Biocorrosion

Proceedings of a joint meeting between the Biodeterioration Society and the French Microbial Corrosion Group

Edited by C.C. Gaylarde and L.H.G. Morton

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BIOCORROSION


13-14th September 1988

Edited by
C.C. Gaylarde and L.H.G. Morton
The Biodeterioration Society
The aims of the Biodeterioration Society are to promote the science and technology of biodeterioration of economic importance.

It is an international society, open to all with a scientific, technological, practical or commercial interest in biodeterioration and biodegradation.

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Foreword

In the fifth in the series of Occasional Publications of the Biodeterioration Society are the proceedings of the second of the highly successful scientific meetings of the society held on the mainland of Europe. This joint meeting of the Biodeterioration Society, the French Microbial Corrosion Group and the International Biodeterioration Research Group was held at the Museum National D'Histoire Naturelle, Paris. The meeting broke new ground for the Biodeterioration Society in having some of the eleven papers and several posters presented in French.

Some eighty delegates from nine countries who were attracted to this conference on Microbial Corrosion enjoyed a meeting which was both scientifically rewarding and socially extremely pleasant. That it was so was largely due to the sterling efforts of our Meetings Secretary, Christine Gaylarde, and to the local organisers, Marie-Francoise Libert and Jean Guzeennec, and their helpers, and to them I offer the thanks of the Biodeterioration Society.

I join with the Editors in expressing my appreciation to the contributors to this volume for providing us with excellent papers, and lastly I thank the Editors themselves, Christine Gaylarde and Glyn Morton, for putting together what is undoubtedly a very valuable addition to the literature on this important area of biodeterioration.

Brian Flannigan
President
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ABSTRACT

Une étude de la corrosion des matériaux métalliques en milieu géothermal a été réalisée grâce à une test branchée directement sur une exploitation géothermique du Bassin Parisien (Coulommiers). De nombreuses nuances d'acier ont pu être soumises à l'action corrosive du fluide (T° - 82°C; salinité - 35g/l) au cours de deux campagnes de tests (24 et 79 jours). Les coupons étaient placés dans plusieurs réacteurs, dont l'un placé en condition stérile par filtration (3 et 0.2μm).

D'après les analyses bacteriologiques réalisées, la densité de cellules est très faible dans le fluide (10⁴ à 10⁵ cellules/ml) et à la surface des coupons corrodiés (10⁴ à 10⁵ cellules/cm²). Ceci laisse supposer une influence négligeable des bactéries au niveau de l'initiation des phénomènes de corrosion dans ce milieu. Cette faible population est composée de bactéries sulfato-réductrices, ainsi que d'autres métabolismes thermophiles non encore totalement identifiés.

La cause principale de la corrosion dans ce milieu géothermique apparaît donc essentiellement liée au caractère agressif du fluide.

GEOTHERMAL CORROSION: METALLOGRAPHIC AND BACTERIAL STUDIES

Corrosion affects low-enthalpy geothermal exploitations. This study aims to characterise and quantify the various types of corrosion that affect the equipment in geothermal loops, with a view to optimising the choice of materials. Several parallel approaches have been taken - studies on metal test-pieces in situ, bacteriological and electrochemical studies on site and in the laboratory.
For the investigations on metal samples, a test pilot was set up and connected in parallel to two installations - Melleray in the Loiret department and Coulommiers in the Seine et-Marne department. Tests lasting from nine to seventy-nine days were carried out under controlled conditions, taking account of flow rates, pressure, temperature and the presence of bacteria. Results backed up observations of corrosion made during exploitation, i.e. a tendency towards generalised corrosion of carbon steel and pitting of stainless steels.

The corrosion results from the presence of dissolved salts and sulphides and from the highly reduced state of the fluids.

Bacteria are rather scarce and have a limited influence on the corrosion potential of these two fluids. The density of bacteria is $10^4$ to $10^5$ cells/ml in the fluids and $10^4$ to $10^6$ cells/cm² on the test piece surfaces. Some bacteria belong to the sulphate-reducer families, but some thermophilic strains are not yet well defined.

Certain wells exploiting the same aquifer (Dogger) present a high content of dissolved sulphide. From this a high scaling potential results. In the deposit the bacterial density reaches $10^9$ cells/g (dry weight). There are some sulphate-reducing bacteria but other strains, not determined in the analyses, contribute to this population.
INTRODUCTION

Le développement de la géothermie-basse enthalpie s’accompagne de certains problèmes techniques liés à la corrosion, qui penalisent la durée de vie des installations. Les différents matériaux métalliques utilisés se sont révélés plus ou moins sensibles à l’action corrosive des fluides géothermaux. Il est donc important de bien connaître le comportement des aciers utilisés actuellement dans les installations géothermiques, ainsi que ceux de nouvelles nuances susceptibles de constituer des solutions de remplacement, compte tenu des impératifs techniques et économiques.

L’exploitation géothermique s’effectuant par l’intermédiaire de forages profonds tubés en acier, il n’est pas aisé d’évaluer la corrosion in situ.

L’accent a été mis sur le rôle des bactéries dans ces phénomènes, étant donné l’origine naturelle du milieu exploité. Pour cela, deux approches ont été menées en parallèle :
- Des tests de corrosion sur éprouvettes placées dans une boucle de surface, en dérivation d’exploitations en fonctionnement.
- L’analyse minéralogique, chimique, biochimique et bactériologique des dépôts sur ces éprouvettes ainsi que ceux présents dans les cuvelages, récupérés en surface au cours de nettoyage de puits.

TESTS DE CORROSION SUR ÉPROUVETTES METALLIQUES

L’unité pilote de corrosion (Honegger et al, 1988) est constituée de trois réacteurs en matériau composite de 0.5m³ de contenance chacun et placés en série; un ensemble de filtration (3µm) et d’ultra-filtration (0.2µm) permettant de maintenir l’un de ces réacteurs à l’abri de la flore bactérienne. Les différents coupons d’acier sont fixés sur des plateaux en PVC et immergés dans les différents réacteurs. Cette unité pilote a été branchée en dérivation de deux installations géothermiques: Melleray (fluide du Trias, 72°C) et Coulommiers (fluide du Dogger, 83°C). La salinité totale des fluides est de l’ordre de 35g/l, sodium et chlorure étant les éléments dominants de la composition chimique. Une pompe volumétrique assurait un débit constant de fluide géothermale de l’ordre de 4-5m³/h dans les réacteurs, à une température presque identique à celle de l’installation.
Cinq campagnes de tests ont été réalisées (9,44 et 73 jours à Melleray; 24 et 79 jours à Coulommiers). Une gamme variée de matériaux métalliques a été sélectionnée pour subir ces tests de corrosion (aciers au carbone et faiblement alliés, fontes, aciers inoxydables, alliages cuivre et nickel, titane), correspondant aux matériaux déjà employés dans les boucles géothermales du bassin parisien, ou à des matériaux susceptibles de représenter des solutions de remplacement.

RÉSULTATS DES TESTS

Les types de corrosion observés sur les coupons des différents matériaux métalliques testés sont en accord avec d'autres observations déjà effectuées en milieu géothermique (Casper & Pinchback, 1980):  
- Les aciers doux (API K55) et faiblement alliés (APS 24), les fontes Ni-Resist (types 1 et D2), les alliages base cuivre (cupro-aluminium) et nickel (Monel K500) sont affectés en priorité par une corrosion généralisée dont nous avons mesuré la vitesse d'attaque par mesures de pertes de poids (Table 1). Lorsque l'on compare l'acier doux K55 et l'acier faiblement allié APS 24, on constate que l'addition d'éléments alliés tend à diminuer la vitesse d'attaque généralisée, mais favorise le développement de corrosions localisées (piqûres).  
- Les aciers inoxydables sont affectés par des corrosions localisées (piqûres, crevasses, fissures, corrosion intergranulaire) plus ou moins sévères selon les nuances considérées (Table 2). Il apparaît que les fluides géothermaux peuvent être considérés comme particulièrement agressifs vis-à-vis de la plupart des aciers inoxydables testés ici, comme les nuances 316L couramment utilisées dans les installations, ou 180 MoT. Seuls, les aciers fortement alliés comme le SANICRO 28 (27% Cr-31% Ni), ou le 290 Mo (29% Cr-4% Mo-Ti) ont montré une bonne résistance à la corrosion localisée, sans toutefois présenter une passivité totale.  
- Le titane est le seul matériau que n'a présenté aucune corrosion.

Dans le cas des matériaux affectés par la corrosion généralisée, on assiste à une stabilisation du taux de corrosion au cours du temps (durée des tests: 9 à 79 jours). Ceci met en lumière un éventuel rôle protecteur de la couche de produits de corrosion (sulfures métalliques) qui se forme à la surface des matériaux. Cette observation, faite à l'échelle des coupons-tests, ne doit cependant pas être transposée directement aux conditions réelles d'une installation, ou la continuité
de la couche de dépôts le long d'un cuvelage par exemple est plus difficile à assurer, et où les conditions hydrodynamiques sont différentes (vitesse d'écoulement beaucoup plus importante que dans l'unité pilote). Ceci est clairement démontré par les différences de vitesses d'attaque obtenues sur des coupons d'acier doux K55 placés dans les réacteurs du pilote (150 à 300 μm/an) et dans la veine fluide de l'installation de Coulommiers (400 μm/an environ). D'autre part, la présence d'une couche de dépôts peut localement aggraver l'attaque des aciers en favorisant l'individualisation de zones confinées propices au développement de la corrosion.

Les produits de corrosion sont constitués majoritairement de sulfures métalliques, dont le soufre provient du fluide et l'ion métal du matériau attaque, avec par exemple: API K55 (mackinawite, pyrrhotite), cupro-aluminium (bornite, chalcopyrite), MONEL K500 (chalcopyrite, heazlewoodite).
### Tableau 1 - Valeur moyenne des pertes d'épaisseur (µm) calculées d'après les pertes de poids mesurées pour les différents matériaux affectés par la corrosion généralisée, lors des tests de Melleray et de Coullomiers.

<table>
<thead>
<tr>
<th>Type</th>
<th>Dénomination</th>
<th>Campagnes de MELLERAY</th>
<th>Campagnes de COULOMIERS</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>9 J.</td>
<td>44 J.</td>
</tr>
<tr>
<td>Acier au carbone</td>
<td>API K55</td>
<td>7-13</td>
<td>20-22</td>
</tr>
<tr>
<td>Acier faiblement allié</td>
<td>APS 24</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fontes</td>
<td>Ni-Resist type 1</td>
<td>6-8</td>
<td>7</td>
</tr>
<tr>
<td></td>
<td>Ni-Resist type D2</td>
<td>8-9</td>
<td>14</td>
</tr>
<tr>
<td>Alliage base Cu</td>
<td>Cupro-aluminium</td>
<td>2-5</td>
<td>3-6</td>
</tr>
<tr>
<td>Alliage base Ni</td>
<td>HONEL K500</td>
<td>2-3</td>
<td>3</td>
</tr>
</tbody>
</table>

### Tableau 2 - Profondeurs maximales des piqures et crevasses relevées sur les coupons de différents aciers inoxydables lors des tests à Melleray (73 jours) et Coullomiers (79 jours). Les valeurs données sont indicatives, sachant que la profondeur des corrosion localisées n'est pas forcément fonction du temps d'attaque.

<table>
<thead>
<tr>
<th>Type</th>
<th>Dénomination</th>
<th>A.F.N.O.R.</th>
<th>Test de 73 jours</th>
<th>Test de 79 jours</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Prof.maxi</td>
<td>Prof.maxi</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>piqures</td>
<td>crevasses</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>(µm)</td>
<td>(µm)</td>
</tr>
<tr>
<td>Ferritiques</td>
<td>180 Hot</td>
<td>26 CDT 18-02</td>
<td>150</td>
<td>380</td>
</tr>
<tr>
<td></td>
<td>250 Ho</td>
<td>22 CDT 29-04</td>
<td>60</td>
<td>30</td>
</tr>
<tr>
<td>Austénitiques</td>
<td>316L Sanicro 28</td>
<td>22 CND 17-12</td>
<td>50</td>
<td>300</td>
</tr>
<tr>
<td></td>
<td></td>
<td>21 NDU 31-27</td>
<td>-</td>
<td>200</td>
</tr>
<tr>
<td>Austéno-ferritiques</td>
<td>SAF 22-05</td>
<td>25 CND 21-08</td>
<td>-</td>
<td>50</td>
</tr>
<tr>
<td></td>
<td>URANUS 3SM</td>
<td>25 CN 23-04</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>URANUS 5SM</td>
<td>25 CND 22-06</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>URANUS 52N</td>
<td>25 CHD 26-06</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Martensitiques</td>
<td>SOLEIL A7</td>
<td>ZNO C1®</td>
<td>180</td>
<td>20</td>
</tr>
<tr>
<td></td>
<td>SOLEIL 95</td>
<td>250 CD15</td>
<td>180</td>
<td>30</td>
</tr>
<tr>
<td></td>
<td></td>
<td>26 CHU 17-04</td>
<td>160</td>
<td>5</td>
</tr>
</tbody>
</table>
Introduction

Outre la corrosion liée à la nature même du milieu et aux conditions hydrodynamiques rencontrées en exploitation, la présence de micro-organismes est susceptible d’initier, par divers mécanismes, des phénomènes de dégradation des matériaux. Cette initiation trouve ses origines dans la présence physique de ces microorganismes sur les surfaces considérées (confinement, chelation de métaux, catalyse enzymatique de certaines réactions électrochimiques), mais aussi dans l’activité de ces microorganismes (dépolarisations anodique et cathodique, formation de métabolites organiques et inorganiques corrosifs, etc).

Le but de ces travaux était de pouvoir procéder à un certain nombre d’analyses biochimiques et microbiologiques destinées à mettre en évidence la présence éventuelle de microorganismes dans le milieu géothermal, pouvant jouer un rôle dans les phénomènes de corrosion.

Ces études bactériologiques concernant le site de Coulommiers ont été effectuées en liaison avec les deux tests de corrosion qui s’y sont déroulés.

Les échantillonnages réalisés à la fin de chacun de ces deux tests concernaient à la fois les coupons métalliques corrodiés et les fluides géothermaux contenus dans les deux types de réacteurs, filtré et non filtré.

Toutes les analyses bactériologiques et biochimiques ont été réalisées par les laboratoires de l’IFREMER (Centre de Brest) et l’Université de Bretagne occidentale.

Méthodes

1. Techniques d’échantillonnage

- Bactéries libres - A chaque niveau de prélèvement, deux échantillons ont été collectés simultanément dans des flacons stériles. Un de ces échantillons a été directement fixé au formaldéhyde (concentration finale 2%) et l’autre traité frais sur le site de prélèvement.
- Bactéries fixées - Chaque surface métallique a été grattée sur sa totalité à l’aide d’un scalpel en plastique stérile. Les reliquats
ont été récupérés dans 10ml d'eau stérile, agités 60mn sur vortex et traités de la même manière que les prélèvements liquides.

2. Numération de la flore bactérienne totale
   Pour chaque échantillon, 5ml ont été colorés à l'acide orange (concentration finale 0.01%) pendant deux minutes, puis filtrés sur un filtre Nucléopore (porosité 0.2μm) préalablement noirci au noir irgalan. Les numérations se font en épifluorescence sur un microscope Olympus BH2, par comptage de 20 champs microscopiques pris au hasard, au grossissement X1000. Certaines surfaces métalliques ont été directement observées au microscope électronique à balayage (JEOL type JSM35), après déshydratation à l'alcool et métallisation.

3. Numération des flares bactériennes viables
   - Flore hétérotrophe - Le nombre de bactéries hétérotrophes a été déterminé à partir de l'échantillon frais, par étalonnage sur milieu gélosé 2216E et à salinité 4% de 0.1ml de l'échantillon à la dilution appropriée. Chaque étalonnage est quadruplé. Respectivement deux étalements de chaque dilution ont été incubés à 22°C en anaérobiose (microflore hétérotrophe anaérobie facultative). Les lectures se font respectivement après 8 jours et 21 jours d'incubation.
   - Bactéries sulfatoréductrices - Milieu Abd & Malek (1958) modifié par l'addition de 1.5g/l d'acétate de sodium.
   Ces milieux sont incubés pendant trois semaines à 55°C et les lectures effectuées à l'aide des tables de McCrady.

RÉSULTATS OBTENUS APRES LE PREMIER TEST DE COULOMMIERS (24 jours)

Les analyses biochimiques réalisées sur le site de Coulommiers à l'issue du premier test illustrent une colonisation faible de l'ensemble des échantillons.

En milieu filtré (réacteur III), il n'apparaît qu'une faible différence de population selon les nuances métalliques rencontrées.
Les résultats des analyses microbiologiques sont reportés dans le tableau 3. Il apparaît que les densités totales de bactéries libres dans ce milieu varient d'un facteur 10, selon que l'eau soit filtrée ou non, respectivement $1.64 \times 10^6$ cellules/ml pour l'eau non filtrée et $2.6 \times 10^4$ cellules/ml après filtration (Table 3).

<table>
<thead>
<tr>
<th>Prélèvement</th>
<th>Flore totale</th>
<th>Bactéries Sulfate-réductrices</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Epifluorescence</td>
<td>Microscope électronique</td>
</tr>
<tr>
<td>Eau réacteur non filtré (1)</td>
<td>$1.64 \times 10^6$</td>
<td>-</td>
</tr>
<tr>
<td>Eau réacteur filtré (1)</td>
<td>$2.6 \times 10^4$</td>
<td>1</td>
</tr>
<tr>
<td>316 L (eau filtrée) (2)</td>
<td>$1.8 \times 10^5$</td>
<td>$4.1 \times 10^5$</td>
</tr>
<tr>
<td>Acier doux (eau filtrée)</td>
<td>$4.6 \times 10^4$</td>
<td>-</td>
</tr>
<tr>
<td>290 Mo (eau filtrée)</td>
<td>$3.7 \times 10^4$</td>
<td>$4.8 \times 10^5$</td>
</tr>
<tr>
<td>290 Mo (eau non filtrée)</td>
<td>$4.27 \times 10^4$</td>
<td>-</td>
</tr>
<tr>
<td>Alliage Cu et Ni (eau non filtrée)</td>
<td>$4.7 \times 10^4$</td>
<td>-</td>
</tr>
</tbody>
</table>

Tableau 3 - Densités bactériennes dans les fluides et à la surface des coupons métalliques

(1) Nombre de cellules observées dans le fluide par ml.
(2) Nombre de cellules fixées à la surface des matériaux après immersion (nombre de cellules par cm²).

Quelque soit la nature du support, les densités totales de cellules fixées sont similaires et varient de $1.8 \times 10^6$ à $3.7 \times 10^4$ cellules/cm². Les observations au microscope électronique à balayage de la surface de l'acier 316L permettent de noter que la majorité des bactéries sont des bacilles de tailles diverses. Observées à différents grossissements, ces surfaces apparaissent finement fracturées et couvertes par un léger dépôt organique et/ou inorganique.
RESULTATS OBTENUS APRÈS LE SECOND TEST DE COULOMMIERS (79 jours)

1. Analyses biochimiques

Les analyses biochimiques ont été réalisées sur une certain nombre de coupons métalliques qui, une fois retirés des différents réacteurs, ont été immédiatement plongés dans l'azote liquide pour traitements ultérieurs. Ces analyses ont été réalisées dans le but de déterminer la biomasses eubactérienne présente sur les différents supports métalliques mais aussi dans le but de rechercher d'autres microorganismes, et plus particulièrement les archeobactéries, groupe bactérien comprenant notamment les bactéries méthanogènes.

Les chromatogrammes réalisés sur les échantillons (sept en milieu filtré, sept en milieu non filtré) illustrent une population eubactérienne relativement faible.

La distribution d'acides gras dérivés des diacyl-phospholipides montre une forte proportion d'acides saturés par rapport aux acides insaturés, qui ne représentent que 10% des acides gras totaux. Cette forte proportion d'acides saturés est une réponse des microorganismes aux conditions extérieures, principalement à la température.

Malgré l'apparente faible biomasse eubactérienne présente sur les divers supports, il convient cependant d'observer que les deux nuances d'acier K55 et APS 24 ainsi que l'alliage de cuivre (cupro-aluminium) se différencient assez sensiblement des autres matériaux par une colonisation plus importante.

D'autres investigations ont également été menées afin de mieux cerner la structure de la communauté bactérienne présente sur les différents substrats et dans le fluide géothermal. Ainsi, des analyses portant non plus sur les constituants de la membrane cytoplasmique bactérienne, mais sur les constituants de la paroi cellulaire et plus spécifiquement sur les acides gras hydroxylés dérivés d'un constituant principal de cette paroi, les lipopolysaccharides, ont été réalisées. Ces analyses par chromatographie en phase gazeuse et couplage chromatographie-spectrométrie de masse, ont confirmé la faible population de bactéries Gram-negative tant sur les différents substrats que dans le fluide géothermal. Ces chromatogrammes se caractérisent à la fois par la présence d'acide gras hydroxylés iso et ante-iso C15:0 et C17:0, composés que l'on trouve principalement chez les bactéries sulfato-réductrices.
La présence d'archéobactéries, qui se distinguent des eubactéries par certain critères biochimiques, et notamment l'absence de diacrylphospholipides dans la membrane cellulaire, a été recherchée. Ces analyses n'ont pas révélé une présence quantitative significative de ce type de microorganismes dans le fluide géothermal ou sur les différents échantillons analysés.

