellulose. Although they are obligately anaerobic organisms, SRB can survive in oxic North Sea waters in numbers of ca 0 to 90/ml (Hardy, 1981). However, anaerobic conditions and the presence of suitable electron donors can result in rapid increases in this SRB population to ca 10^6/ml (Wilkinson, 1982).

During off-shore oil drilling, penetration of the oil-bearing rock strata releases the pressure of the gases dissolved in the oil. This energy and that of the formation water drive the crude oil from the rock reservoir up to the storage tanks on the platform or in the derrick legs. This initial flow rate diminishes rapidly and as a consequence a considerable proportion (20 to 30 %) of the crude oil remains in the reservoir adsorbed to the rock pore walls. This loss of oil is unacceptable and in order to maximize the profit from industrial exploitation secondary oil recovery is used.

Secondary oil recovery consists of injecting, under pressure, large amounts of filtered sea water into the oil reservoir. This water displaces residual oil which is still bound in the rock after the fall-off in the initial well pressure. As rock strata vary in permeability, such injection water may fail to penetrate and flush oil from the denser layers. In principle this problem can be overcome by the addition of a viscous compound to the injection water which can block the more porous strata. The biopolymer xanthan has been recommended for this purpose at a concentration of ca 0.1 % w/v.

Xanthan is a high molecular weight polymer commercially produced from the plant pathogen *Xanthomonas campestris*. It consists of a linear backbone of β glucose residues -similar to cellulose-with trisaccharide side chains of:
1) acetylated mannose
2) glucuronic acid
3) pyruvylated mannose.

Although this molecule is not attacked by SRB, it was thought that other heterotrophic organisms associated with them in the marine environment could degrade xanthan to the lower molecular weight compounds used by SRB. Therefore we investigated the effect of xanthan addition on the degradation rate of organic matter, electron transport system (ETS) activity and on SRB development in marine sediments.

ETS ACTIVITY

Degradation of organic material in a sediment occurs by aerobic respiration, in the presence of oxygen and by fermentation/anaerobic respiration in its absence. As fermentation end products are respired somewhere in the system (e.g; by SRB), xanthan decomposition can be considered to occur *via* aerobic and anaerobic respiration (Vosjan, 1982). All respiring organisms possess a respiratory chain and the quantity of electrons passing along the electron transport system per unit of time is a useful measure of the rate of respiration. ETS activity can be measured using the artificial electron acceptor INT (2-(p-iodophenyl)-3-(p-nitrophenyl)-5-phenyl tetrazolium chloride) which is reduced to INT-formazan. The quantity of this can be determined by its absorption at 490 nm. As a large abundance of electron donors (NADH, NADPH and succinate) are used in the assay the rates obtained give the metabolic potential of the sediment (Vosjan, 1982).

MATERIALS AND METHODS

Intertidal sediment from the River Forth estuary (S.E. Scotland) was collected at low tide using Perspex core tubes. The top 10 cm of a series of cores were combined and carefully mixed, to avoid as far as possible, the inclusion of air. Homogenized sediment was distributed in 17 g amounts into sterile 28 ml bottles and the following additions made (2 ml):

1) sterile sea water
2) xanthan
3) acetyl-free xanthan
4) pyruvate-free xanthan
} final concentrations 0.1 % w/v

5) glucuronic acid — final concentration 0.03 % w/v

Acetyl- and pyruvate-free xanthans were prepared as described by Dentini *et al.*, 1984). Unmodified xanthan was supplied by Sigma.

The bottles were incubated at 25°C either aerobically or under an atmosphere of 85 % N_2/10 % H_2/5 % CO_2. Initially, and after 5 days incubation, SRB shake tube counts were performed on the samples as described by Postgate (1979). ETS activity after incubation was determined on cell-free extracts of the sediments using the method of Olanczuk-Neyman and Vosjan (1977). INT was supplied by Sigma, homogenization was for 2 minutes and incubation of the assay mixture was for 10 minutes at 25°C. Any chemical reduction of the INT was determined by the method of Pamatmat *et al.* (1981).

RESULT AND DISCUSSION

SRB counts and ETS activity for the sediments are shown in table 1. ETS activity measured in unamended sediment was comparable to that reported for similar intertidal sediments in the Dutch Wadden Sea (Vosjan and Olanczuk-Neyman, 1977). Addition of xanthan, acetyl-free and pyruvate-free xanthan to aerobically and anaerobically incubated sediments lead to a marked increase in ETS activity. This was reflected by an increase

Sample	ETS activity μl O_2/g/h	SRB bacteria/g
Aerobic :		
No addition	5.5	3.3×10^2
O.1 % w/v xanthan	8.7	8.2×10^4
O.1 % w/v acetyl-free xanthan	8.1	4.1×10^4
0.1 % w/v pyruvate-free xanthan	9.0	2.5×10^4
0.03 % w/v glucuronic acid	6.4	4.9×10^3
Anaerobic :		
No addition	5.8	3.3×10^3
0.1 % w/v xanthan	7.8	8.2×10^5
0.1 % w/v acetyl-free xanthan	7.3	1.6×10^6
0.1 % w/v pyruvate-free xanthan	7.2	4.1×10^5
0.03 % w/v glucuronic acid	5.8	1.6×10^5

Table 1. Electron transport system (ETS) activity and SRB numbers in xanthan supplemented River Forth estuary sediments, after 5 days incubation at 25°C.

All values are expressed per g dry sediment

in SRB numbers of up to 250-fold in amended sediments with a concomitant production of sulphide (indicated by sediment blackening). As the ETS assay used also measures mineralization by sulphate reduction (Olanczuk-Neyman and Vosjan, 1977) it would apear that part of the increase in ETS activity in these sulphate-rich sediments,on supplementation with xanthan, was due to a stimulation of SRB. Under anaerobic conditions, the addition of deacetylated and depyruvylated xanthans to the sediments caused a lower stimulation in degradation than with unmodified xanthan. It is thought that naturally occurring xanthanase enzyme complexes attack the trisaccharide side chains of the xanthan molecule to yield mannose, glucuronic acid, pyruvylated and acetylated mannose (Cadmus *et al.* 1982). Under marine conditions these side chains protect the β $(1 \rightarrow 4)$ glucose backbone from hydrolysis by cellulases (Rinaudo and Milas, 1980). As SRB isolated from xanthan enrichments of these sediments cannot utilize xanthan, glucuronic acid or mannose it is thought that SRB growth was occurring on "xanthanase" liberated acetate, pyruvate and fermented mannose and glucuronic acid. Hence, the decrease in stimulation with the modified xanthans may have been due to a decrease in available substrates for SRB growth (i. e. acetate and pyruvate).

CONCLUSION

Xanthan has several advantages as a mobility control agent in enhanced oil recovery. These include :

1) pseudoplasticity (i.e. : shear thinning properties)
2) thermal stability
3) salt compatibility

However, the date presented above indicate that despite the implicit benefits,the risks of SRB proliferation which the addition of xanthan brings with it, militates against its use in sulphate-rich injection waters.

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