A l'ensemble de ces analyses biochimiques, il conviendra cependant d'ajouter la très grande similitude observée entre certains chromatogrammes effectués sur les échantillons les plus colonisés et la distribution d'acides gras observée sur une culture de bactéries sulfatoreductrice Desulfitomaculum geothermicum récemment isolée et purifiée dans certains puits géothermiques (Daumas, 1987). Cette bactérie se caractérise par une distribution d'acides gras de la membrane cytoplasmique tout-à-fait particulière (J. Guezennec, communication personnelle), avec notamment la présence de composés inhabituels dont la structure complète n'a pas encore été totalement déterminée. Des investigations en ce sens sont actuellement en cours afin de relier, le cas échéant, la présence de ces microorganismes au comportement électrochimique de certain matériaux.

2. Analyses chimiques

Les analyses réalisées par chromatographie en phase gazeuse n'ont révélé que la présence en faible quantité d'acides gras volatils, avec comme principaux composés, les acides acétiques, propioniques et butyriques.

Par ailleurs, lors de la séparation en différents classes des lipides extraits des surfaces métalliques analysées, il a été observé la présence en quantité non négligeable de produits peu polaires. Des analyses effectuées sur ces produits ont permis de déterminer qu'il s'agit d'alcanes, mais surtout qu'ils étaient constitués également d'une grande quantité de composés non identifiés. Des analyses plus détaillées ont alors été effectuées sur la fraction aliphatique de ces composés et ont permis de distinguer une fraction alcane C14 à C30, avec comme composé majoritaire le nC17, et une seconde fraction de composés indéterminés, en proportion élevée, ne correspondant pas aux alcanes ramifiés couramment rencontrés dans le pétrole.
Dans certains puits (Zone nord de Paris), une quantité importante de dépôts s'accumule au cours de l'exploitation, ce qui a nécessité des opérations de curage (Sainson & Honegger, 1986; Menjoz & Honegger, 1986). Cette zone est caractérisée par une concentration importante de sulfures dans le fluide (>30mg/l).

1. Nature et structure des dépôts

La présence de sulfures de fer est massive (plus de 70% quels que soient le site et le rôle du puits, producteur ou injecteur): pyrite, pyrrhotite et mackinawite sont les principaux constituants. Les hydroxydes, hydroxychlorures et carbonates de fer (sidérite) sont systématiquement observés mais en moindre importance. La calcite apparaît surtout dans les échantillons de fond de puits (Honegger & Criaud, 1986).

Il n'y a pas de phase amorphe de FeS dans les dépôts étudiés. D'autre part, on observe une grande homogénéité dans la nature et la morphologie des sulfures de fer, quelle que soit la granulométrie (de 0.1μ à plusieurs centimètres d'épaisseur). De nombreux éléments métalliques (Cr, Ni, Cu, As, Mo) sont présents dans les dépôts à l'état d'impuretés, mais sans constituer de phases sulfurées exprimées.

Les sections polies montrent une structure rythmique, dans laquelle mackinawite, pyrite, pyrrhotite, hydroxydes de fer et parfois carbonates sont intimement associés soit en couches, soit en agglomérats. La chronologie des précipitations n'est pas toujours aisée à préciser, car de nombreuses figures de dissolution-recristallisation apparaissent. Quelques grains ont manifestement été transportés par le fluide et peuvent avoir grossi et s'être associés avant de se fixer sur les parois des tubages: les agencements en cocarde de certaines observations en sont les témoins.

Ceci met en évidence le caractère dynamique des phénomènes qui conduisent aux dépôts observés: précipitation initiale sans doute de mackinawite et de produits de corrosion (Fe₂(OH)₃Cl), évolution des phases minérales par vieillissement et migration d'éléments ... Les zones de confinement sont également des milieux privilégiés (conditions physico-chimiques extrêmes, migration d'ions ...) pour l'apparition de phases secondaires. Les différentes couches peuvent également refléter les variations...
saisonnières des régimes d'exploitation des puits: débit, température et pression de reinjection entre été-hiver, jour-nuit. Il faut noter toutefois que l'agencement des dépôts en couches de compositions et structures différentes est classiquement décrit dans la littérature relative à la corrosion par H₂S dans différents milieux (Honegger et al., 1988), la croissance du film du sulfures étant généralement attribuée à un mécanisme de diffusion de Fe²⁺ à travers le dépôt.

2. Analyses biochimique et bactériologique

Au cours du nettoyage du puits de production de la Courneuve nord, les analyses ont été réalisées sur trois échantillons en tête de puits:
- fluide géothermal produit,
- dépôt dont l'origine se situe à -450m de profondeur,
- dépôt dont l'origine se situe à -600m de profondeur.

Résultats:

L'analyse de lipides révèle la présence d'acides gras d'origine bactérienne tels les acides saturés iso et anté-iso C15:0 et C17:0, mono-insaturés C18:1ω7C et cyclopropaniques cyclo C17:0 et C19:0. La mise en évidence de certains biomarqueurs tels les acides iso et anté-iso C17:1ω7 est à relier à la présence de bactéries sulfato-réductrices sur les divers échantillons analysés, sans que toutefois cette microflore apparaisse abondante.

Les densités bactériennes observées dans les prélèvements de fluide et de dépôts à différentes profondeurs sont reportées dans le tableau 4.

<table>
<thead>
<tr>
<th>Prélèvement</th>
<th>Flore totale</th>
<th>Sulfato-réductrice</th>
<th>Sulfo- oxydantes</th>
<th>Hétérotrophe aérobie Anaérobie</th>
</tr>
</thead>
<tbody>
<tr>
<td>Eau forage*</td>
<td>2.3 x 10⁴</td>
<td>2.5 x 10⁴</td>
<td>10</td>
<td>-</td>
</tr>
<tr>
<td>Dépôt -450 m</td>
<td>1.65 x 10³</td>
<td>-</td>
<td>428</td>
<td>-</td>
</tr>
<tr>
<td>Dépôt -600 m</td>
<td>1.47 x 10³</td>
<td>8 x 10³</td>
<td>40</td>
<td>400</td>
</tr>
</tbody>
</table>

Tableau 4 - Densités bactériennes exprimées en nombre de cellules par gramme de sédiment sec, sauf * qui est exprimée en nombre de cellules/ml.
Il apparaît que la densité totale de cellules en suspension dans le fluide est assez faible (2.3 x 10⁴ cellules/ml). Elle est beaucoup plus importante dans les sédiments réduits, prélèves à -450 et -600 m de profondeur, respectivement 1.65 x 10⁹ et 1.47 x 10⁸ cellules par gramme de dépôt sec.

Quelque soit le prélèvement, les densités de cellules viables, correspondant aux différents métabolismes recherchés, sont très faibles, et, sauf pour les bactéries sulfato-réductrices, elles sont inférieures aux seuils de détection choisis. D'autres métabolismes non recherchés dans cette étude paraissent contribuer de façon importante à la microflore bactérienne analysée dans les divers échantillons.

**CONCLUSION**

Les tests de corrosion ont mis en évidence le caractère particulièrement agressif des fluides géothermaux utilisés pour cette étude, même vis-à-vis des aciers inoxydables fortement alliés.

Les densités totales de cellules en suspension dans les différents fluides analysés sont relativement faibles. Il en est de même en ce qui concerne la colonisation des éprouvettes métalliques: le facteur biologique ne semble pas jouer un rôle important dans la corrosion sur le site de Coulommiers.

Par contre, dans une zone où le fluide présente un potentiel important de dépôt, il apparaît une grande différence entre les densités totale et viable observées; ce qui laisse supposer que ce sont des cellules mortes et/ou que les métabolismes recherchés dans cette étude préliminaire ne sont pas appropriés.

Dans ce cas, il n'est pas encore possible de conclure si le développement de ces bactéries se trouve seulement facilité par la couche de sulfures de fer, ou si ces microorganismes sont à l'origine ou participent activement à la croissance de ces dépôts.


A wide range of aerobic and anaerobic bacteria have been isolated from the surface of 90/10 copper-nickel and its associated biofilm. Many of these are involved in the complex of sulphur transformations known to occur in microbial communities but particularly in pathways involving thiosulphate. This ion is a product of both SRB activity and of atmospheric oxidation of reduced sulphur compounds and is highly active in the corrosion process on copper alloys as well as ferrous alloys. The microbial activities will be considered in relation to copper alloy corrosion processes.
Evaluation of the cement degradation induced by the metabolic products of two fungal strains


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Abstract

During their metabolism, microorganisms can produce organic acids able to induce the degradation of cement. Two acidifying fungal strains had been isolated from a soil sample. One is an Aspergillus strain which produces gluconic and oxalic acids. These acids induce (after eight months of contact) a dissolution of portlandite (without leaching of calcium), an increase in the cement porosity (+ 8.2%), a loss of the bending strength (- 78%). The second strain, unidentified, produces gluconic and citric acids, responsible (in the same period) for a very important dissolution of portlandite, a creation of ettringite crystals, an increase in the porosity (+ 6.6%), a loss in the bending strength less important than in the other case.

Au cours de leur métabolisme, les microorganismes peuvent excréter des acides organiques corrosifs pour le ciment. Deux souches fongiques acidifiantes ont été isolées d'un échantillon de sol. L'une est une souche d'Aspergillus qui produit des acides gluconique et oxalique. Ces deux acides provoquent (après 8 mois de contact) une dissolution de la portlandite (sans lixiviation de calcium), une augmentation de la porosité du ciment (+ 8.2%), une perte de la résistance mécanique en flexion (- 78%). La seconde souche, non identifiée, produit des acides gluconique et citrique, responsables (dans la même période) d'une dissolution très importante de portlandite, d'une création de cristaux d'ettringite, d'une augmentation de la porosité (+6.6%), d'une perte moins importante que dans le cas précédent de la résistance en flexion.
INTRODUCTION

Cement and concrete are amongst the matrices studied for the coating of low- and intermediate level radioactive wastes, with a view to their long term underground storage. The aim of this coating is to prevent radionuclide dispersion into the biosphere. The waste repositories can be geological formations sustaining microbial life (West et al., 1982) and it is therefore necessary to evaluate the biodegradability of this material.

Artificial Portland Cement contains, after hydration of the silicates, mainly crystals of hydrated calcium silicate (C-S-H) and portlandite (Ca(OH)$_2$) (Dubois, 1977; Drex, 1964). These crystals delimit pores in which an interstitial liquid phase circulates. This liquid phase contains Ca and OH ions in equilibrium with the portlandite crystals.

Examples of deterioration of stones or cement (mainly in sewer systems) have often been described. In the sewer systems, this degradation is due to the development of sulfooxidizing bacteria (Parker, 1945a and 1945b; Milde et al., 1983) which are autotrophic bacteria; but heterotrophic microorganisms can also induce degradation (Jaton, 1974). In this case it is the production of organic acids which causes the alteration. Fungi are heterotrophic microorganisms, they often grow on the surface of stone or other materials and they can produce carboxylic acids (Strzelczyk, 1981).

Three wild strains of microorganisms able to grow near cement (in alkaline conditions) and to produce organic acids have been isolated. An experimental system (figure 1) is used to bring into contact the cement and the microbial cultures. The aim of this paper is to report the modifications shown by the cement samples in contact for eight months with:

(i) an *Aspergillus niger* strain,

(ii) an unidentified fungal strain. The third strain (*Pseudomonas* strain) is also tested in the same experiments but the results obtained are less significant and not presented here.
Figure 1

Experimental system used for simulation of contact between a cement sample and a microbial culture

- Microbial culture
- Peristaltic pump
- Determination of acids, pH, glucose
- Cement sample

1 mm
MATERIALS AND METHODS

Microbial Strains and Cultures

The strains used are two fungal strains isolated from a natural soil sample (1) and a natural granitic mineral sample (2). The first strain has been identified; it is an *Aspergillus niger* strain (Fassatovia, 1986). The second strain is not, as yet, identified because it doesn’t form spores. The culture medium used contains glucose (20 g/l) as substrate; the pH is 5.6.

Cement Sample

The cement used in this study is an artificial Portland cement. The sample is a disk (thickness 4 mm; diameter 70 mm).

Analysis performed before the cement-culture contact: the acids produced from the decomposition of the glucose by the strain are determined by high-performance liquid chromatography (Guenant et al, 1982). The consumption of glucose (by enzymatic method) and the medium pH are followed.

Analysis performed after the cement-culture contact: Calcium release: the medium passed through the cement is periodically collected. Its calcium content is measured by atomic absorption spectrometry. Physico-chemical analysis of cement: after the contact, the modifications induced in the cement are followed by determination of pore volume and pore size (measured after a full desiccation, by intrusion of mercury); determination of the Portlandite crystal content by a thermo-gravimetric method; determination of the bending strength (three points method); identification of some constituents (like quartz, portlandite, ettringite, C-S-H, non hydrated silicates) by X-ray diffraction (Lea, 1970). Before this last test the samples are powdered and sifted (0.063 μ).
RESULTS

Microbial Growth and Acid Production

The two fungal strains utilise glucose as substrate and produce organic acids as metabolites.

- Aspergillus Niger strain. The pH of the medium falls from 5.6 to 2.1 during the metabolism of glucose. The acids produced (determined by HPLC) are mainly gluconic and oxalic acids. The decomposition of 111 mmol of glucose goes with the production of 15 mmol of gluconic acid and 30 mmol of oxalic acid.

- Fungal strain. The pH of the medium falls during growth from 5.6 to 3.5. The acids produced are gluconic (11 mmol) and citric acid (13 mmol).

These acids are known for being able to solubilize mineral elements such as Ca, Si, Al, Fe (Berthelin, 1976; Berthelin and Domergues, 1972).

With the experimental system used, the mycelia can grow on to the cement samples. We can observe an important growth of the Aspergillus strain on the cement surface.

Calcium Release

The release of calcium produced by the organic acids is compared to the release from a control, in contact with the culture medium (pH 5) without acids or microorganisms (figure 2). The culture medium (pH 5) causes a calcium leaching of 60 mg during the first month. In the same period, the sample in contact with the fungal strain loses 100 mg of calcium and that in contact with the Aspergillus loses 6 mg. The quantity of calcium released is not in proportion with the quantity of acids. It is the nature of the acids which determine the amount of calcium solubilized from the cement (Berthelin, 1972; Berthelin, 1976); this calcium comes from either the interstitial liquid phase or the Portlandite crystals. The calcium oxalate is sparingly soluble and precipitates in the pores. This precipitation can also prevent the entry of the gluconic acid into the pores. On the contrary, the association of gluconic and citric acids causes an important release of calcium.
Physico-chemical Modifications of the Cement Sample

Variation of the porosity

The total pore volume measured with mercury can be divided into smaller volumes, depending on the pore family. The porosity of the samples in contact with the microbial cultures is compared to the porosity of a control as for the calcium release (Table 1). However, in this case the control sample was not in contact with a culture medium. It is generally admitted that an increase in the number of pores of the second family (0.9 > d > 0.6 \( \mu m \)) corresponds with a dissolution of portlandite crystals. The contact with the organic acids and the mycelium of the Aspergillus strain causes an increase of the total porosity of the sample: + 8.26\%.

We can observe that only the number of the small pores (d < 0.009 \( \mu m \)) does not increase. More important increases are observed for the second and third family of pores (+ 4.42\% and + 3.3\%). This indicates a dissolution of portlandite, and another phenomenon. For the sample in contact with the acids and the mycelium of the fungal strain, we can also notice an increase of the total porosity (+ 6.6\%). It is mainly the number of pores which have a diameter between 0.06 and 0.9 \( \mu m \) that increase less than in the other sample (+ 0.6\% and + 2.46\%).

**TABLE I**

Porosity of the cement sample in contact for eight months with the two fungal cultures and of the control (the porosity is expressed as the percentage of pore volume compared with the total solid and pore volume)

<table>
<thead>
<tr>
<th>Pore diameter</th>
<th>Pore volumes (%) of the sample in contact with</th>
<th>Fungal strain</th>
<th>Aspergillus</th>
<th>Control</th>
</tr>
</thead>
<tbody>
<tr>
<td>D&gt;0.9 ( \mu m )</td>
<td>1.37</td>
<td>3.18</td>
<td>0.72</td>
<td></td>
</tr>
<tr>
<td>0.9&gt;d&gt;0.06 ( \mu m )</td>
<td>7.26</td>
<td>5.95</td>
<td>1.53</td>
<td></td>
</tr>
<tr>
<td>0.06&gt;d&gt;0.009 ( \mu m )</td>
<td>11.03</td>
<td>14.33</td>
<td>11.03</td>
<td></td>
</tr>
<tr>
<td>d&lt;0.009 ( \mu m )</td>
<td>6.81</td>
<td>6.66</td>
<td>6.58</td>
<td></td>
</tr>
<tr>
<td>Total porosity</td>
<td>26.48</td>
<td>28.13</td>
<td>19.87</td>
<td></td>
</tr>
</tbody>
</table>
X-ray diffraction

The spectra of X-ray diffraction for the sample in contact with the Aspergillus strain and the fungal strain are in figures 3 and 4 respectively. They are compared with the spectra for a control sample not in contact with micro-organisms, acids or culture medium. In the spectra we can recognize the peaks of the portlandite calcium silicate ($C_2S$ and $C_3S$). The method used does not allow the quantification of the different constituents. The sample in contact with the Aspergillus strain (gluconic and oxalic acids) shows a decrease of the portlandite crystals and no change for the other crystals. The sample in contact with the fungal strain (which produces gluconic and citric acid), on the contrary, shows two kind of aberration: (i) an important loss of portlandite; (ii) an important appearance of ettringite crystals (calcium sulfoaluminate). This last phenomenon has been observed on cement corroded by sulfooxidizing bacteria (Morgan et al, 1983). These new ettringite crystals can be responsible for the fact that the number of big pores does not increase in this sample: the ettringite crystals are bigger than the portlandite ones. But the amount produced is not enough to create microfissures.

Figure 2

Release of calcium from the sample in contact with the two fungal strains and from the control.
Figure 3

X-ray diffraction spectrum of the control and the sample in contact with the *Aspergillus* strain.

**Control**

**Sample in contact with the *Aspergillus* strain**
Sample in contact with the fungal strain

Ettringite
Ca(OH)$_2$

Ettringite, Ca(OH)$_2$

Ettringite, C-S-H; C-S

Ettringite

Ca(OH)$_2$

Ettringite

Ca(OH)$_2$

Control

Ettringite

Ca(OH)$_2$

C-S-H; C-S

Ettringite

Ca(OH)$_2$

X-ray diffraction spectrum of the control and the sample in contact with the fungal strain.

Figure 4
Content of portlandite

Thermogravimetric analysis allows measurement of the portlandite by following the weight loss of the cement sample between 420 and 480°C. In this range, the Ca(OH)\(_2\) is broken up into CaO and H\(_2\)O. The results available concern the samples brought into contact for four months with the microbial cultures. The sample in contact with the *Aspergillus* strain contains 9.88% W/W of portlandite that corresponds with a loss of 29% in comparison with the control. The sample in contact with the fungal strain and its acids contains after four months 9.04% W/W of portlandite. This indicates a loss of 36%. If we correlate these results with those of X-ray diffraction, we can evaluate the loss of portlandite for the samples in contact for eight months with the strains: about 45% with the *Aspergillus niger* strain and about 70% with the fungal strain.

Bending Strength

The bending strength is expressed by the strength which, applied on the surface (on three points) of the sample, causes its breakage. The control is a sample which was not in contact with the culture medium or acids. When it is brought into contact with oxalic and gluconic acids produced by the *Aspergillus* strain and with the mycelium grown on the surface, the cement shows a loss in the bending strength. This loss is particularly marked from four months (-55%). At eight months, this loss exceeds 78%. For the sample brought into contact with the fungal strain, we can say that the structural and chemical modifications induced in the cement lead to a loss of the bending strength less important than that observed with the *Aspergillus* strain. This is perhaps due to the creation of ettringite crystals in the big pores rigidifying the structure.

**TABLE 2**

Bending strength (measured by the three points method) of cement sample in contact with microbial strain. The strength is expressed in Newtons.

<table>
<thead>
<tr>
<th>Time of contact (months)</th>
<th>Microbial strains</th>
<th>Control</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td><em>Aspergillus</em></td>
<td>Fungal strain</td>
</tr>
<tr>
<td>0</td>
<td>420</td>
<td>-</td>
</tr>
<tr>
<td>1</td>
<td>382</td>
<td>420</td>
</tr>
<tr>
<td>2</td>
<td>205</td>
<td>350</td>
</tr>
<tr>
<td>8</td>
<td>100</td>
<td>-</td>
</tr>
</tbody>
</table>
DISCUSSION

In the soil, the groundwater can transport organic or inorganic matter and microorganisms far from the initial point (Dommergues and Mangenot, 1970). The organic acids produced by heterotrophic microorganisms are known for being able to generate physico-chemical modifications in the cement (Lea, 1970). At the laboratory, an experimental system is used to simulate the running of groundwater on cement samples. It was established that the organic acids can solubilize mineral elements like Ca, Si, Al, Fe ... but the acid concentration is less important than the nature of the acid (Berthelin, 1972; Berthelin, 1976; Krumbein, 1972).

In our experiments two strains are tested which produce both gluconic acid and another acid: oxalic acid for an Aspergillus strain and citric acid for an unidentified fungal strain.

The association of gluconic and oxalic acids induces the solubilization of portlandite (\(\text{Ca(OH)}_2\)) but without calcium leaching. The calcium oxalate precipitates in the cement pores and the calcium concentration in the interstitial liquid phase decreases. To restore the equilibrium between the solid (\(\text{Ca(OH)}_2\)) and dissociated (\(\text{Ca}^{2+} + \text{OH}^-\)) portlandite forms, crystals are dissolved. The loss of portlandite is apparently responsible for the fall of the bending strength of the material. The new calcium oxalate crystals don't take the place of the portlandite. Furthermore, the effects of the fungal growth directly on the cement remain to be evaluated.

The association of gluconic and citric acid gives an important portlandite dissolution, in addition to the calcium leaching. New ettringite crystals appear in the cement but the mechanism of their formation is not explained. These crystals allow that the total porosity of the cement does not increase too much and that the loss of the bending strength is not very important.

In conclusion, we can say that it is not only the pH and the acid concentration of the water which must be considered, but also the nature of the acid, to predict the behaviour of the cement and the alteration induced. We can also say that microorganisms are able to produce enough acids for modifying the constituents of the cement. In anaerobic conditions, the bacteria can produce an important quantity of acids, (Dommergues and Mangenot, 1970) not tested in this study.
ACKNOWLEDGEMENTS

We wish to thank Miss G Moine and Miss C Peyre (DRDD/SESD/SECBO) for providing the physio-chemical analysis and Mrs V Celi (DRDD/SDFM/SEATN) for her technical assistance.
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DETECTION OF SULPHATE-REDUCING BACTERIA

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ABSTRACT

New media allowing the detection of sulphate-reducing bacteria (SRB) are described in this paper. Detection is more sensitive and more rapid than with kits routinely used in the ELF AQUITAINE group. The basal medium contains two carbon sources (lactate and acetate) and a sulfur free reducing agent (titanium citrate).

A concentrated nutrient medium having the same characteristics is used in an automated SRB detection system elaborated for the PLATINE project (central unmanned production platform). The automated detector is described here.

Des kits de détection des bactéries sulfato-réductrices (BSR) plus sensibles que les divers kits utilisés dans le group ELF AQUITAINE ont été mis au point. Ils se caractérisent par l'addition de deux sources de carbone (lactate et acétate) et l'utilisation d'un agent réducteur non souillé (citrate de titane).

Un milieu nutritif concentré ayant les mêmes caractéristiques est utilisé dans le détecteur automatique de BSR conçu dans le cadre du projet PLATINE (plateformes inhabitées). Cet automate est décrit dans cet article.
INTRODUCTION

The development of sulphate-reducing bacteria (SRB) in industrial plants where water is present is responsible for considerable economic losses due to corrosion of installations or pollution of products (Cord-Ruwisch et al., 1987; Obuekwe and Westlake, 1987; Postgate, 1984). Efficient detection of these microorganisms is necessary to monitor and control their development by appropriate means (Hamilton, 1983; Postgate, 1984).

Although very rapid detection kits which use antibodies raised against APS reductase were recently developed by the Du Pont Company (Tatnall et al., 1988), their relatively low sensitivity and high price limit their use mainly to cases of heavy contamination where very rapid diagnosis and treatment are necessary. Routine checking for SRB development can be done by the usual culturing techniques.

The most widespread method of detection uses conventional anaerobic broth bottle inoculation as described in the API RP 38 recommended practice (American Petroleum Institute, 1965). Samples are inoculated in an adequate growth medium containing iron and sulphate. The production of $H_2S$ during the growth of SRB causes a blackening of the medium by formation of a FeS precipitate. Unfortunately, the composition of one particular medium may select certain strains, impeding the detection of metabolically or physiologically different ones.

Our experience with the manipulation of varied strains of SRB, both from laboratory collections or from the field, has shown that the sensitivity of this method could be improved if: (1) the basal medium was complemented with another carbon source in addition to the commonly used sodium lactate, (2) the reducing agent was sulfur free, since we have observed that some strains can be inhibited by sulfur containing reducers like cysteine. Such a reducer could also eliminate false positive responses obtained with some microorganisms. The SEBR medium A defined in this paper is compared with different test-kits used in the Elf-Aquitaine group.

Our teams are implicated in an SNEAP (Societe Nationale Elf Aquitaine Production) project called "PLATINE". PLATINE is a research project aimed at determining specifications of a central unmanned production platform, yearly (or half-yearly) visited, and remotely controlled from the shore. An automated SRB detection system was conceived for continuous
monitoring of SRB development in the water injection process. We devised an adapted detection medium to be used in this system which takes into account the preceding considerations. The automated detector is briefly described in this paper.

MATERIALS AND METHODS

Bacterial Strains

Growth of mesophilic and thermophilic strains, from fresh or salt water, was tested by the various detection kits. The strains were obtained from international collections or were isolated from industrial ecosystems. Their characteristics are listed in Table 1.

Detection Kits

The kits we have tested are listed in Table 2, with their origin and main characteristics. Commercial kits (D,F) were used according to the manufacturers' instructions.

The compositions of the SEBR media A and B differ slightly. Media A1 and B1 were devised for the detection of fresh water strains whereas A2 and B2 were devised for the detection of salt water strains. Their compositions are given in Table 3. Before dispensing the media into sterile penicillin bottles, reducing agent and FeSO₄ solutions were added according to the following procedures:

Medium A: A solution of filter-sterilised titanium citrate was added to a 2% final concentration (10 ml of 15% titanium chloride in 100 ml sodium citrate 0.2 M, pH 6.0, stored anaerobically in the dark). The pH was adjusted to 7.4 with sterile 2N NaOH. Then a solution of 2.5% filter sterilised FeSO₄ was added to 2% final concentration.

Medium B: A solution of 10% filter sterilised ascorbic acid was added to 0.2% final concentration. The pH was adjusted to 7.4 with sterile 2N NaOH. Finally, a solution of 2.5% filter sterilised FeSO₄ was added to 2% final concentration.

All steps were performed under anaerobiosis in an anaerobic glove box.

Medium C is a modified Postgate B medium (Postgate, 1984) routinely used in the Elf-Aquitaine group. It is prepared under strictly anaerobic conditions. Thirteen g/l NaCl were included in medium C2 but not in medium C1.
TABLE 1: Bacterial strains and their characteristics

<table>
<thead>
<tr>
<th>Strain</th>
<th>Origin</th>
<th>Growth medium</th>
<th>Carbon source</th>
<th>NaCl g/l</th>
<th>Optimal temperature</th>
</tr>
</thead>
<tbody>
<tr>
<td>SEBR 422</td>
<td>Tanganyika Lake</td>
<td>SEBR A (1)</td>
<td>Lactate</td>
<td>0</td>
<td>30</td>
</tr>
<tr>
<td>SEBR 513</td>
<td>DSM 1926 (Desulfovibrio desulfuricans)</td>
<td>DSM N° 163</td>
<td>Lactate</td>
<td>25</td>
<td>30</td>
</tr>
<tr>
<td>SEBR 583</td>
<td>DSM 574 (Desulfotomaculum nigrificans)</td>
<td>DSM N° 63</td>
<td>Lactate</td>
<td>0</td>
<td>55</td>
</tr>
<tr>
<td>C 20</td>
<td>Oil production well</td>
<td>SEBR A (1)</td>
<td>Lactate</td>
<td>30</td>
<td>55</td>
</tr>
<tr>
<td>SEBR 615</td>
<td>Cooling water system</td>
<td>SEBR A (2)</td>
<td>Acetate</td>
<td>0</td>
<td>30</td>
</tr>
<tr>
<td>SEBR 885</td>
<td>DSM 2034 (Desulfbacter postgatei)</td>
<td>DSM N° 193</td>
<td>Acetate</td>
<td>7</td>
<td>30</td>
</tr>
<tr>
<td>SEBR 886</td>
<td>DSM 2032 (Desulfobulbus propionicus)</td>
<td>DSM N° 194</td>
<td>Propionate</td>
<td>1</td>
<td>37</td>
</tr>
</tbody>
</table>

(1) without acetate and Fe SO₄
(2) without lactate and Fe SO₄
TABLE 2: Origin and characteristics of detection kits

<table>
<thead>
<tr>
<th>Origin</th>
<th>Medium</th>
<th>Method</th>
</tr>
</thead>
<tbody>
<tr>
<td>A This paper</td>
<td>SEBR A1 or A2 see text</td>
<td>Anaerobic broth bottles</td>
</tr>
<tr>
<td>B This paper</td>
<td>SEBR B1 or B2 see text</td>
<td>Anaerobic broth bottles</td>
</tr>
<tr>
<td>C Elf Aquitaine Group</td>
<td>Cl or C2. See text (Modified Postgate B) (5)</td>
<td>Anaerobic broth bottles</td>
</tr>
<tr>
<td>D Williams Brothers (a)</td>
<td>API.RP 38 (1)</td>
<td>Anaerobic broth bottles</td>
</tr>
<tr>
<td>E Microtest SR (b)</td>
<td></td>
<td>Deep agar tubes</td>
</tr>
<tr>
<td>F Pasteur Lyon Novotec (c)</td>
<td></td>
<td>Aerobic broth tubes</td>
</tr>
</tbody>
</table>

(a) Laboratories of Williams Brothers Engineering Company Resource Science Park. 6600 South Yale Avenue, Tulsa, Oklahoma 74136 - USA.

(b) France Organo Chimique. 52, rue Bichat, 75010 Paris - France.

(c) Pasteur Lyon Novotec. Route de Saint Bel, Domaine du Poirier, Lentilly, 69210 L'Arbresle, France.
TABLE 3: Composition of detection media SEBR A and B (for 1 l medium)

<table>
<thead>
<tr>
<th></th>
<th>Fresh water strains (A1, B1)</th>
<th>Salt water strains (A2, B2)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CaSO₄</td>
<td>-</td>
<td>1g</td>
</tr>
<tr>
<td>CaCl₂·2H₂O</td>
<td>1g</td>
<td>-</td>
</tr>
<tr>
<td>MgSO₄·7H₂O</td>
<td>1g</td>
<td>2g</td>
</tr>
<tr>
<td>MgCl₂</td>
<td>-</td>
<td>2g</td>
</tr>
<tr>
<td>NaCl</td>
<td>-</td>
<td>30g</td>
</tr>
<tr>
<td>CaCO₃</td>
<td>-</td>
<td>0.15g</td>
</tr>
<tr>
<td>Na₂SO₄</td>
<td>2g</td>
<td>1g</td>
</tr>
<tr>
<td>KHCO₃</td>
<td>0.2g</td>
<td>-</td>
</tr>
<tr>
<td>FeSO₄ (0.4% solution)</td>
<td>1ml</td>
<td>1ml</td>
</tr>
<tr>
<td>MoPS</td>
<td>3g</td>
<td>3g</td>
</tr>
<tr>
<td>Sodium lactate</td>
<td>6ml</td>
<td>6ml</td>
</tr>
<tr>
<td>Sodium acetate</td>
<td>2g</td>
<td>2g</td>
</tr>
<tr>
<td>Yeast extract</td>
<td>1g</td>
<td>1g</td>
</tr>
<tr>
<td>Resazurin (0.1% solution)</td>
<td>1ml</td>
<td>1ml</td>
</tr>
</tbody>
</table>

pH is adjusted to 7.8
Heat sterilization for 20min at 120°C
Automated SRB Detector Medium

The concentrated medium used in the automated SRB detector has the following composition: for 1 litre of distilled water, yeast extract, 10 g; \( \text{NH}_4 \text{Cl}, 10 \text{ g}; \) sodium lactate, 60 ml; sodium acetate, 20 g; resazurin, 10 ml of a 0.1% solution, pH 7.8. After autoclaving, 40 ml of a titanium citrate solution, prepared as described above, were added. The pH was adjusted to 7.4 with sterile 2N NaOH, and a solution of 2.5% filter sterilised FeSO_4 was added to 5% final concentration. One millilitre of medium was dispensed in each sterile test tube. All steps were performed under strictly anaerobic conditions.

SRB Growth Tests

Each strain was inoculated in its optimal growth medium (Table 1) and incubated at the correct temperature. When maximal growth was obtained, the bacterial concentration was estimated by counting under the microscope. The cultures were then diluted either in anaerobic physiologic buffer (for fresh water strains) or in anaerobic synthetic sea water (for salt water strains). One millilitre of each dilution was inoculated into three kits of each type, and the inoculated kits were incubated at the adequate temperature. The incubation time necessary to turn the bottles or tubes black was registered.

RESULTS AND DISCUSSION

Comparison of the Detection Kits

(1) The Preliminary Tests:

Two SRB strains from fresh water and two from salt water were chosen for a preliminary comparison of the efficiency of the kits in promoting and detecting SRB development. In both cases, one of the two strains is thermophilic. The results are given in Table 4. It appears that aerobic procedures using deep agar (E) or broth (F) tubes are quite inefficient for the detection of even large inocula. The best results were obtained with detection kits SEBR A1 A2, which could detect small inocula of strains SEBR 422 and SEBR 513 (< 10 cells). Although detection kits C and D are almost as sensitive as kit SEBR A, they require longer periods of incubation for small inocula. Results are also often more consistent with SEBR A kits, since all three kits were always positive except at highest dilutions.
TABLE 4: Incubation time (days) necessary for the detection of SRB (first set)

<table>
<thead>
<tr>
<th>Inoculum (cells/ml)</th>
<th>SEBR 422</th>
<th>SEBR 513</th>
<th>SEBR 583</th>
<th>C 20</th>
</tr>
</thead>
<tbody>
<tr>
<td>$10^6$</td>
<td>1/2/1/10-11/17(1/3)</td>
<td>2-3/1/-</td>
<td>NT/NT/8(1/3)</td>
<td>NT/-</td>
</tr>
<tr>
<td>$10^5$</td>
<td>2/2/2/14(1/3)/-</td>
<td>2/4/-</td>
<td>1/1/-</td>
<td>2/-</td>
</tr>
<tr>
<td>$10^4$</td>
<td>2/2/2/-/-</td>
<td>3/5-6/-</td>
<td>1/1(2/3)/-</td>
<td>2/-</td>
</tr>
<tr>
<td>$10^3$</td>
<td>2/3/2-3/-/-</td>
<td>3/7-16/-</td>
<td>1/1(2/3)/-</td>
<td>2/-</td>
</tr>
<tr>
<td>$10^2$</td>
<td>3/3(2/3)/3/-/-</td>
<td>3/6-7/-</td>
<td>1(1/3)/1(2/3)/-</td>
<td>3(2/3)/-</td>
</tr>
<tr>
<td>10</td>
<td>3/3(2/3)/3/-/-</td>
<td>4/11-24/-</td>
<td>-/10(1/3)/-</td>
<td>-/-</td>
</tr>
<tr>
<td>&lt;10</td>
<td>3(2/3)/7(1/3)/-/-</td>
<td>4-5/7-17/-/-</td>
<td>-/-/-</td>
<td>3(1/3)/-</td>
</tr>
</tbody>
</table>

NT: not tested; - all three kits were negative after 32 days of incubation.

Numbers in parenthesis indicate that 1 or 2 of the three tests were positive after the indicated incubation time. The others were negative after 32 days.
With all types of detection kits, thermophilic SRB were most often detected after one to three days of incubation. After that, most of the media turned brownish and precipitated, indicating a degradation of one or more of the components.

(2) Complementary Laboratory Tests:
Since our preliminary experiments were performed with conventional lactate metabolizing strains, we decided to test the response of kits A, C and D against several metabolically unrelated strains. Detection kit SEBR B, for which ascorbic acid was used as the reducing agent instead of titanium citrate as in SEBR A, was also tested. The results are reported in Table 5.

SEBR 615, an acetate metabolizing strain, was efficiently detected in all four types of kit, but incubation time was longer with kit D than with other kits. Kit D did not allow efficient detection of the three other strains.

The thermophilic SEBR 583 strain was tested again in this experiment. No significant differences were observed with kits Al, Bl and Cl.

The slowly growing strain SEBR 885 was detected only with the largest inoculum by kits B2 and D, whereas medium SEBR A2 allowed a 100 fold more sensitive detection after an incubation period of three to four weeks.

The propionate metabolizing strain SEBR 886 (Desulfobulbus propionicus) was detected in kit SEBR A1, even with low inocula. Detection kit Cl was less sensitive and longer incubation times were needed. Detection kits B1 and D are very poorly sensitive.

(3) Field Experiments:
Detection kits A and B were supplied to different on-field operators in the Elf-Aquitaine Group for comparison with their usual detection kits. It was observed that kit A was often more efficient than kit B. It proved to be more rapid and sensitive, and to give more consistent responses than the different test kits commonly used in our group. In one case, samples were taken in different parts of an oil production plant and inoculated into detection kits A1, B1, Cl and D. The results, given in Table 6, showed that the best results were obtained with detection kits A1 and B1.
TABLE 5: Incubation time (days) necessary for the detection of SRB (second set)

<table>
<thead>
<tr>
<th>Inoculum cells/ml</th>
<th>SEBR 615</th>
<th>SEBR 583</th>
<th>SEBR 885</th>
<th>SEBR 886</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>A1</td>
<td>B1</td>
<td>C1</td>
<td>D</td>
</tr>
<tr>
<td>10^6</td>
<td>NT</td>
<td>NT</td>
<td>NT</td>
<td>NT</td>
</tr>
<tr>
<td>10^5</td>
<td>NT</td>
<td>NT</td>
<td>NT</td>
<td>NT</td>
</tr>
<tr>
<td>10^4</td>
<td>2</td>
<td>2</td>
<td>2</td>
<td>3</td>
</tr>
<tr>
<td>10^3</td>
<td>2</td>
<td>2</td>
<td>2</td>
<td>4</td>
</tr>
<tr>
<td>10^2</td>
<td>2-3</td>
<td>2</td>
<td>2</td>
<td>4</td>
</tr>
<tr>
<td>10</td>
<td>3</td>
<td>3</td>
<td>3</td>
<td>5(1/3)</td>
</tr>
<tr>
<td>&lt;10</td>
<td>3(1/3)</td>
<td>-</td>
<td>3(1/3)</td>
<td>6(1/3)</td>
</tr>
</tbody>
</table>

Same legend as table 4
TABLE 6: Incubation time (days) necessary for the detection of SRB (field experiment)

<table>
<thead>
<tr>
<th>Sample</th>
<th>A1</th>
<th>B1</th>
<th>C1</th>
<th>D</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sample 1</td>
<td>5(1/3)</td>
<td>6-9</td>
<td>-</td>
<td>NT</td>
</tr>
<tr>
<td>Sample 2</td>
<td>4-5</td>
<td>6-9</td>
<td>6(1/3)</td>
<td>NT</td>
</tr>
<tr>
<td>Sample 3</td>
<td>4</td>
<td>6-9</td>
<td>9-17</td>
<td>NT</td>
</tr>
<tr>
<td>Sample 4 no dilution</td>
<td>7-11</td>
<td>6-12</td>
<td>12(1/3)</td>
<td>9-11(1/3)</td>
</tr>
<tr>
<td>dil. $10^{-1}$</td>
<td>9</td>
<td>9(1/3)</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>dil. $10^{-2}$</td>
<td>9(1/3)</td>
<td>9(1/3)</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>dil. $10^{-3}$</td>
<td>-</td>
<td>9(1/3)</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

Same legend as Table 4
(4) Conclusion:

It appears from these laboratory and field trials that the detection of SRB by the conventional culturing method could be improved if the carbon sources and the reducing agent were selected to allow the growth of metabolically different SRB strains. The addition of sodium acetate to sodium lactate widens the detection spectrum to include slowly growing acetate metabolizing bacteria such as Desulfobacter postgatei. The addition of a very efficient reducer, titanium citrate (redox potential of test kit A is about -600 mV) allows a more rapid detection of most strains, probably by shortening the lag-time.

The addition to the same medium of another non sulphur-containing reducer, ascorbic acid, has proven to be less efficient. Moreover, the necessary low redox potential in kit B is not correctly maintained after long periods of storage (data not shown). Since the titanium citrate reducer is not a sulphur-containing compound, growth inhibition of some strains by sulphur-containing reducing agents like cysteine, which sometimes occurs, was not observed. The major drawback of titanium citrate is that it is rapidly degraded at high temperature, and therefore must be added after heat sterilization of the medium.

Automated SRB Detector

For pressure maintenance purposes, large quantities of water are injected into the oil-bearing formations. The water is filtered and de-aerated in order to reduce formation plugging and electrochemical corrosion, and consequently an anaerobic ecosystem favourable for SRB development is created. Bacterial growth can be controlled by chemical injections and/or physical treatments, and monitoring of the process needs controls for the presence of SRB; in most cases, this is done by culturing methods.

In the context of an unmanned oil platform, but also to reduce manual intervention in conventional plants, an automated SRB detector was devised.

(1) Characteristics of the Detector:

The apparatus was designed to be capable of functioning without direct human intervention for a period of one year, taking one sample once a day at each of two sampling points located on the water injection line. The volume of each sample is ten millilitres, injected into a culture tube.
Figure 1

Automated SRB Detector
containing 1 ml of concentrated nutrient medium, which has the following composition: for 1 litre of distilled water, yeast extract, 10 g; \( \text{NH}_4\text{Cl} \), 10 g; sodium lactate, 60 ml; sodium acetate, 10 g; resazurin, 10 ml of a 0.1% solution in water. After heat sterilisation, the titanium citrate solution (4% final concentration) and the FeSO\(_4\) solution (5% final concentration) are added following the previously mentioned procedure.

All samples are incubated for a period of twenty-one days and examined each day in a spectrophotometer for bacterial development and anaerobiosis. Bacterial development is indicated by blackening of the medium due to formation of FeS precipitate. Lack of anaerobiosis can be detected by the pink colour of the resazurin indicator.

(2) Description:

The detector is composed of (figure 1):

- 1 programmable manipulator-arm (Zymark Co) (1)
- 1 set of spatial references for the arm positions (13)
- 1 storage area (3) of tubes (11) pre-filled with concentrated nutrient medium
- 1 storage area (2) of anaerobiosis control tubes
- 2 identical stations (5) for sampling water to be analysed
- 1 station for screwing and unscrewing the tube caps (6)
- 1 deposit site for placing the caps (13)
- 1 incubation station (4)
- 1 double beam spectrophotometer (7)
- 1 storage area for tubes after utilization (40)
- 1 airproof bell filled with argon (9)
- Siphons of protection against air intrusion (15)

The apparatus and all the processes are computer controlled.

(3) Simplified Functioning Principles:

(a) Daily Sampling:

- The arm takes a sampling tube by its cap in the storage area, sets it on the screwing/unscrewing station, which holds the tube firmly, then turns the tube to unscrew the cap.
- The arm sets down the cap, seizes the tube which is then freed by the screwing/unscrewing station.
• The arm carries the tube to the station for water sampling, which liberates 10 ml of water.
• The arm sets the tube on the screwing/unscrewing station which grips it.
• The arm takes the cap.
• The arm and the station together recap the tube.
• After mixing, the arm puts the tube in the culture rack which is maintained at the chosen temperature.

(b) Monitoring of SRB Development:

Each day, the arm takes each tube in the culture area for colourimetric examination. Upon detection of a black iron sulphide precipitate, or after twenty-one days of culture, the tube is put in a second storage area. The results of the measurements are sent to the calculator monitoring the water injection process for eventual action.

(4) Surveillance of Failures:

The apparatus monitors:

• The good functioning of the water injection process on which it is installed: absence of oxygen in the sampled water (the contrary would indicate some failure in the process of water deaeration).
• The good functioning of the apparatus itself: anaerobiosis of the atmosphere filling of the tube during sampling correct functioning of the photometer correct screwing of the caps onto the tubes good functioning of the manipulator arm.

(5) Trials:

The SRB detector was tested in the laboratory by injection of dilutions of a laboratory bacterial strain into synthetic sea water. The highest dilution tested contained two to five living bacteria per ml; it gave positive responses after 48 hours of incubation (data not shown). The apparatus is now being tested in a pilot unit of the entirely automated injection process conceived for the PLATINE project. The preliminary results correlate with data obtained with anaerobic broth bottles SEBR A.
ACKNOWLEDGEMENTS

We greatly thank M. Fontimpe, M. I. Marley, V. Mercier, J. Protin and J. Sourbe for their excellent technical assistance, and T. E. Pou and J. P. Seailes (CECA) for preparing and giving us kit C.

REFERENCES


STUDY OF BIOCORROSION INHIBITORS WITH ELECTROCHEMICAL METHODS

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ABSTRACT

Using polarization curves and evolution of polarization resistance of steel versus time, we have tested inhibiting efficiency of different substances: - cationic (cetylpyridinium chloride - CPCL, cetyl trimethylammonium bromide - CTAB, bipyridilium chloride - BP) - anionic (sodium dodecylsulfate - SDS) and non-ionic (TRITON X100) against corrosion of steel by sulfate reducing bacteria metabolic products (sulfide, acetate and carbonate species) in synthetic solutions. When cationic surfactants are adsorbed on iron sulfide film, they turn it into a more effective barrier to corrosion. Best inhibiting effects are obtained with CPCL which is also a good biocide. TRITON is less effective than cationic surfactants and SDS does not show inhibiting effects on the corrosion by sulfide species.

A l'aide des tracés voltampérométriques et en suivant l'évolution de la résistance de polarisation de l'acier en fonction du temps, nous avons testé l'efficacité inhibitrice de différentes substances: - cationique (chlorure de cetylpyridium - CPCL, bromure de cétyle triméthy ammonium - CTAB, chlorure de bipyridilium - BP) - anionique (dodécy1 sulfate de sodium - SDS) et non-ionique (TRITON X100) vis-à-vis de la corrosion de l'acier par les métabolites des bactéries sulfato-réductrices (sulfures, carbonates, acétates) en solutions synthétiques. Les surfactants cationiques en s'adsorbant sur le film de sulfure de fer renforcent son effet protecteur. Le CPCL est le plus efficace, c'est aussi un bon biocide. Le Triton est moins efficace que les tensio-actifs cationiques et le SDS ne présente aucun effet inhibiteur de la corrosion de l'acier par les espèces sulfurées.
INTRODUCTION

The active participation of sulfate reducing bacteria (SRB) in the corrosion process of steel under anaerobic conditions is well-known. The mechanisms by which these organisms cause or accelerate corrosion have been a matter of controversy. The most widely accepted mechanistic model is the depolarisation theory but the participation of other metabolic species (sulfides, phosphides) in the corrosion process cannot be excluded (Iverson and al, 1983; Costello, 1974; Weimer and al, 1988). Using electrochemical methods (polarisation curves, E vs time) we have shown that bacterial metabolic products (sulfide, carbonate and acetate) induce corrosion of steel (Sinicki and al, 1984, 1987). The iron sulfide film formed on steel is protective but crevices and pits induce passivity breakdown that produces a galvanic cell between the bare metal and the protective film; then passivating of pits is impossible (Gaboriau-Soubrier, 1985). The behaviour of steel is the same in inoculated medium as in the synthetic sulfide solutions: Substances acting both as biocide and inhibitor should have good efficiency against corrosion induced by SRB.

In this work we have tested inhibiting efficiency of different classes of inhibitors. Bactericidal activity has been checked with the best corrosion inhibitors (Gaboriau-Soubrier, 1985).

MATERIALS AND METHODS

- Tests are all conducted at 25°C, pH 7.5 in polarization cell as previously described (Sinicki and al, 1984; Gaboriau-Soubrier, 1985). Solutions are deaerated before immersion of coupons and cells are incubated under a protective N₂ atmosphere.

- Metal coupons (A48 steel supplied by Creusot-Loire) are embedded in a thermo-setting resin; only a circular area of 40 mm² is used for tests. Preparation of coupons has been described elsewhere.
Synthetic solutions contain sodium sulfide ($10^{-3}$ M), sodium acetate ($2.10^{-3}$ M) and sodium carbonate ($2.10^{-3}$ M). Inhibitors are added and pH is adjusted under N$_2$ atmosphere just before immersion of specimens.

Polarization curves are drawn with a Princeton Applied Research potentiostat, Model 173, coupled with a computer using the corrosion software PAR 332. Each polarization run is conducted on a fresh coupon sample. All potential values are relative to the saturated calomel electrode (SCE). The working electrode is polarized at the slow scan rate of 0.013 mVsec$^{-1}$. Polarization resistance measurements are made in a scanning potential range +20 mV versus E$_{corr}$, at the scan rate of 0.1 mVsec$^{-1}$.

RESULTS

Figs. 1 to 4 show the anodic and cathodic polarization curves recorded on immersion of coupons in synthetic solutions ($S^2-10^{-3}$ M + $CH_3COO-2.10^{-3}$ M + $CO_3^2-2.10^{-3}$ M), with or without inhibitors.

In sulfide solutions, E$_{corr}$ is included in the range -0.77/-0.80 V/SCE. Except for SDS, when inhibitors are added, the corrosion potential moves in the positive direction (noble potential). The potential shift observed increases with the surfactant concentration. Lower current density values are obtained in the presence of surfactant compared to the non-inhibited test. The substances tested show preferential inhibition of the anodic reaction (anodic inhibitors).

Inhibitive efficiency

By extrapolating the cathodic and anodic Tafel lines to the corrosion potential, corrosion current values are obtained. These results are compared with polarization resistance values (Rp) in order to determine whether Rp values can give inhibitive efficiency (IE) of inhibitors (CTAB, CPCL, TRITON, BP) (Suzuki and Sato, 1981). Results are given in table 1.
Fig. 1: Polarization curves in sulfide ($10^{-3}$M)/carbonate ($2.10^{-3}$M)/acetate ($2.10^{-3}$M) solutions: 1) blank test with (or without) SDS $10^{-3}$M 2) with CTAB $10^{-3}$M 3) with CPCL $10^{-3}$M.
Fig. 2: Cathodic (a) and anodic (b) polarization curves in sulfide/carbonate/acetate solutions with increasing concentration of CPCL:
1) blank test  2) CPCL $10^{-5}$M  3) CPCL $10^{-4}$M
(3a: slow, 3b: fast evolution of $E_{corr}$ with time on immersion)
Fig. 3: Cathodic (a) and anodic (b) polarization curves in sulfide/carbonate/acetate solutions with increasing concentration of BP salt:
1) blank test  2) BP $10^{-6}$M  3) BP $5.10^{-4}$M  4) BP $2.10^{-3}$M.
Fig. 4: Cathodic (a) and anodic (b) polarization curves in sulfide/carbonate/acetate solutions with increasing concentration of TRITON: 1) blank test 2) TRITON $10^{-5}$M 3) TRITON $10^{-4}$M.
TABLE 1: Corrosion parameters and inhibitive efficiency of inhibitors, on immersion of coupons

<table>
<thead>
<tr>
<th>Inhibitor</th>
<th>Anodic Tafel icorr, μA.cm(^{-2})</th>
<th>Cathodic Tafel icorr, μA.cm(^{-2})</th>
<th>Polarization resistance Rp(^{-1})×10(^{-2}) Ω(^{-1}).cm(^{-2})</th>
</tr>
</thead>
<tbody>
<tr>
<td>S(^2-)CO(_3)(^2-)</td>
<td>10</td>
<td>10</td>
<td>7.1</td>
</tr>
<tr>
<td>+CH(_3)COO-**</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CTAB 10(^{-5}) M</td>
<td>1.4</td>
<td>86-</td>
<td>1.8 to 1</td>
</tr>
<tr>
<td>CPCL 10(^{-5}) M</td>
<td>2.6</td>
<td>74</td>
<td>2</td>
</tr>
<tr>
<td>10(^{-4}) M</td>
<td>0.9-0.2**</td>
<td>91 to 98</td>
<td>0.7 to 0.2</td>
</tr>
<tr>
<td>10(^{-3}) M</td>
<td>0.4</td>
<td>96</td>
<td>0.7 to 0.2</td>
</tr>
<tr>
<td>BP 10(^{-4}) M</td>
<td>10</td>
<td>0</td>
<td>8.3 to 5</td>
</tr>
<tr>
<td>5.10(^{-4}) M</td>
<td>4</td>
<td>60</td>
<td>3.8 to 1.7</td>
</tr>
<tr>
<td>2.10(^{-3}) M</td>
<td>3.4</td>
<td>66,</td>
<td>1.3</td>
</tr>
<tr>
<td>TRITON 10(^{-5}) M</td>
<td>1</td>
<td>90</td>
<td>2.5 to 0.6</td>
</tr>
<tr>
<td>10(^{-4}) M</td>
<td>0.8</td>
<td>92</td>
<td>1.1 to 0.2</td>
</tr>
</tbody>
</table>

* \(S^{2-}\)10\(^{-3}\) M, CO\(_3\)\(^2-\)2.10\(^{-3}\) M, CH\(_3\)COO\(^-\)2.10\(^{-3}\) M pH 7.5

** icorr = 0.9 μA.cm\(^{-2}\), IE = 91% (fig 2, curve 3a);

icorr = 0.2 μA.cm\(^{-2}\), IE = 98% (fig 2, curve 3b)
With CTAB and CPCL, a good correlation is observed between inhibitive efficiency calculated with Rp and those obtained by extrapolation of Tafel lines.

For the same surfactant concentration, inhibitive efficiency is higher with CPCL than with CTAB and solubility of CTAB is less interesting ($T_K(\text{CTAB}) = 22^\circ\text{C}$). So we have not studied its efficiency versus concentration though it is also a good biocide. IE of CPCL increases with the concentration but the maximum efficiency is attained with $3.10^{-4}$ mole.L$^{-1}$ of CPCL (Sinicki and al, 1987).

**Evolution of polarization resistance versus time**

The evolution of Rp versus time is reported in Fig 5. With lower concentration of CPCL ($10^{-5}$ M), after seven days of exposure, the metal surface is overlaid with a black, bright and adherent iron sulfide film. When the concentration of CPCL is beyond $3.10^{-4}$ M, the film formation is inhibited but few pits appear during exposure causing the Rp decrease.

![Image of Fig 5](image-url)
With BP concentration up to $10^{-4}$, the corrosion potential moves in the positive direction as with CPCL; but after some days of exposure Rp values are low, though slightly higher than in blank test solutions (Fig 6). Samples are partly bright (sample surface does not blacken), but extended crevices are formed under localized FeS deposits. During exposure, the inhibiting effect does not increase with the BP concentration as expected on immersion.

![Graph](image)

Fig. 6: Rp change with time in sulfide/carbonate/acetate solutions with increasing concentration of BP: 1) blank test 2) $10^{-4}$ to $5.10^{-4}$ M BP

In the test solutions of TRITON, the Rp values of steel coupons increase with the concentration during exposure, but we observe a very large variability of coupon behaviour for equal concentrations of surfactant. Sometimes TRITON completes the ferrous sulfide scale formed on the metal surface and at other times the surfactant adsorbed on steel prevents the FeS film formation, but pits are detected in the two cases. As shown in Fig 7, inhibition by TRITON is weak when compared to CPCL. Rp values are low as expected with the results obtained on immersion of coupons.

Among the inhibitors studied here, only the BP salt does not foam at the tested concentrations. This parameter should be taken into account for industrial applications.
Biocidal efficiency

The ability of CPCL and CTAB to inhibit sulfate reducing bacterial growth has been checked in the concentration range from 5 to 40 mg.l⁻¹ (Gaboriau-Soubrier, 1985). The inhibiting effect on bacterial metabolism was noted by measuring the amount of ATP versus time contact. Bacteriostatic effect is adequate for concentrations from 10 mg.l⁻¹.

BP and TRITON do not inhibit SRB growth with the concentrations tested for corrosion inhibition.
DISCUSSION

Anionic surfactant (SDS) is ineffective against corrosion by SRB metabolic products. HS⁻ ions are adsorbed on steel preferentially to dodecyl sulfate anions.

Non-ionic surfactant (TRITON X100) is weakly inhibitive when compared to cationic CPCL and the behaviour of steel coupons is not reproducible.

Cationic surfactants seem the most effective, except for BP. With this salt, we observe a preferential adsorption of the surfactant (potential shift on immersion) but crevices appear and Rp values are low. Its efficiency does not seem better than that of non-ionic surfactants. It should be interesting to modify the BP salt formula to improve inhibiting and biocidal efficiency of this compound.

With CPCL and CTAB, we observe a preferential adsorption of surfactants which inhibit the FeS film formation. With CPCL, Rp values are higher and best efficiency is obtained with the concentration 3.10⁻⁴ mole.l⁻¹. At this concentration, we have shown that both CPCL and CTAB are bactericidal (Gaboriau-Soubrier, 1985). According to Iofa and al (1964), HS⁻ ions chemisorb on iron to form a monolayer. Cationic surfactants adsorb on this monolayer, complete it and prevent the formation of the FeS film.

Electrochemical methods allow the comparison of inhibiting properties of inhibitors against corrosion of steel by SRB metabolic products. The model of sulfide/acetate/carbonate synthetic solutions is easy to implement so as to carry our comparative tests.

ACKNOWLEDGEMENT

This work has been carried out with the financial support of ELF FRANCE. Mr. Coste (IUT Perpignan) is also gratefully acknowledged for supplying the BP salt.
REFERENCES


In anaerobic environments, a significant amount of corrosion is thought to be mediated by sulphate-reducing bacteria which are responsible for biological sulphate reduction to sulphides. The main metabolic pathways, hydrogen oxidation and sulphate reduction, are catalysed by proteins such as hydrogenase and sulphite reductase, the two key enzymes of anaerobic biocorrosion by sulphate-reducers. We have recently reported that hydrogenase activity of Desulfovibrio vulgaris Hildenborough is stable for months even in the absence of viable cells. Sulphite reductase specific activity was also stable in aged cultures of the same strain and there was only a 20% decrease in the specific activity in a one-month old culture as compared to a 24 hours old culture. A change in the kinetic patterns of hydrogenase activity was noticed during ageing of the cells.

To evaluate the importance of the depolarizing phenomenon induced by the enzymatic activities (hydrogenase and sulphite reductase) of aged D. vulgaris Hildenborough cells, the ability of such cells to oxidize cathodic hydrogen (from mild steel coupons) for reduction of sulphite was determined. Cathodic hydrogen oxidized was measured in terms of sulphite reduced based on the equation:

\[ \text{HSO}_3^- + 3\text{H}_2 \rightarrow \text{HS}^- + 3\text{H}_2\text{O} \]

In terms of enzymatic activity, old cultures of D. vulgaris are potentially as corrosive as growing cells.
Les bactéries sulfato-réductrices (BSR) sont responsables de phénomènes de corrosion de fer ou d'acier, dans des environnements anaérobies par divers mécanismes :

- L'accélération des réactions électrochimiques à la surface du métal, c'est-à-dire consommation d'hydrogène, et transformation du Fe dessous en sulfure de fer par l'intermédiaire des sulfures résultant du métabolisme bactérien.

- La production de composés acides corrosifs comme l'hydrogène sulfuré ou les phosphures.

Ces réactions métaboliques (oxidation de l'hydrogène et réduction des sulfates) sont catalysées par des protéines d'oxydo-réduction comme l'hydrogénase et la sulfite réductase des BSR.

Nous avons montré récemment (Chatelus et al 1987) que l'activité hydrogénase de D. vulgaris Hildenborough est stable plusieurs mois, même en l'absence de cellules viables. Les mêmes expériences ont été réalisées sur la stabilité de la sulfite réductase. L'activité spécifique de la sulfite réductase d'une culture de D. vulgaris Hildenborough s'est révélée stable, même dans de vieilles cultures: une baisse de seulement 20% de l'activité est notée après un mois. Le vieillissement des cellules provoque une modification du profil de cinétique des activités enzymatiques. Parallèlement, nous avons mesuré la capacité de telles cultures à oxyder l'hydrogène cathodique d'un coupon de métal. Cette oxydation de l'hydrogène cathodique est mesurée en fonction de la réduction des sulfites selon l'équation :

\[ \text{HSO}_3^- + 3\text{H}_2 \rightarrow \text{HS}^- + 3\text{H}_2\text{O} \]

Les résultats nous permettent de conclure que des cellules âgées de D. vulgaris sont potentiellement aussi corrosives que des cellules en phase de croissance.
INTRODUCTION

In anaerobic environments, corrosion has been attributed to the development of microorganisms, mainly sulphate-reducing bacteria (SRB) (Hamilton, 1985). The SRB are obligate anaerobes that use sulphate as terminal electron acceptor, reducing it to sulphide. The corrosive activities of these organisms have been attributed to several mechanisms (King and Miller, 1971):

- the stimulation of the cathodic part of the corrosion cell by removal of the polarising hydrogen,
- the stimulation of the anodic reaction by metal dissolution induced by sulphides produced during bacterial growth,
- the production of corrosive products like hydrogen sulphide or organic and mineral acids.

The main metabolic pathways of SRB are hydrogen oxidation and sulphate reduction which are catalysed by proteins such as hydrogenase and sulphite reductase. These are the two key enzymes implicated in the anaerobic corrosion of metals by SRB. Hydrogenase effects cathodic depolarisation by catalysing reversibly an energy-producing reaction in which hydrogen is oxidised with the concomitant reduction of sulphate to sulphite. This last step is catalysed by the enzymes of sulphate reduction such as bisulphite reductase, following these equations (LeGall and Fauque, 1988):

\[
3H_2 \rightleftharpoons 6H^+ + 6e^- \text{(hydrogenase)}
\]

\[
HSO_3^- \rightarrow HS^- + 3H_2O \text{ (bisulphite reductase)}
\]

We have recently demonstrated (Chatelus et al 1987) that hydrogenase activity of Desulfovibrio vulgaris Hildenborough is stable for months even in the absence of viable cells. These observations show that detrimental enzymatic activities may be present in anaerobic environments independent of viable cells. In this study similar experiments were conducted to determine the stability of sulfite reductase in aged cells of D. vulgaris Hildenborough. In addition the ability of enzymatic activities (hydrogenase and sulphite reductase) of aged cells to oxidise cathodic hydrogen from mild steel coupons for reduction of sulphite is demonstrated.
MATERIALS AND METHODS

Organisms

Desulfovibrio vulgaris Hildenborough (DSM644, NCIB 8303) and D. desulfuricans (ATCC 27774) were obtained from the culture collection of the laboratory.

Medium and Growth Conditions

Cells were grown at 32°C (37°C for D. desulfuricans) with lactate (30 mM) as carbon and energy source or with H₂ as energy source and acetate (5 mM) and CO₂ as carbon sources in serum bottles using the strict anaerobic techniques described by Balch and Wolfe (1976) and Daniels et al (1986). The medium used for growth consisted of the following components (g/l) in distilled and deionized water: KH₂PO₄ 0.4, K₂HPO₄ 0.2, NaCl 0.6, NH₄Cl 0.5, MgCl₂·6H₂O 0.1, CaCl₂·2H₂O 0.05, Na₂SO₄ 2.1, Yeast extract 1.0, resazurin 0.001. 10 ml/l of trace minerals solution (Brandis and Thauer, 1981) was also added. The pH of the medium was adjusted to 6.8 to 6.9 with NaOH while bubbling the medium with argon or with Na₂CO₃ while bubbling the medium with N₂-CO₂ (80:20 v/v). After sterilisation the medium was reduced with Na₂S·9H₂O (0.5 mM, final concentration) and inoculated with 5 to 10% inoculum. Enumeration of viable cells was made by the most probable number method (Battersby et al 1985) in lactate-sulphate medium.

Enzyme Assays

The culture (2 litre) was centrifuged at 4°C (31500 x g) and the pellet was suspended in 100 mM potassium phosphate buffer, pH6.5 with a final protein concentration of 20 - 30 mg/ml for sulphite reductase activity measurement and pH 7.2 with a final protein concentration of 4 - 5 mg/ml for hydrogenase activity measurement. A manometric assay was utilised for the determination of activities of sulphite reductase (Schedel et al 1975) and hydrogenase (Umbreit et al 1972). Hydrogenase activity was measured as H₂ uptake under an hydrogen atmosphere using benzyl viologen (10 mM) as electron acceptor.
The sulphite reductase assay requires the generation of reduced methyl viologen (1.4·mM) by an excess of hydrogenase under hydrogen atmosphere and the reduced due serves as electron donor to the sulphite reductase. Specific activities are expressed as nmoles of hydrogen consumed per minute per mg protein. Hydrogenase activity in hydrogen production was determined by measuring hydrogen evolution from dithionite-reduced methyl viologen (1 mM) (Peck and Gest 1956).

Corrosion Experiments

Mild steel coupons (surface area 3 cm²) served as source of hydrogen. The coupons were treated with dilute HCl (2 M) for about 2 min to remove surface corrosion products and rinsed immediately with distilled water. The coupons were then washed with acetone, dried and added to serum bottles. The bottles containing coupons were made anaerobic as described previously (Daniels et al 1986) and autoclaved.

The ability of aged cells to utilise cathodic hydrogen for reduction of sulphite was determined using the two-bottle system described recently (Rajagopal et al 1988). Bottle 1 contained 50 ml of medium under N₂-CO₂ atmosphere and 4-5 metal coupons and bottle 2 contained 50 ml of 1,2 or 3 months old culture (protein content was adjusted in all the cases to approximately 300 μg/ml). The cultures were thoroughly flushed with N₂-CO₂ (80:20 v/v) to remove H₂S in the gas phase, before adding to bottle 2. The two bottles had been fused through their neck with a glass tubing and had provisions on the sides for closing the bottles with black butyl rubber stoppers and for other manipulations. Sulphite (10 mM) was added to bottle 2 and the bottles were gently shaken. Sterile medium, aged cultures with added sulphite but with no iron in bottle 1 and heat killed cells with added sulphite and with iron in bottle 1 served as controls. At periodic intervals Fe³⁺ (in bottle 1), sulphite and dissolved sulphide (in bottle 2) were determined.

Weight Loss Experiments

Pre-weighed mild steel coupons (4) were surface sterilised with 90% alcohol and added to a 9-month old culture (300 ml) of D. vulgaris grown in lactate sulphate medium. Sulphite (10 mM) was added to the medium and after one month metal coupons were removed from the cultures, cleaned...
ultrasonically in 4% sodium citrate and weighed. Weight loss of coupons from culture medium with no added sulphite and from sterile medium served as controls. Corrosion rate is expressed as mg per day per dm$^2$ (mdd).

Chemical Analyses

Merck (E. Merck, Darmstadt, FRG) Spectroquant$^R$ for rapid colorimetric analyses were used for determination of Fe$^{++}$ and sulphide. Sulphite was determined colorimetrically (Truper et al 1964) and protein was determined by a modified Lowry method (Markwell et al 1978). Molecular hydrogen was measured with a gas chromatograph equipped with a thermal conductivity detector and a molecular sieve, type 5A column. Fluorimetric measurements of sulphite reductase were made as described before (Duriez et al 1988). Lactate was measured by high pressure liquid chromatography according to Guerrant et al (1982).
RESULTS AND DISCUSSION

Enzymatic activity of aged *D. vulgaris* resting cells

Two days, one, two and nine-month aged cultures grown on lactate sulphate medium were centrifuged and enzymatic activities were assayed with resting cells. The results are shown in Table 1 and, as previously reported (Chatelus et al 1987), specific activity of hydrogenase was preserved with time indicating that the depolarization reaction can occur independent of viable cells. After one month the number of viable cells was 18 per ml as compared to $2.5 \times 10^8$ per ml in 48 h old culture and in spite of this decrease in cell numbers the enzymatic activity was stable. Several hydrogenases have been purified and localised in *D. vulgaris* Hildenborough (Gow et al 1986, Lissolo et al 1986). Periplasmic hydrogenase is supposed to be involved in the depolarizing phenomenon whereas the membrane-bound hydrogenases are responsible for hydrogen production.

A lower hydrogenase activity in $H_2$ consumption (theoretically responsible for the depolarization reaction) was noticed as compared to the activity of hydrogenase in $H_2$ production. However these reactions are reversible (Adams et al 1981), and thus the hydrogenase activity noticed in production could be responsible for the removal of the protective layer of $H_2$ attached to the metal surfaces (Hardy 1983).

Sulphite reductase activity exhibits a remarkable stability with time unlike hydrogenase activity and the low activity of sulphite reductase noticed here is probably because of low diffusion of sulphite into the cell (this activity was four times higher in the case of disrupted cells; L. Duriez unpublished results). The enzyme concentration determined by fluorimetric measurement (Duriez et al 1988) in the 9 month old culture was comparable to the total protein content confirming the resistance of sulphite reductase to denaturation as opposed to other proteins.

The kinetics of $H_2$ production and consumption by hydrogenase are shown in fig. 1 and fig. 2 respectively. The kinetics of $SO_3$ reduction by sulphite reductase are depicted in fig. 3. A decrease in functional stability of hydrogenase (during 20 min under functioning conditions) was noticed with time of ageing cells, whereas the specific activity is slightly modified (fig. 1 and 2). This rapid decrease was not observed in the case of the kinetics of sulphite reductase (fig 3), as the reaction
**TABLE 1**

Hydrogenase and sulfite reductase specific activity of *D. vulgaris* Hildenborough aged resting cells

The hydrogen evolution in production was mediated using methylviologen, H₂ uptake using benzyl viologen; Sulphite reductase concentration was determined by fluorimetry according to Duriez et al (1988).

(nd : not determined)

<table>
<thead>
<tr>
<th>48 hours</th>
<th>1 month</th>
<th>2 months</th>
<th>9 months</th>
</tr>
</thead>
<tbody>
<tr>
<td>H₂ uptake</td>
<td>1.6</td>
<td>1.32</td>
<td>0.59</td>
</tr>
<tr>
<td>µmoles H₂/min/mg of protein</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>H₂ production</td>
<td>4.3</td>
<td>4.23</td>
<td>3.72</td>
</tr>
<tr>
<td>µmoles H₂/min/mg</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sulphite reductase</td>
<td>6.6</td>
<td>4.6</td>
<td>2.1</td>
</tr>
<tr>
<td>nanomoles H₂/min/mg</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sulphite reductase concentration/total protein concentration</td>
<td>5.3x10⁻⁶</td>
<td>3x10⁻⁶</td>
<td>4x10⁻⁶</td>
</tr>
<tr>
<td>Most number probable bacteria per milliliter</td>
<td>2.5x10⁸</td>
<td>18</td>
<td>2</td>
</tr>
</tbody>
</table>
Figure 1. \( H_2 \) production of aged resting cells of \textit{D. vulgaris} Hildenborough as a function of time. (▲) 48 hours, (●) 1 month, (□) 12 months.
Figure 2. \( \text{H}_2 \) uptake of aged resting cells of \textit{D. vulgaris} Hildenborough as a function of time. (▲) 48 hours, (●) 1 month, (□) 12 months.
Figure 3. Sulphite reductase activity of aged resting cells of *D. vulgaris* Hildenborough as a function of time (▲) 48 hours, (●) 1 month, (□) 12 months. The $\text{H}_2$ uptake is proportional to sulphite reductase activity expressed per mg of proteins.
was not limited due to the presence of excess amounts of hydrogenase. This
decrease with time of the kinetic pattern of hydrogenase activity under
functioning conditions probably indicates a fatigue phenomenon of the
enzyme during the oxido-reduction reactions rather than denaturation of
the proteins with ageing. This was not observed with sulphite reductase.

Utilization of Cathodic Hydrogen for Reduction of Sulphite by aged Cells
of *D. vulgaris*

In order to evaluate the importance of the depolarizing phenomenon induced
by the enzymatic activities (hydrogenase and sulphite reductase) of old
cells of *D. vulgaris* Hildenborough, the ability of these cells to oxidise
cathodic hydrogen from mild steel coupons for reduction of sulphite was
determined.

One or two or three month old cells of *D. vulgaris* Hildenborough, grown
with lactate as energy and carbon source (fig 4) or with hydrogen as
energy source and acetate and CO₂ as carbon sources, were able to utilize
cathodic hydrogen for reduction of sulphite (data not shown). Before
beginning the experiment it was made sure that there was no remaining
lactate or sulphate in the medium. There was no reduction of sulphite in
the control medium or with heat killed cells in the presence of mild steel
coupons or by aged cells in the absence of mild steel coupons (data not
shown). The results in fig 4 show the amount of cathodic hydrogen
oxidized in terms of sulphite reduced based on the equation:

\[ \text{HSO}_3^- + 3H_2 \rightarrow 3H_2O \]

The oxidation of cathodic hydrogen was faster during the first 4 - 5 days
and continued slowly thereafter. During later stages of the experiment
(beyond 6 - 8 days) H₂S produced reacted with Fe²⁺ in bottle 1 and poisoned
the cathodic reaction, apparently by formation of a sulphide film. There
was always slight production of hydrogen (1.5 to 2.0 n moles/l) from
metal coupons in control medium and it accumulated in the aged cultures
without added sulphite or heat killed cells with added sulphite. The
activity of the aged cultures to oxidise cathodic hydrogen for reduction
of sulphite declined with age of the cultures, probably due to decrease in
the total activity of the enzymes. A two month old culture of
*D. desulfuricans* (ATCC 27774) was also able to utilize cathodic hydrogen
for reduction of sulphite (data not shown).
Figure 4. Oxidation of cathodic hydrogen from mild steel by one (O), or two (▲) or three (△) months old cells of *D. vulgaris* Hildenborough for reduction of sulphite. (□) H₂ production in control medium. Results are shown based on the equation

\[
\text{HSO}_3^- + 3 \text{H}_2 \rightarrow \text{HS}^- + 3 \text{H}_2\text{O}
\]
In another experiment, the ability of nine months aged cells of *D. vulgaris* Hildenborough to utilize cathodic hydrogen directly in contact with metal coupons was tried. The dissolved sulphide in the cultures reacted with the metal coupons and poisoned the cathodic reaction. Also, due to formation of FeS precipitate it was difficult to monitor Fe$^{++}$ formation and sulphite reduction. The corrosion rates of metal coupons with aged cells in the presence of sulphite or in its absence are given in Table 2. The results indicate an increase in the corrosion rate of the coupons due to the utilization of cathodic hydrogen for reduction of sulphites to sulphides.

In anaerobic environments, cathodic hydrogen utilization by SRB will result in concomitant sulphide production. These two processes, hydrogen removal and sulphide generation, are known to contribute to the overall corrosion of steel (Hardy et al 1983). Most of the corrosion observed in SRB cultures had been attributed to the sulphides (Smith and Miller 1975). Recent studies (Pankhania et al 1986 and Rajogopal et al 1988) have reported coupling of growth of SRB with oxidation of cathodic hydrogen from mild steel according to the classical theory of Von Wolzogen Kuhr and Van der Vlught (1934). *D. vulgaris* Hildenborough stored at growth temperatures for ten months have been found to retain 50% of the hydrogenase activity of a 1-day culture indicating that the depolarisation phenomenon can occur independent of viable cells (Chatelus et al 1987). The data provided in this study indicate that both hydrogenase and sulphite-reductase activities are stable in aged cells of *D. vulgaris* and that these enzymes are capable of carrying out the depolarisation phenomenon. Sulphite reductase activity exhibits much more stability that the hydrogenase activity and these results indicate that non-viable cells can continue the process of microbial induced corrosion by the production of highly corrosive sulphides.

**TABLE 2**
Corrosion rates of metal coupons (3 cm$^2$) in presence of 9 months aged cells of *D. vulgaris* Hildenborough.
Mean values of triplicate determinations.

<table>
<thead>
<tr>
<th></th>
<th>Control (without SO$_3^-$)</th>
<th>With SO$_3^-$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Corrosion rate</td>
<td>0.4</td>
<td>0.8</td>
</tr>
<tr>
<td>mg/dm$^2$/day</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

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REFERENCES


Copper and copper alloys (principally 90/10 copper-nickel) are used as antifouling surfaces in the marine environment. These surfaces leach toxic metal ions which retard the colonisation of fouling communities. Resistant bacteria do eventually become established and are often found buried within the corrosion products on these alloys. Isolation of bacteria from these surfaces has shown that different species of bacteria immobilised copper in a variety of ways. By electron microscopy and energy dispersive X-ray microanalysis it has been shown that two species of bacteria immobilised copper within a tightly adherent mucilage sheath whilst other species immobilised copper intracellularly and within lipopolysaccharide between the two cell membranes. Copper uptake studies have shown that the most resistant species exclude this toxic ion from the cell. These results are discussed in relation to the build up of bacteria seen on these surfaces in the marine environment.
les ions cuivre à l'intérieur d'un mucilage épais et adhèrent tandis que d'autres l'immobilisaient de façon intracellulaire et à l'intérieur de lipopolysaccharide présents entre les deux membranes. Des études approfondies ont montré que les espèces les plus résistantes excluaient cet ion de la cellule. Ces résultats sont discutés en relation avec la croissance de bactéries observées sur ces surfaces dans l'environnement marin.
BACTERIAL POLYSACCHARIDES AND CORROSION

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ABSTRACT

Bacterial polysaccharides have been implicated in corrosion as lipopolysaccharides (LPS) in the cell wall, enhancing cell adhesion to the metal surface, and as extracellular polymeric substances (EPS), incorporating into biofilms and encouraging differential aeration cell formation.

The initial colonisation and subsequent biofilm formation on mild steel surfaces in pure and mixed cultures of Pseudomonas fluorescens and Desulfovibrio desulfuricans was studied using fluorescence microscopy, scanning electron microscopy and EDAX analysis. Adhesion of cells to the metal surface after 1 h has been shown by the use of specific sugar antagonists and glycolytic enzymes to be related to the presence of N-acetyl glucosamine and, in the case of P. fluorescens only, glucose on the cell surfaces. The inhibition of adhesion by specific antibodies to LPS indicated that these surface sugars were probably present as polysaccharide side chains of LPS.

In spite of the initial more rapid adhesion of P. fluorescens cells, D. desulfuricans produced a complete biofilm on the metal surface more quickly. Within six weeks mild steel surfaces incubated with D. desulfuricans, either alone or in combination with P. fluorescens, were completely obscured by a biofilm consisting of bacterial cells, EPS and corrosion products. After the same period, P. fluorescens incubated metal surfaces showed only patchy accumulations of cells with no apparent EPS or corrosion products and large areas of bare metal. EDAX analysis demonstrated the presence of large amounts of sulphur in the D. desulfuricans biofilm and lesser quantities in biofilms on mild steel incubated with mixed cultures of D. desulfuricans and P. fluorescens, thus confirming the production of sulphide-containing corrosion products in these two environments.
Les polysaccharides bactériens interviennent dans les problèmes de corrosion sous forme des lipopolysaccharides (LPS), constituant la paroi, permettant l'adhésion de la cellule aux surfaces métalliques, et sous forme de polymères extracellulaires.

La colonisation initiale à la surface d'un acier doux et la formation ultérieure du biofilm par des cultures pures ou mixtes de *Pseudomonas fluorescens* et *Desulfovibrio desulfuricans* sont ici étudiées au moyen de la microscopie de fluorescence, de la microscopie électronique à balayage et de l'analyse EDAX. Au moyen de sucres spécifiques antagonistes et d'enzymes glycolytiques, l'adhésion des cellules sur une surface métallique est corrélée à la présence de N-acetyl glucosamine et dans le cas de *Pseudomonas fluorescens* seul à la présence de glucose à la surface de la cellule. L'inhibition de l'adhésion par des anticorps spécifiques des LPS indiquent que ces sucres de surface sont présents au sein des structures lipopolysaccharidiques. Malgré l'adhésion initiale plus rapide des cellules de *P. fluorescens*, les cellules de *D. desulfuricans* produisent un biofilm à la surface métallique plus rapidement. Après six semaines d'incubation de coupons d'acier avec des cellules de *D. desulfuricans*, ceux-ci sont complètement recouverts par un biofilm constitué de bactéries, de polymères extracellulaires et de produits de corrosion. Dans le cas des cellules de *P. fluorescens*, les coupons métalliques présentent une répartition inégale de cellules, sans polymères extracellulaires ni produits de corrosion et de larges surfaces de métal nu. L'analyse EDAX indique la présence de grandes quantités de soufre au sein du biofilm, de *D. desulfuricans* et de plus faibles quantités dans les biofilms de cultures mixtes de *D. desulfuricans* et *P. fluorescens*. Ceci confirme la production de produits de corrosion contenant du sulfure dans ces deux environnements.
Polysaccharides are important constituents of bacterial surface structures, forming varying proportions of capsules, Gram positive cell walls and Gram negative outer membranes. With the latter, polysaccharides, in the form of lipopolysaccharides (LPS), comprise the interface between the bacterial cell and its surrounding environment (Fig. 1). The polysaccharide side chains of LPS project from the cell surface and facilitate interactions between the cell and adjacent cells (Peterson & Quie, 1981) or with extracellular materials such as cations (Leive et al, 1968). Such interactions could be important in the corrosion of metals, which is an electrochemical phenomenon occurring at the metal/solution/cell interface (Videla, 1988). LPS could enhance corrosion in two ways: by chelating metal ions, thus allowing further dissolution of the solid metal (i.e. by altering the equilibrium of the equation $M \rightleftharpoons M^+$) and by acting as adsorption structures, allowing adhesion of bacterial cells to the metal surface with resulting oxygen concentration cell formation and subsequent corrosion (Tiller, 1983). It has been shown that the attachment of cells of the sulphate-reducing bacterium Desulfovibrio vulgaris to a metal surface is necessary for the production of the rapid corrosion associated with this group of organisms (Gaylarde and Johnston, 1980). In addition, Gaylarde and Videla (1987) have demonstrated the severe pitting corrosion occurring on mild steel beneath adherent colonies of the marine bacterium Vibrio alginolyticus. There can be little doubt that the adhesion of bacterial cells and the concomitant build-up of a biofilm can induce rapid metal corrosion.

Microbial biofilms are complex structures consisting not only of bacterial cells but also of extracellular materials (waste metabolic products and excreted polymers) and, on a metal surface, corrosion products. Excreted polymers (extracellular polymeric substances, EPS) are mainly polysaccharide in nature and have a range of important biological functions including attachment, protection and nutrient uptake (Costerton et al, 1978). It has been shown that bacterial EPS can selectively bind metal ions (see, for example, Friedman and Dugan, 1968). Mittleman and Geesey (1985) have suggested that a bacterial exopolymer, which they had demonstrated to bind copper, could form copper-concentration cells on a copper alloy surface, thus promoting oxidation of less noble metals. Ford et al (1987) have isolated a thermophilic bacterium from corroded nickel tubing and shown that the exopolymers produced by this organism bind copper and,
Figure 1
Diagrammatic representation across a Gram Negative Bacterial Outer Membrane and of a Typical Lipopolysaccharide Molecule

- Environment
- Periplasmic space
- Cytoplasmic membrane
- Cell interior

KEY
- Metal cation
- Protein
- Phospholipid
- Lipoprotein
- Lipopolysaccharide

- Lipid A
- Sugar backbone and core
- Variable sugar side chain
furthermore, accelerate the corrosion of various metals, probably by the formation of copper concentration cells (Little et al., 1987). Later work by Ford and his co-workers (Ford et al., 1988) has demonstrated that exopolymers from different bacteria and even from the same bacterial species under varying conditions have different metal-binding abilities. In particular, exopolymer from planktonic Thermus sp. binds much less copper than that from sessile organisms. This Thermus sp. has also been shown to accelerate corrosion. The LPS of D. vulgaris has been shown to bind ferrous ions selectively (Bradley et al., 1984) and similar corrosion mechanisms may be postulated in this case. Further evidence for the involvement of EPS in corrosion comes from David White's group. They have shown that the excreted polysaccharide from the marine bacterium *Pseudomonas atlantica* produces enhanced corrosion rates of stainless steel over control rates (White et al., 1985).

It is apparent then that bacterial polysaccharides may be important in corrosion processes in a number of ways -

(a) As LPS in the external leaflet of the outer membrane of Gram negative cells
   (i) enhancing adhesion of cells to the metal surface
   (ii) selectively binding metal cations and so accelerating corrosion

(b) As EPS excreted from bacterial cells
   (i) enhancing adhesion of the excreting cells and of other cells in the vicinity to the metal surface to form a biofilm
   (ii) chelating metal ions or otherwise promoting corrosive processes.

This presentation reports the initial results of experiments undertaken to define the importance of LPS and EPS of two widely differing bacterial genera in adhesion to metal surfaces and the concomitant corrosion. The bacteria chosen are the anaerobic *Desulfovibrio desulfuricans*, New Jersey, a member of the sulphate-reducing bacteria group and hence an important corrosion-inducing species (Postgate, 1984), and the aerobic *Pseudomonas fluorescens*, a ubiquitous bacterium often found as a primary coloniser of surfaces in aqueous environments and belonging to a genus previously associated with metal corrosion (Obuekwe et al., 1981; White et al., 1985).
METHODS

D. desulfuricans New Jersey (NCIMB 8313) was grown in Postgate's Medium C (Postgate, 1984).

P. fluorescens, isolated from a contaminated metal-working fluid, was grown on nutrient agar and resuspended in Medium C for adhesion experiments. For studies on biofilm formation the cells were grown in Medium C.

Specific antibodies were raised in New Zealand White rabbits by intramuscular injection with extracted LPS from either species of bacteria. Two injections were given at weekly intervals and the serum separated from blood taken one week after the last injection. Immunoglobulins were partially purified from the sera by salt precipitation (Siew, 1987) and were freeze-dried.

The influence of the immunoglobulins on the adhesion of bacterial cells to mild steel was investigated by incubating stubs composed of mild steel (BS970) in Medium C plus approximately $10^8$ bacterial cells/ml with or without cell pre-treatment for 1.5 h with 400 µg/ml immunoglobulin or salt-precipitate from normal rabbit serum. The numbers of cells adhering after 1 h were determined by fluorescence microscopy of specimens stained with 0.001% acridine orange.

Biofilm formation on mild steel was studied by SEM and EDAX. Stubs were incubated as above in Medium C containing one or both bacterial genera (inoculum $10^5$ cells/ml of each type) for up to eight weeks. At defined intervals, stubs were removed, rinsed in cacodylate buffer, fixed in glutaraldehyde and post-fixed in osmium tetroxide. After dehydration through an isopropanol-water series, samples were freeze-dried, mounted on aluminium stubs and sputter-coated with gold ready for observation in the SEM. EDAX was performed on unfixed and uncoated samples using a Super-Mini SEM equipped with an energy-dispersive X-ray analyser (Lewell Electronics Ltd).
It has previously been reported (Gaylarde and Beech, 1988) that the adhesion of P. fluorescens to mild steel is inhibited by the lectins concanavalin A and wheat germ agglutinin and that this inhibition is reversed by the sugars glucose and N-acetyl glucosamine. The inference drawn from these results, that P. fluorescens adhesion is associated with the presence of glucose and N-acetyl glucosamine on the cell surface, was substantiated by the results of enzyme treatments. Glucosidase and N-acetyl glucosaminidase both reduced P. fluorescens adhesion to mild steel. In the case of D. desulfuricans New Jersey, similar experiments indicated that N-acetyl glucosamine only was involved in this adhesion.

For both bacterial species, protease treatments did not affect adsorption, suggesting that the sugars involved in adhesion are not present as glycoproteins, but rather as LPS or EPS associated with the bacterial cells (Gaylarde and Beech, 1988). Glucose and N-acetyl glucosamine have been shown to be the main sugar components of the EPS of Pseudomonas spp. (O'Neill et al, 1983; Wrangstad et al, 1986) and glucosamine has been detected in the LPS of Desulfovibrio sp. (Siew, 1987), hence both LPS and EPS are good candidates for adsorption-relation molecules.

In the present study, immunoglobulins precipitated from antisera raised against the LPS of each of the bacterial species and of the related sulphate-reducing bacterium Desulfovibrio vulgaris Woolwich were shown to inhibit adhesion of homologous or related cells to mild steel (Tab. 1). The adsorption of D. desulfuricans cells was reduced by treatment with antibodies against the LPS of either D. desulfuricans or D. vulgaris, but not by treatment with normal rabbit serum or with antibodies raised against P. fluorescens LPS. Similarly, P. fluorescens adhesion was inhibited by antibodies to homologous LPS, but not by anti-Desulfovibrio LPS or normal rabbit serum.

These results would seem to indicate that the structures important in adhesion of P. fluorescens and D. desulfuricans to mild steel are the polysaccharide side chains of LPS. However, Sutherland (1985) has stated that it is often difficult to distinguish between truly structural polysaccharide, present as LPS in the outer membrane, and excreted polysaccharide (EPS) in transit through the cell wall. It is therefore
TABLE 1: Numbers of bacterial cells adhering to mild steel within one hour following preincubation of cells with specific antibodies for 1.5 hours.

<table>
<thead>
<tr>
<th>Bacterial species</th>
<th>Preincubation with:</th>
<th>Mean number of cells adhering to $0.03\text{mm}^2 \pm \text{S.D.}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>D. desulfuricans</td>
<td>Medium C only</td>
<td>48.05 ± 7.83</td>
</tr>
<tr>
<td></td>
<td>NRS</td>
<td>50.7 ± 12.4</td>
</tr>
<tr>
<td></td>
<td>A-LPS$_{PF}$</td>
<td>49.9 ± 5.98</td>
</tr>
<tr>
<td></td>
<td>A-LPS$_{DD}$</td>
<td>33.4 ± 8.9</td>
</tr>
<tr>
<td></td>
<td>A-LPS$_{DV}$</td>
<td>31.4 ± 8.21</td>
</tr>
<tr>
<td>P. fluorescens</td>
<td>Medium C only</td>
<td>50.02 ± 8.76</td>
</tr>
<tr>
<td></td>
<td>NRS</td>
<td>50.12 ± 6.88</td>
</tr>
<tr>
<td></td>
<td>A-LPS$_{PF}$</td>
<td>29.0 ± 4.37</td>
</tr>
<tr>
<td></td>
<td>A-LPS$_{DD}$</td>
<td>49.7 ± 8.2</td>
</tr>
<tr>
<td></td>
<td>A-LPS$_{DV}$</td>
<td>50.7 ± 7.02</td>
</tr>
</tbody>
</table>

NRS: Normal rabbit serum
A-LPS$_{PF}$: Antibody to P. fluorescens LPS
A-LPS$_{DD}$: Antibody to D. desulfuricans LPS
A-LPS$_{DV}$: Antibody to D. vulgaris LPS
important to isolate and analyse EPS from the two bacterial species used in this study and this is at present under way.

Any EPS produced will form part of the biofilm produced by the bacteria. SEM studies of biofilm formed on mild steel stubs incubated with pure or mixed cultures of bacteria gave widely differing results. In the early stages of colonisation, P. fluorescens attached more readily to the mild steel surface than D. desulfuricans (Gaylard and Beech, 1988), but after six weeks P. fluorescens incubated stubs were only patchily colonised, with areas of bare metal still visible (Fig. 2). There was little apparent biofilm. On the other hand, D. desulfuricans incubated stubs viewed after six weeks showed an almost complete covering of biofilm consisting of bacterial cells, EPS and corrosion products (Fig. 3). EDAX analysis showed that the major elements in this biofilm were iron and sulphur, presumably present as iron sulphides. High quantities of sulphur were also detected in biofilms formed in mixed cultures of D. desulfuricans and P. fluorescens, but not in biofilms on mild steel surfaces incubated with P. fluorescens alone. This confirms that P. fluorescens, a non-sulphate-reducing bacterium, does not induce the production of corrosive iron sulphides. In view of earlier work showing enhanced sulphide film formation and associated corrosion in mixed as compared with pure cultures of SRB (Gaylard and Johnston, 1982; Gaylard and Videla, 1987), it is interesting to note that in the current study sulphur is present in larger quantities in biofilms formed in the presence of pure D. desulfuricans than in those produced in mixed Pseudomonas-Desulfovibrio cultures. This serves to emphasise the importance of the species of associated bacteria in mixed SRB-induced corrosion, as has been previously demonstrated (Gaylard and Johnston, 1986).

EDAX analysis also showed that the content of phosphorus in the biofilm varied with environment. It has been suggested that the corrosive activity of SRB is due to the production of a reduced phosphorus compound (Iverson and Olson, 1983). In the present study, phosphorus was found to be present in greatest amount on mild steel stubs which had been incubated in Medium C without any bacterial cells. This does not appear to confirm the relationship of phosphorus to the intense corrosion induced by SRB.
Figure 2
Scanning electron micrograph of mild steel surface after 4 weeks' incubation in Medium C plus P. fluorescens. Note large areas of bare metal. Occasional inorganic crystals are also seen.

Figure 3
Scanning electron micrograph of mild steel surface after 4 weeks' incubation in Medium C plus D. desulfuricans. Note large numbers of bacterial cells and string-like EPS. Clumps of granular corrosion product are also visible.
CONCLUSION

The early-colonisation of mild steel by *D. desulfuricans* and *P. fluorescens* is associated with cellular surface structures containing N-acetyl glucosamine and, in the case of *P. fluorescens*, glucose. These structures are probably the polysaccharide side chains of LPS, but may occur in excreted polysaccharides (EPS) present transiently in the cell outer membranes.

Following initial cell adhesion to the mild steel, *D. desulfuricans* rapidly causes the build-up of a biofilm consisting of bacterial cells, EPS and corrosion products. This biofilm completely obscures the metal surface. *P. fluorescens*, in spite of an initial rapid adherence, produces an incomplete biofilm over the same timescale. Patchy accumulations of cells with no apparent EPS or corrosion products are found on the mild steel surface. Work is at present under way to quantify the degree of corrosion associated with these adherence phenomena.
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DETECTION OF BIOFILMS ASSOCIATED WITH PITTING CORROSION OF COPPER PIPEWORK IN SCOTTISH HOSPITALS

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ABSTRACT

A survey was undertaken of the water quality and plumbing systems of two Scottish hospitals experiencing corrosion of the copper pipework. Waters were also concentrated aseptically from various sites within the hospitals and one metre lengths of copper pipework were withdrawn from the hot water supply for microbiological examination. Extensive pitting corrosion was evident at several sites and a copious biofilm was observed by scanning electron microscopy. Microbiological analysis of the black tubercles covering the perforated areas indicated the presence of anaerobic sulphate-reducing bacteria (SRB) and a variety of aerobes, including *Pseudomonas* and *Alcaligenes* spp., pink-pigmented facultatively methylotrophic bacteria and fungi. Fewer SRB and fungi were detectable in the thinner biofilm associated with the site experiencing minor corrosion but no pitting. The water quality of both the badly and less corroded sites was similar but the hot water supply of the latter was maintained at 60°C whilst the former rarely exceeded 50°C.

Une enquête sur le terrain a été entreprise au niveau de la qualité des eaux et des systèmes de distribution de plusieurs hôpitaux de Glasgow connus ou suspectés pour avoir des problèmes de corrosion au niveau des canalisations en cuivre. Les eaux de plusieurs sites hospitaliers sont concentrées stérilement et un canalisation d’un mètre de long est prélevée du circuit d’eau chaude pour examen microbiologique. On remarque une importante corrosion par piqûres à plusieurs endroits et un biofilm abondant est observé en microscopie électronique à balayage. Les analyses microbiologiques des amas noirs au niveau des surfaces perforées indiquent la présence de bactéries anaérobies sulfatoréductrices (BRS) et
d'une flore aérobie comprenant des *Pseudomonas* et des champignons. Les eaux concentrées contiennent aussi des BSR et la même flore aérobie bien que peu de champignons soient détectés. Un plus grand nombre de BSR et de champignons ont été trouvés au sein d'un fin biofilm présent sur un site expérimenté où seulement une corrosion mineure sans piqûres était observée. La qualité des eaux des deux sites plus ou moins corrodes est similaire bien que la première soit maintenue à 60°C tandis que la seconde excède rarement 50°C.
INTRODUCTION

Copper pipework has a twenty-five year guarantee against manufacturing defects and under normal operating conditions is expected to last the lifetime of the building. Many millions of metres of copper plumbing tubes continue to give excellent service supplying hot and cold water in both domestic and industrial buildings. However, it is now apparent that the plumbing systems of a few institutional buildings in certain soft water areas have suffered severe deterioration. In particular, pitting corrosion has resulted in perforation of copper tube in such diverse areas as Scotland, Saudi Arabia and West Germany, although corrosion in the latter country appears to be of a modified form to that experienced elsewhere. The corrosion process was so well established in some Scottish hospitals that renewed sections of the plumbing system suffered identical pitting in a matter of months. This unique corrosion problem cannot be explained simply in terms of chemical corrosion per se and suspicion has fallen on the possible involvement of microorganisms, as exemplified by the role of biofilms in corrosion of the steel legs of drilling platforms in the sea (Hamilton, 1985). In this process, sulphate-reducing bacteria (SRB) in a complex microbial consortium use sulphate in the water as an electron acceptor for anaerobic growth, liberating hydrogen sulphide in the biofilm which attacks the steel and establishes a corrosion cell.

The extent of corrosion of copper tubing in Scottish hospitals is not known but replacement costs could be very high. Moreover, the escape of aerosolised water sprays through perforated tubing poses a potential health risk, particularly if Legionella spp. were present in the water or able to grow in biofilms (Colbourne and Dennis, 1988; Keevil et al., 1988) adjacent to the perforations. Even in the absence of such pathogens, aquatic bacteria may, when aerosolised, be involved in processes giving rise to humidifier fever and other lung infections. Indeed, any biofilm which is resistant to the toxic effects of copper may well harbour and protect potential pathogens which are normally inhibited by this metal (Gadd and Griffiths, 1978; Falkinham et al., 1984; States et al., 1984).

Growth of individual bacterial species or complex microbial consortia and the development of biofilms is markedly affected by the local environment, including changes in nutrient availability, pH, temperature, oxygen concentration, redox potential and metals concentration (Ellwood et al., 1982; Keevil et al., 1987, 1988; Glenister et al., 1988). Accordingly, the aim of this investigation was to assess the water quality and plumbing systems of two Scottish hospitals and establish physico-chemical and microbiological parameters of importance to the corrosion process.
MATERIALS AND METHODS

Water Supply and Sampling
The soft, peaty water was supplied from an upland catchment (Scottish loch). Water at various points in the plumbing of the hospitals was collected for chemical analysis (Anon, 1979) and determination of assimilable organic carbon (AOC; Stanfield and Jago, 1987). A continuous monitor was installed in one hospital close to the calorifier to study Eh, pH, temperature and dissolved oxygen fluctuations over a 24-hour period. In addition, 10 l samples of the incoming mains supply (hydrant), the stores supply (first tap off the holding tank) and water from the calorifiers (first tap off calorifier) or representative of that flowing through the rest of the distribution system (furthest tap in system) were concentrated aseptically by passage through 0.2 µm nylon membrane filters followed by resuspension in 50 ml of the filtrate. Swabs of various taps, cold water holding tanks and calorifier outlets were suspended in 10 ml of the local water supply. The hot water flow to suspect sections of copper pipework was stopped and one metre lengths were cut and immediately sealed at one end with a bung covered with sterile plastic sheet. The tube was filled to the brim with water from that part of the supply and sealed.

Microbiological Analysis
The sealed tubes were reopened in an anaerobic cabinet containing an atmosphere of 80% N₂:10% H₂:10% CO₂ to exclude oxygen that can kill any strict anaerobes present in the samples. The waters were aseptically decanted into sterile containers and some of the pipes were seen to contain large black nodules which were clearly associated with perforations through the metal. These were scraped off with a sterile dental probe and resuspended by vortex mixing in either 5 ml of the membrane-filtered water (Colbourne et al., 1988) obtained from the site or sterile Page’s (1967) amoebal saline which was found to be better in the present study for maintaining the viability of the aquatic bacteria. The concentrated waters, resuspended swabs and biofilm were plated (0.1 ml) onto modified Postgate’s (1984) Medium agar (to isolate SRB), mineral salts agar containing 0.2% (v/v) methanol (to isolate methylo trophic microorganisms; Colby and Zatman, 1973), minimal R2A agar (to isolate the majority of aerobic and anaerobic microbes and reduce the risk of substrate shock preventing recovery of oligotrophs; Reasoner and Geldreich, 1985) and BCYE agar (to isolate more fastidious aerobes, legionellae and anaerobes; Pasculle et al., 1980). Dilutions of
samples were also inoculated into SRB anaerobic broth (Micran, Aberdeen) in triplicate to determine the concentration of SRB by the most probable number (MPN) technique. Biofilm was also streaked directly onto the agar media without resuspension. All samples were incubated at 30°C, both anaerobically in 80% N₂:10% H₂:10% CO₂, and aerobically in 5% CO₂ in air. The number and morphology of colony forming units (cfu) were ascertained after three, six and nine days incubation. The isolated bacteria were tentatively identified by their biochemical reactivity using the API (API 20NE, API-Biomerieux, Basingstoke) and automated Vitek (MacDonald Douglas Bactomatic) database systems. Those colonies suspected of being Legionella spp. after growth on BCYE agar (by morphology and purple colouration) were subcultured onto GVPC Legionella Selective Agar (Dennis et al., 1984). L. bozemanii was confirmed by fluorescence of colonies under UV irradiation and reaction with a specific antiserum raised in guinea pigs.

Scanning electron microscopy of suspected biofilms was performed according to the method of Keevil et al., (1987) on 1 cm diameter disks which had been cut out along the lengths of the recovered pipe. These disks were also observed for corrosion under a binocular microscope at low magnification. The waters and resuspended biofilms were observed at 1000 x magnification after Gram staining for the presence of microorganisms.

RESULTS

Pipe Corrosion

The interior surfaces of the copper pipework from the first hospital (hospital X) were covered with a heavy film (Fig.1a,b). Large black tubercles in the tubing taken approximately 100 metres from the calorifier (site 1; Fig.1c) were clearly associated with extensive pitting corrosion (Fig.1d), leading to perforations through the tubing and escape of water. Fewer and smaller tubercles with no perforations were observed in tubing from hospital X at a point less than 60 metres from the calorifier (site 2). The pipework of the second hospital (hospital Y) is supplied with water from the same public supply and it also contained a heavy deposit (not necessarily biofilm; see later) but there was little or no evidence of tubercle formation or corrosion (site 3; Fig.1e,f).
Chemical Analysis

The chemical quality of the water supplying the two hospitals was typical of an upland surface source with a natural colour from the dissolved humic acids of peaty soil (Table 1). The low mineral content and poor buffering capacity resulted in a variable pH of 7.4 - 9.3. These variations are most probably associated with lime dosing at the water treatment works and the passage through the distribution network but they might also indicate changes within the plumbing itself. Apart from pH, water chemistry remained reasonably constant throughout the plumbing but copper concentrations increased approximately 30-fold in the hot water at hospital X where pitting corrosion was severe and 60-fold at hospital Y experiencing little corrosion in the sample observed. These increases may have been due to corrosion processes in the pipework but the copper concentrations are still well within EC limits. Carbon available for microbial growth (AOC) was abundant in the incoming water supply (Table 2). This value increased from approximately 7.8 in the hydrant water supplying the two hospitals to 12.4 in the holding tank of the first hospital which may reflect concentration of organic debris in the tank. By comparison, the AOC of London water (lowland, hard surface water) is typically only 3.0. The AOC decreased to values of 4.8 and 1.4 in the plumbing systems of hospitals X and Y, respectively. This depletion may indicate prolific biofilm activity assimilating the available carbon.

Temperature and Oxygen Analysis

The site survey was undertaken in late autumn so the temperature of the hydrant and tank samples was only 10°C, sufficient for survival but not growth of microorganisms (Table 1). A continuous monitoring system revealed that the temperature of the hospital X hot water rarely exceeded 50°C and was more often at 40°C for many hours (Fig.2). This is contrary to the DHSS Code of Practice (1988) stating the need for maintaining a water temperature of at least 50°C. Even when the first outlets downstream from the calorifier were at 50°C the temperature at outlets more distant decreased (Table 1). In the case of hospital X which experienced severe corrosion the temperature was only 43°C at the distant tap outlet after flushing and was as low as 28°C when no water was flowing. A similar low temperature was noted at the distant outlet in hospital Y when no hot water was flowing. Perhaps significantly, however, the temperature of the flowing hot water was always above 56°C and little or no corrosion of pipework was evident. The generally low temperatures at hospital X experiencing corrosion were complemented with marked variation in the dissolved oxygen concentration: a sharp fall
Table 1. Chemical analysis of the waters in the plumbing systems of two hospitals experiencing severe (x) or minor (y) pitting corrosion

<table>
<thead>
<tr>
<th></th>
<th>Hospital x</th>
<th></th>
<th></th>
<th>Hospital y</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Hydrant</td>
<td>Tank off</td>
<td>1st tap</td>
<td>Hot tap</td>
<td>1st tap</td>
<td>Hot tap</td>
</tr>
<tr>
<td></td>
<td></td>
<td>calor.</td>
<td>site 1</td>
<td>site 2</td>
<td>site 3</td>
<td>site 3</td>
</tr>
<tr>
<td>Temperature - initial</td>
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<td>28</td>
<td>17</td>
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<td></td>
<td>after flush</td>
<td>-</td>
<td>51</td>
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<td>Colour (Hazen)</td>
<td>13</td>
<td>14</td>
<td>13</td>
<td>13</td>
<td>13</td>
<td>12</td>
</tr>
<tr>
<td>mg/l Solids</td>
<td>81 (81)</td>
<td>44 (44)</td>
<td>50 (50)</td>
<td>46 (46)</td>
<td>37 (37)</td>
<td>33 (33)</td>
</tr>
<tr>
<td>Hardness (CaCO₃)</td>
<td>&lt;20</td>
<td>&lt;20</td>
<td>&lt;20</td>
<td>&lt;20</td>
<td>&lt;20</td>
<td>&lt;20</td>
</tr>
<tr>
<td>Carbon dioxide</td>
<td>0.1</td>
<td>0.1</td>
<td>0.1</td>
<td>0.2</td>
<td>0.2</td>
<td>1</td>
</tr>
<tr>
<td>Organic carbon</td>
<td>1.6</td>
<td>1.6</td>
<td>1.6</td>
<td>1.6</td>
<td>1.6</td>
<td>1.6</td>
</tr>
<tr>
<td>Dissolved oxygen</td>
<td>11.2</td>
<td>11.3</td>
<td>12.6</td>
<td>11.7</td>
<td>12.3</td>
<td>8.4</td>
</tr>
<tr>
<td>Oxidised nitrogen</td>
<td>&lt;0.2</td>
<td>0.2</td>
<td>0.2</td>
<td>&lt;0.2</td>
<td>&lt;0.2</td>
<td>&lt;0.2</td>
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<tr>
<td>Chloride</td>
<td>&lt;5</td>
<td>5</td>
<td>5</td>
<td>&lt;5</td>
<td>&lt;5</td>
<td>&lt;5</td>
</tr>
<tr>
<td>Sulphate</td>
<td>3</td>
<td>4</td>
<td>3</td>
<td>3</td>
<td>4</td>
<td>3</td>
</tr>
<tr>
<td>Sulphide</td>
<td>&lt;0.003</td>
<td>&lt;0.003</td>
<td>&lt;0.003</td>
<td>&lt;0.003</td>
<td>&lt;0.003</td>
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</tr>
<tr>
<td>KMnO₄ oxidisability</td>
<td>2.4</td>
<td>2.6</td>
<td>2.3</td>
<td>1.7</td>
<td>2.4</td>
<td>1.7</td>
</tr>
<tr>
<td>Reactive phosphorus</td>
<td>&lt;0.1</td>
<td>&lt;0.1</td>
<td>&lt;0.1</td>
<td>&lt;0.1</td>
<td>&lt;0.1</td>
<td>&lt;0.1</td>
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<tr>
<td>Reactive SiO₂</td>
<td>&lt;0.5</td>
<td>&lt;0.5</td>
<td>&lt;0.5</td>
<td>&lt;0.5</td>
<td>&lt;0.5</td>
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<tr>
<td>Calcium</td>
<td>5</td>
<td>4</td>
<td>4</td>
<td>3</td>
<td>3</td>
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<tr>
<td>Magnesium</td>
<td>0.6</td>
<td>0.7</td>
<td>0.6</td>
<td>0.6</td>
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<tr>
<td>Sodium</td>
<td>3.3</td>
<td>3.3</td>
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<tr>
<td>Potassium</td>
<td>0.3</td>
<td>0.3</td>
<td>0.3</td>
<td>0.3</td>
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<td>0.3</td>
</tr>
<tr>
<td>µg/l aluminium</td>
<td>33</td>
<td>35</td>
<td>33</td>
<td>39</td>
<td>48</td>
<td>26</td>
</tr>
<tr>
<td>Copper</td>
<td>10 (25)</td>
<td>10 (10)</td>
<td>139 (46)</td>
<td>111 (63)</td>
<td>332 (77)</td>
<td>78 (44)</td>
</tr>
<tr>
<td>Iron</td>
<td>64 (25)</td>
<td>74 (35)</td>
<td>60 (33)</td>
<td>94 (38)</td>
<td>201 (33)</td>
<td>96 (45)</td>
</tr>
<tr>
<td>Lead</td>
<td>&lt;5</td>
<td>&lt;5</td>
<td>&lt;5</td>
<td>5</td>
<td>&lt;5</td>
<td>&lt;5</td>
</tr>
<tr>
<td>Manganese</td>
<td>10 (&lt;10)</td>
<td>10 (&lt;10)</td>
<td>10 (&lt;10)</td>
<td>24 (&lt;10)</td>
<td>10 (&lt;10)</td>
<td>10 (&lt;10)</td>
</tr>
<tr>
<td>Zinc</td>
<td>12 (10)</td>
<td>20 (12)</td>
<td>10 (10)</td>
<td>16 (13)</td>
<td>25 (13)</td>
<td>69 (54)</td>
</tr>
</tbody>
</table>

The data are expressed as total dissolved plus insoluble concentrations. Numbers in parentheses refer to the dissolved concn. only, were appropriate. aThe solids and iron concn. of the hospital y mains supply were 32 (32) mg/l and 105 (32) µg/l, respectively.
Table 2. Microbiological and assimilable organic carbon analyses of the waters from various sites within the plumbing systems of hospitals experiencing severe (x) or minor (y) corrosion.

<table>
<thead>
<tr>
<th>Aerotolerant spp: (cfu/ml)</th>
<th>Hospital x</th>
<th>Hospital y</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Hydrant Tank</td>
<td>Tank</td>
</tr>
<tr>
<td>Aerotolerant spp: (cfu/ml)</td>
<td>280*</td>
<td>300</td>
</tr>
<tr>
<td>Legionella spp. (cfu/ml)</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Fungi (cfu/100ml)</td>
<td>2</td>
<td>7</td>
</tr>
<tr>
<td>Sulphate reducing bacteria (cfu/100ml)</td>
<td>6</td>
<td>0</td>
</tr>
<tr>
<td>Assimilable organic carbon (ATP x 10^-10 g/l)</td>
<td>7.4*</td>
<td>12.4</td>
</tr>
</tbody>
</table>

No coliforms or Pseudomonas aeruginosa were detectable in the waters. *The number of aerotolerant spp. and the ADC value in the mains water of hospital y were 50 cfu/ml and 8.1, respectively.
Figure 2  Continuous monitoring of the temperature (\(\bullet\)) and dissolved oxygen concentration (\(\circ\)) of the hot water supply at hospital X over a 24 hour time course
occurred overnight for 12 out of 24 hours when little water was used, establishing anaerobic conditions in the water column (Fig. 1).

Microbiological Analysis
The incoming cold water to both hospitals contained low counts of bacteria (Table 2). The composition of the aerobic microbial flora after up to nine days incubation on agar media comprised mainly slow-growing, Gram-positive streptococci, micrococci, and Gram-negative rods and bacilli which were predominantly white, yellow or red in colouration. Using the API and Vitek databases, some of the Gram-negative species were tentatively identified as *Alcaligenes*, *Achromobacter* and *Flavobacterium* spp., (white colonies) and *P. paucimobilis* (yellow colonies). *Legionellae* were recovered from the base of the vertical calorifier in hospital Y; the temperature of the preflush water was only 27°C and a high count of viable bacteria was detected in this sample. After prolonged flushing this count decreased but *Legionella bozemanii* was recovered. Presumably this had been released from biofilm near the outlet by turbulence (Colbourne and Dennis, 1988). Low numbers of fungi were present in samples from the hydrant and tanks at both hospitals. Blackening of SRB broth and anaerobic growth on SRB agar confirmed the presence of low numbers of SRB, probably *Desulfovibrio* spp., in all of the water column samples. The MPN determination was possibly an underestimate of the SRB concentration since more organisms were apparently detected at the higher dilutions. The could be due to dilution of a toxic substance, such as the relatively high copper concentration in the water.

A similar complex flora was detected in swabs (biofilm/deposits) from various sites but samples taken from the taps and tank sediments in both hospitals were negative for SRB. However, pink-pigmented facultative methylotrophs, able to grow aerobically on either R2A or methanol agar, were detected in biofilms but not in the water supply. These bacteria were probably of the genus *Methylobacterium* (Hood et al., 1988). Their niche within the complex consortia might have been established due to release of C1 compounds from AOC by the focal activity of biofilm.

When the black tubercles associated with perforations in the tubing of hospital X were recovered anaerobically, the presence of many SRB was indicated by the rapid blackening of the indicator medium. These anaerobic bacteria were also visualised as discrete colonies when the
films were streaked directly onto Postgate's Medium agar. Many aerobic species were found, including fungi which could not be detected (visually or by culture) in the water itself. Scanning electron microscopy of the pitted surfaces revealed a copious biofilm (Fig.3a) which under increased magnification was seen to contain a complex community of rod and coccoid bacteria covered with extracellular polymeric material (Fig.3b). Their presence was confirmed by Gram stain of biofilm homogenised in water. By contrast, no black tubercles (Fig.1f) were visible in the tubing taken from hospital Y and fewer viable SRB and fungi were detected. No perforations were observed at low magnification (Fig.1e). Scanning electron microscopy revealed a much thinner biofilm with few rods and cocci (Fig.3c,d). Their presence was again confirmed in homogenised samples by Gram's stain.

DISCUSSION

Chemical analysis alone of the water supplying the Scottish hospitals could not readily explain the corrosion being experienced. By contrast, the survey indicated a link between the presence of biofilm and pitting corrosion of copper tubing. The more copious the biofilm and the greater the numbers of organisms, the more severe the corrosion. Intriguingly, McEvoy (1985) has described how a fungal isolate from fuel tank sludge, Cladosporium resinae, is capable of corroding steel and cupronickel alloys. Although identification of the organisms found in these biofilms has yet to be completed it is apparent that Pseudomonas and Alcaligenes spp. are present. This is of particular interest since such aquatic species are common in treated waters and produce exopolysaccharides. The production of these polymers may explain why the biofilm consortia were covered in polymeric material. Recent work by Geesey et al., (1987) has shown that microbial exopolymers can sequester copper, even when purified from the bacteria producing them. This sequestration seems to result in corrosion of the copper surface.

Perhaps as importantly, polymer formation helps to consolidate a biofilm and establish diffusion gradients whereby anaerobes can survive in the layers of the film (near the metal surface) which are depleted of oxygen by the metabolism of aerobic species in the upper layers. Biofilm consolidation can also be encouraged by the presence of fungi whose hyphae spread throughout the biofilm and tenaciously trap bacteria in a mesh-like structure. Interestingly, some of these fungi are resistant
to comparatively high concentrations of copper (McEvoy, 1985), making them attractive candidates as early pioneer colonisers of copper tubing. Thus, it is possible to speculate on the steps which might be involved in biofilm formation and corrosion of copper tubing in soft water areas. The following tentative scheme is proposed:

1. Most surfaces acquire a "pellicle" of absorbed material from the water supply which may modify the physico-chemistry of the surface. Pellicle formation was observed on the copper tube in the present survey. Soft, upland catchment waters such as those supplying the Glasgow area contain natural organic substances derived from the soil, e.g. humic acids (Thurman, 1985). Moreover, the Scottish water contained assimilable organic carbon at levels above those found in harder, lowland river waters and aerobes such as Pseudomonas spp. may use the polyphenolic humic acids present as nutrients. Colbourne (1979) and others (see Andreoni and Bestetti, 1988) have shown that pseudomonads in water can utilise a wide range of organic substances as sole carbon sources; the concentration for growth being very low. The pseudomonads produce exopolymers and attach to copper surfaces whose inhibitory effects may be rendered inert due to the pellicle. Polysaccharide production is also believed to protect bacteria against the inhibitory effects of copper. The bacteria may be joined by other species such as fungi which can be copper tolerant and thus a biofilm matrix forms. At this stage, polysaccharide production may sequest copper and initiate an "aerobic corrosion" process.

2. Metabolism by aerobic members of the biofilm consortium reduces the oxygen concentration in the surrounding water (perhaps as demonstrated at hospital X) and permits the proliferation of strictly anaerobic species, such as SRB. This activity also depletes the oxygen within the deeper layers of the biofilm and permits the anaerobes to establish near the metal surface. Sulphate metabolism by the SRB produces hydrogen sulphide which forms copper sulphides and stimulates a corrosion potential, establishing the "anaerobic corrosion" process (Hamilton, 1985).
Depending on the physico-chemical interactions of the biofilms, these aerobic and anaerobic corrosion processes may take place sequentially or simultaneously. This proposed hypothesis is currently being evaluated in a continuous culture biofilm model using waters and biofilm inocula obtained from the badly corroded sites in hospital X (Keevil et al., 1988).

Of great interest is the observation that biofilm formation and corrosion was less in the hospital Y plumbing system and this correlated with the elevated temperatures at which the hot water was maintained. If biofilm formation is indeed responsible for the corrosion then it should be possible to apply preventative measures to minimise the problem. Regular cleaning is clearly important but this will not prevent viable bacteria re-colonising the systems. Regular physical "pigging" would help control the extent of biofilm formation, particularly if combined with the application of dispersants (organic acids or alkalis) but this is not practicable in the tortuous plumbing of institutional buildings. Another option may be to utilise the apparent correlation observed at hospital Y between increased water temperatures and biofilm reduction. The easiest procedure would be to "pasteurise" the distribution system intermittently. For example, the water of large institutional buildings is hardly used at night and could be heated to perhaps 70°C for 1 hour or pumped from top to bottom at 60°C, as recommended by the DHSS Code of Practice (1988). The finding of Legionella bozemanii at the bottom of a calorifier, itself running at 60°C at the outlet, shows the need to open all outlets in the system, if only for a few minutes each, to ensure heat penetrates any deadlegs.

This heating procedure may be less manually intensive than cleaning alone. The rate of biofilm buildup reoccurring between treatments might be judged by incorporating a continuous oxygen concentration monitor into the system and watching for waters becoming anoxic, as happened overnight at hospital X. If other measures are taken to reduce the nutrient input to the hot water systems (e.g. tank cleaning, reduced storage capacity, water treatment) then the frequency of heat treatment could be reduced still further.

In conclusion, it is recommended that the temperature of the hot water supply be maintained at between 50 and 60°C, as specified by the DHSS Code of Practice (1988).
Figure 1

Interior surfaces of copper pipework supplying hot water to two Scottish hospitals. Hospital X experienced severe corrosion at site 1, furthest from the calorifier (a) with extensive tubercle formation (viewed down the length of the tube (c)) and pronounced perforations (d). Tubercle formation and pitting was less evident at site 2, closer to the calorifier (b) and at hospital Y (e,f), where water temperatures were higher than at site 1. Marker bars denote 10mm.
Figure 3  Scanning electron micrographs of biofilm on the internal surfaces of copper pipework supplying hot water to hospital X (a,b) and hospital Y (c,d). Marker bars denote 10mm.
ACKNOWLEDGEMENT

We are grateful to IMI Yorkshire Copper Tube Ltd., Liverpool and the International Copper Research Association, Washington for funding this work and to Dr. P.J. Dennis, Dr. A.B. Dowsett, Miss A.A. West and Mrs. E.R. Elphick for their excellent technical assistance.

REFERENCES


INFLUENCE DE LA PROTECTION CATHODIQUE SUR LA CROISSANCE DES BACTERIES SULFATO-REDUCTRICES ET SUR LA CORROSION D'ACIERS LES SEDIMENTS MARINES

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IFREMER, Centre de Brest, BP 70, 29263 Plouzane, France.

ABSTRACT

La durée de vie des structures métalliques immergées en milieu marin est souvent liée à la mise en place d'une protection cathodique réalisée par anodes sacrificielles ou encore par courant imposé. Cependant, ce type de protection induit la formation de quantités non négligeables d'hydrogène susceptible d'être utilisé par un certain nombres de micro-organismes tels les bactéries méthanogènes ou sulfato-réductrices comme source potentielle d'énergie.

Une expérience a été réalisée dans le but d'étudier les relations possibles entre différents potentiels de protection, la croissance de bactéries sulfato-reductrices et la corrosion d'aciers doux placés dans des sédiments marins à deux températures; la température ambiante (10°C) et une température supérieure (35°C). Des analyses chimiques, bio-chimiques, microbiologiques ainsi que des études électrochimiques incluant des tracés de courbes de polarisation et diagrammes d'impédance ont été réalisées sur des périodes d'exposition de 1 à 3 mois et pour des potentiels imposés de -800, -900, -1000 et -1100mV/Ag.AgCl.

Les résultats obtenus indiquent une évolution de la structure de la communauté bactérienne à la surface des échantillons ou dans les premiers millimètres de sédiments au dessus des plaques. Cette évolution bactérienne, et principalement celle de bactéries sulfato-réductrices utilisant l'hydrogène cathodique comme donneur d'électrons, est de nature à remettre en cause certains critères de protection cathodique habituellement préconisés en milieu marin et un potential de -900mV/Ag.AgCl apparaît ainsi insuffisant pour protéger efficacement et durablement des structures métalliques placées dans un tel environnement.
INFLUENCE OF CATHODIC PROTECTION ON GROWTH OF SRB AND ON THE CORROSION OF MILD STEEL IN MARINE SEDIMENTS

In order to protect offshore structures from marine corrosion, cathodic protection is widely applied via sacrificial anodes or impressed current. In aerated seawater, steel is considered to be protected when a potential of -850mV/Cu./CuSO_4 is achieved. However in many cases of sediments this potential must be lowered due to the presence and activity of microorganisms and more especially sulphate-reducing bacteria (SRB). SRB are obligate anaerobes using sulphate as electron acceptor with resultant production of sulphide. Some of them are also able to use hydrogen as energy source causing depolarization of steel surfaces.

An experiment was performed in order to analyze the possible existing relation between SRB activity and use of different cathodic potentials applied to mild steel in marine sediments. These experiments were performed at two temperatures, 10°C and 35°C.

Analytical techniques included detection of lipid biomarkers and electrochemical techniques. Results indicated an evolution of the bacterial community structure both on steel surfaces and in the sediment surrounding the plates. Thus when cathodic protection breaks down or is in some way faulty over extended periods of time, biologically induced anaerobic corrosion can be expected to cause significant corrosion problems.
INTRODUCTION

La protection cathodique des structures métalliques placées en milieu marin peut être réalisée soit par l'utilisation d'anodes sacrificielles (Zinc, magnésium ou-aluminium) soit encore par courant imposé. Un potentiel de -850mV/Cu.CuSO₄ (Booth and Tiller, 1968) est généralement considéré comme suffisant pour assurer une bonne protection des ouvrages et placer les aciers habituellement utilisés à cet effet, dans des conditions de protection durables. Cependant, dans certaines sédiments marins, ce potentiel doit être très sensiblement abaissé du fait de la nature anaérobie du milieu et de la présence et activité de micro-organismes tels les bactéries sulfato-réductrices et méthanogènes. En se référant à des paramètres thermodynamiques et notamment au système Fe/S/H₂O (Norvath & Novak, 1964), ainsi qu'à "l'expérience" en ce domaine, un potentiel de -950mV/Cu.CuSO₄ apparaît comme nécessaire pour assurer une bonne protection des ouvrages.

Cependant la protection subséquente d'hydrogène cathodique à la surface des matériaux, peut avoir pour conséquences, outre les problèmes de fragilisation, l'utilisation par un certain nombre de microorganismes, de cet élément comme source d'énergie. C'est ainsi le cas de certaines bactéries sulfato-réductrices ouvrent considérées comme responsables d'initiation de corrosions localisées sur des ouvrages exposés en condition anaérobie.

Cette recherche a donc porté sur la relation possible entre la protection cathodique et la croissance de bactéries sulfato-réductrices dans des sédiments marins et les conséquences sur la corrosion d'aciers doux placés dans de telles conditions. Ces expérimentations ont été menées à deux températures, la première à la température de 10°C et la seconde à une température de 35°C.

Le développement des microorganismes en différentes zones du sédiment a été suivi à l'aide de méthodes microbiologiques et biochimiques, la corrosion des alliages étant quant à elle déterminée par voie électro-chimique avec tracés de courbes de polarisation, suivi de potentiels ou densités de courant en fonction du temps, et tracés de diagrammes d'impédances. Des analyses chimiques portant notamment sur les produits de corrosion, ont complété ces analyses.
MATERIELS ET METHODES

Des échantillons d'aciers doux de dimension 70 x 70 x 4 mm ont été placés dans différents bacs en polypropylène et recouverts de 5 cm de sédiments marins fraîchement prélevés de la Station marine de Ste. Anne du Fortzic à Brest.

Ces échantillons ont été polarisés respectivement aux potentiels de -800, -900, -1000 et -1100 mV/Ag.AgCl sur des périodes de 1, 2 et 3 mois. De manière identique, des échantillons de même dimension ont été placés sans protection dans des bacs et recouverts de sédiments marins pour les mêmes périodes d'exposition (Figure 1). Les évolutions du potentiel libre et des densités de courant des échantillons ont été suivies journellement par l'intermédiaire d'une centrale d'acquisition de données.

Les analyses et observations biochimiques et microbiologiques ont été réalisées pour chaque période d'exposition, en trois zones (Figure 2); une zone 1 correspondant à la surface des échantillons y compris les produits de corrosion adhérents à cette surface, une zone 2 correspondant aux premiers millimètres au dessus des sédiments et une zone 3 correspondant au reste du sédiment, exception faite de la couche de surface.

Les essais réalisés à la température de 35°C l'ont été par la mise en place de rubans chauffants fixés sur les échantillons, la face en contact avec l'élément chauffant étant recouverte d'une résine époxyde. Des mesures de température réalisées en différents points du sédiment et à divers temps d'exposition ont permis de déterminer une température de 35 à 37°C dans les premiers millimètres au dessus des échantillons chauffés. La température de 10°C correspond quant à elle à celle de l'eau de mer au moment des expérimentations.

Les analyses biochimiques ont été réalisées en étudiant les lipides bactériens. A l'aide des acides gras dérivés des diacylphospholipides de la membrane cytoplasmique bactérienne ou de ceux dérivés des lipopolysaccharides de la paroi cellulaire de ces microorganismes (White et al., 1979 et Guezzennec, 1985), il est possible d'obtenir de précieuses informations sur la biomasse bactérienne ainsi que sur la structure de la population bactérienne. Ces techniques biochimiques présentent outre une grande sensibilité, l'avantage de la non sélectivité des microorganismes et de la non destruction des interactions entre microcolonies pouvant mener à une sous-estimation appreciable de la biomasse bactérienne réellement présente.
Figure 1 : Schéma du montage

Figure 2 : Zones de prélèvement
RESULTATS ET DISCUSSIONS

Les analyses microbiologiques réalisées sur le sédiment avant mise en place des échantillons et de la protection cathodique, révèlent une population bactérienne majoritairement aérobie avec une microflore sulfato-réductrice peu abondante. Ces résultats sont confirmés par l'analyse des lipides bactériens avec une présence réduite en iC17:1w7c, 10 MeCl6:0 et cyclo Cl9:0 (Tableaux 1 et 2).

A température ambiante, le potentiel libre des échantillons non protégés cathodiquement évolue selon 3 phases distinctes; une première phase où les potentiels fluctuent autour d'une valeur de -800mV/Ag.AgCl, une seconde phase correspondant à une évolution de ces potentiels vers des valeurs moins électronégatives et une dernière phase correspondant à une stabilisation de ces valeurs autour de -650mV/Ag.AgCl (Figure 3). Cette évolution en 3 phases correspond au développement des bactéries sulfato-réductrices à l'interface métal-sédiment et à une activité de ces micro-organismes avec comme conséquence la formation d'une couche passivante. Cette croissance de bactéries sulfato-réductrices est mise en évidence par une évolution très sensible dans la zone 2 mais surtout au niveau des plaques, des différents marqueurs de ce métabolisme bactérien avec prédominance de bactéries du genre *Desulfovibrio* caractérisées par des teneurs importantes en acide iC17:1w7c.

L'évolution des potentiels libres est sensiblement différente à température plus élevée où l'on observe, après une courte phase de stablisation, une augmentation régulière en fonction du temps, évolution parallèle au développement de bactéries sulfato-réductrices dans les différentes zones prélèvement (Figure 4).

Les densités de courant en fonction du temps varient selon les potentiels imposés. À température ambiante, ces valeurs se situent entre $2 \times 10^6$ et $5 \times 10^5 \mu A/m^2$ selon les potentiels considérées. Les fluctuations de ces densités de courant, observées principalement aux potentiels les moins cathodiques peuvent être attribuées à la formation et à l'instabilité de certains produits de corrosion sur les surfaces (Figure 5). Il est à noter à -800 et -900mV/Ag.AgCl une augmentation de ces densités de courant après 20 jours de polarisation tandis qu'aux potentiels plus cathodiques, ces valeurs diminuent progressivement en fonction de temps. Cette diminution est le résultat de la formation de dépôts calcomagnésien.
<table>
<thead>
<tr>
<th>Microflore aérobie</th>
<th>1.4 $10^6$ bact/g poids sec</th>
</tr>
</thead>
<tbody>
<tr>
<td>anaérobie</td>
<td>4.3 $10^3$ bact/g poids sec</td>
</tr>
<tr>
<td>Bactéries sulfato-réductrices</td>
<td>2 $10^3$ bact/g poids sec</td>
</tr>
</tbody>
</table>

Tableau 1 : Analyses microbiologiques de sédiments au temps to.

<p>| | | |</p>
<table>
<thead>
<tr>
<th></th>
<th></th>
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<tbody>
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</tr>
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<td>aC17:0</td>
</tr>
<tr>
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<td>C17:1w8</td>
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Tableau 2 : Distribution d'acides gras dans le sédiment au temps to.
Figure 3 : Evolution du potentiel libre (t = 10°C)

Figure 4 : Evolution du potentiel libre (t = 35°C)
Figure 5 : Evolution des densités de courant $t = 10^\circ C$

Figure 6 : Evolution du potentiel après arrêt de polarisation (3 mois) $t = 10^\circ C$
sur les surfaces, conséquence de l’alcalinisation du milieu par réduction de l’oxygène ou des protons et donc, de la diminution de la surface active de ces échantillons.

L’évolution de la demande de courant enregistrée à -800 et -900mV/Ag.AgCl est à relier à l’augmentation de la biomasse bactérienne et principalement de la microflore sulfato-réductrice analysée après 1 mois d'expérimentation.

Cette augmentation de la demande en courant est également notée pour le potentiel de -900mV à 35°C tandis qu’au potentiel le plus cathodique (-1100mV/Ag.AgCl), ces densités restent stables tout au long des expériences. Cette stabilisation peut être liée à la formation plus rapide d’un dépôt homogène de calcium et de magnésium sous forme de calcite et brucite. Les valeurs de densités de courant observées à température ambiante et à 35°C vont dans le sens d’une prédominance du carbonate de calcium et par ailleurs, l’augmentation de température favorise la précipitation de ce sel (Wolfson and Hartt, 1981).

Lors du retrait des échantillons à l’issue du premier mois d’exposition, ceux préalablement polarisés à -800 et -900mV/Ag.AgCl sont recouverts de produits de corrosion et principalement de dépots de sulfures de fer adhérents aux surfaces, tandis que ceux polarisés aux potentiels les plus cathodiques apparaissent partiellement recouverts d’un dépôt de sels de calcium et magnésium identifié comme étant majoritairement du carbonate de calcium. Ces observations se retrouvent lors des essais réalisés à 35°C avec cependant moins de sulfures sur les échantillons polarisés à -900mV/Ag.AgCl et à l’opposé, un dépôt calco-magnésien plus homogène à -1100mV/Ag.AgCl.

Les analyses biochimiques et microbiologiques relevant à température ambiante une population bactérienne plus abondante comparativement au temps T₀, avec notamment, une densité de bactéries sulfato-réductrices mise en evidence par la présence de marqueurs biochimiques tels les acides iC17:1w7c, aC17:1w7c, 10MeC16:0, cycloC17:0 et C17:1w6 caractéristiques ou abondants chez ce type de microorganismes (White et al., 1986; Parks and Taylor, 1983). À -800mV et -900V/Ag.AgCl, cette croissance de population sulfato-réductrice se traduit par une augmentation de la demande en courant cathodique.
Aux autres potentiels considérés, la biomasse est également importante mais il faut également noter la présence d’indicateurs de bactéries sulfato-réductrices sur les surface d’échantillons polarisés à -1100mV/Ag.AgCl (Therene and Guezennec, 1987).

A la fin de l’expérimentation réalisée à température ambiante, soit après 3 mois sous protection cathodique, les échantillons non polarisés ainsi que ceux polarisés à -800 et -900mV/Ag.AgCl sont recouverts d’une couche de produits de corrosion, couche hétérogène constituée de sulfures de fer et de sable, tandis qu’aux deux autres potentiels considérés, les échantillons sont recouverts de dépôts calco-magnésien avec cependant présence de tâches de sulfure de fer sur les surfaces polarisées à -1000mV/Ag.AgCl.

A 35°C, les échantillons sous protection cathodique sont recouverts de dépôts calco-magnésien quelque soient les potentiels considérés. Aucune trace visible de corrosion n’est observée sur ces différents échantillons.

Les analyses biochimiques réalisées dans les différentes zones à température ambiante, montrent une population bactérienne proportionnelle au potentiel de protection avec une biomasse plus importance dans la zone 2 pour le potentiel le plus cathodique. Cette augmentation de population bactérienne se retrouve également au niveau des principaux marqueurs bactériens considérés dans cette étude et plus particulièrement ceux liés à la présence de bactéries sulfato-réductrices.

Il apparaît donc que le potentiel de protection et l’hydrogène produit cathodiquement favorisent dans la zone 2, c’est à dire dans le sédiment adjacent aux surfaces, la croissance du population bactérienne et plus particulièrement celle de bactéries sulfato-réductrices utilisant cet hydrogène comme source d’électrons pour leur métabolisme.

Les diagrammes d’impédance réalisés 24h et 15 jours après arrêt de la polarisation pour des périodes d’exposition de 3 mois ainsi que le suivi des potentiels de ces mêmes échantillons (Figure 5), confirment cette croissance de bactéries sulfato-réductrices et leur action sur le comportement des matériaux. Ces diagrammes d’impédance sont caractérisés par la présence de deux demi-boucles capacititives, la première aux hautes fréquences reliée à la présence de film et dépôt sur les surfaces, et une seconde aux basses fréquences, correspondant à la résistance de transfert de charge.
Au potentiel libre, à -800 et -900mV/Ag.AgCl, cette résistance de transfert n'est plus mesurable dès le premier mois pour les deux premiers potentiels, et après 2 mois pour -900mV/Ag.AgCl, les échantillons étant alors recouverts d'une épaisse couche de produits de corrosion. À -1000 et -1100mV/Ag.AgCl, la première demi-boucle observée aux hautes fréquences correspond à la formation d'un dépôt calco-magnésien avec des valeurs de capacité de double couche proches de celles trouvées dans des études précédentes, la seconde boucle aux basses fréquences indiquant une diminution de la résistance de transfert.

Le potentiel libre déterminé après arrêt de polarisation montre une évolution rapide les échantillons les plus polarisés vers des valeurs proches de -650mV/Ag.AgCl comparables à celles observées pour les échantillons non polarisés après 3 mois d'exposition (Figure 6). Cette évolution des potentiels correspond à une activité de bactéries sulfato-réductrices mise en évidence par les analyses biochimiques et microbiologiques et la formation d'une couche passivante de sulfures de fer. Cette corrosion des alliages ainsi que la présence de sulfures de fer est confirmée lors du retrait des échantillons du milieu sédimentaire avec notamment l'observation de corrosions localisées sur les échantillons préalablement polarisés à -1000 et -1100mV/Ag.AgCl.

A la température de 35°C, après 1 mois sous protection cathodique, les analyses microbiologiques et biochimiques montrent une population bactérienne abondante principalement au niveau des surfaces, que ce soit au potentiel de -900 ou -1100mV/Ag.AgCl. Cette importante population bactérienne se retrouve également après 3 mois d'exposition avec des densités bactériennes atteignant 10⁹ bactéries/cm² au potentiel le plus cathodique.

Les échantillons sont recouverts à la fin de l'expérimentation d'une couche de dépôts calcomagnésien aux deux potentiels étudiés, dépôts plus homogènes que ceux observés à température ambiante.

L'évolution des potentiels des échantillons laissés alors sans polarisation ainsi que celle des diagrammes d'impédance réalisés 24h et 15 jours après arrêt de polarisation, apparaît peu différente de celle observée pour les essais à température ambiante. L'évolution des potentiels libres vers des valeurs plus nobles apparaît cependant plus rapide notamment au potentiel de -900mV/Ag.AgCl. L'allure des
Figure 7 : Evolution des diagrammes d'impédance

A : 24 heures après arrêt de polarisation (3 mois)
B : 15 jours
diagrammes d'impédance montre simplement une diminution de la résistance de transfert pour les échantillons laissés sans protection 15 jours, principalement au potentiel de -900mV/Ag.AgCl.

Les densités de courant, tout comme dans l'expérimentation, conduit à plus basse température, favorisent la formation de carbonate de calcium comparativement à celle de l'hydroxyde de magnésium sous sa forme la plus couramment rencontrée, la brucité. Par ailleurs, l'augmentation de température va dans le sens d'une accélération du processus de précipitation des sels de calcium dont l'aspect dense et compact sur les surfaces leur confère un certain caractère protecteur.

CONCLUSIONS

De ces expérimentations en sédiment marin, il en ressort qu'à température ambiante, un potentiel de protection -900mV/Ag.AgCl apparaît insuffisant pour assurer une protection complète et durable de structures placées dans un tel environnement. Par ailleurs, si des polarisations inférieures à cette valeur assurent une bonne protection des ouvrages, il n'en reste pas moins vrai que l'hydrogène produit cathodiquement favorisé dans la zone de sédiments adjacente aux surfaces polarisées, la croissance de bactéries et principalement de bactéries sulfate-réductrices utilisant cet hydrogène comme source d'électrons. Cette croissance se traduit lors de l'arrêt de la protection par une évolution rapide des potentiels libres des échantillons, une corrosion localisée des aciers considérés et subséquemment la formation de couche passive sur les surfaces.

A une température plus élevée (35°C), le phénomène n'apparaît différent qu'au niveau de la densité de population bactérienne et sulfato-réductrice importante favorisée par une température proche de l'optimum de croissance de ces microorganismes et par la localisation de cette microflore présente principalement au niveau des surfaces polarisées au détriment de la zone 2. Toutefois, pour les périodes d'expérimentations considérées, il n'en résulte que de faibles conséquences sur la corrosion des alliages. La nature des dépôts calco-magnésien présents sur les surfaces et les phénomènes de redissolution de ces dépôts lors de l'arrêt de polarisation sont à prendre en considération pour interpréter les différences de résultats obtenus aux deux températures.
Les évolutions similaires obtenues dans les deux séries d'expériences, pour la biomasse bactérienne et la microflore de bactéries sulfato-réductrices confirment l'étroite dépendance de ces microorganismes vis-à-vis des sources de carbone nécessaires pour leur croissance. Par ailleurs les analyses biochimiques réalisées grâce à l'étude des lipides bactériens et caractérisation de marqueurs spécifiques à ces bactéries, permettent d'analyser une relation entre les bactéries utilisant l'hydrogène cathodique et celles ne possédant pas l'hydrogénase nécessaire à ce métabolisme.

Il convient cependant d'ajouter, que si, dans cette experimentation une relation entre l'hydrogène cathodique et la croissance de bactéries sulfato-réductrices a pu être mise en évidence à deux températures d'autres métabolismes et microorganismes, non considérés dans cette étude, utilisant l'hydrogène et/ou vivant en symbiose avec ces bactéries peuvent également initier des corrosions sur les surfaces et participer aux processus de dégradation des matériaux.
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EVALUATION OF BIOFILMS BY ADVANCED ELECTROCHEMICAL MONITORING

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ABSTRACT

The determination of the extent of biofouling on surfaces exposed to water which is prone to microbial proliferation is traditionally achieved by visual inspection, which may involve shutdown, or by the use of retrievable biofilm fouling probes. Both techniques are retrospective and the effectiveness of remedial measures cannot be evaluated until the next inspection. In the interim period, corrosion damage, a reduction in heat transfer efficiency, reduced flow or a combination of these conditions may persist. There is thus a recognised industrial need for rapid evaluation of the fouling and/or corrosion status of pipework and process equipment handling microbially active water, some of which may not be easily accessible.

Electrochemical monitoring techniques have been applied to continuously characterise the corrosion environment, including biofilm effects, and to evaluate the efficiency of water treatment chemicals. The monitoring systems are based on a combination of recently developed electrochemical techniques which are applied to suitable probes. We have used steel probes which are active.

These techniques have been principally used to evaluate the overall rate of metal loss due to corrosion but can also indicate the morphology of attack, i.e. they can characterise pitting, crevice, or stress corrosion cracking. The techniques are now being tested for their ability to indicate the presence and contribution to corrosion of surface films such as those formed by organic coatings, passive oxides, corrosion products, or biofilms and this paper outlines the success to date.
La détermination du colmatage biologique de surfaces exposées aux eaux prédisposées au développement des bactéries est habituellement réalisée par un examen visuel ou par l'utilisation de sondes. Les deux techniques sont rétrospectives et l'efficacité des traitements ne peut être évaluée qu'à l'examen suivant. Pendant cette période transitoire, des dégâts dus à la corrosion, une modification des transferts de chaleur, et/ou une réduction du débit peuvent persister. Il y a donc un besoin industriel de méthodes d'évaluation rapide du colmatage et/ou de l'état de corrosion des canalisations et des circuits d'eaux souvent peu accessibles contenant des microorganismes.

Des techniques de suivi électrochimiques ont été appliquées pour caractériser en continu les milieux de corrosion, y compris l'effet des biofilms et pour évaluer l'efficacité des traitements chimiques des eaux. Les systèmes de suivi sont basés sur une combinaison de différentes techniques électrochimiques développées récemment appliquées aux sondes.

Ces techniques ont été principalement utilisées pour évaluer la vitesse de la perte de poids due à la corrosion mais aussi pour observer le type d'attaque, c'est-à-dire piqûres, crevasses ou corrosion sous contraintes. Les techniques sont maintenant testées pour évaluer la présence et le rôle de films: revêtements organiques, couches d'oxydes, produits de corrosion ou biofilms. Cet article met en valeur les résultats actuels.
BACKGROUND

The Oil Industry has long been aware of the problem caused by biofilms; API and NACE documents from the early '60s (e.g. API RP-38 and NACE TPC-3) are evidence of this. The increased activity in this technology area appears to have arisen because the Industry has moved offshore and/or has begun to exploit deeper reservoirs. Both these aspects imply higher costs per barrel, fewer wells per field (hence higher cost per shutdown) and a more aggressive exploitation of the reservoir. Equally of note is the progress made by the Industry to reduce "general" corrosion.

Corrosion was once accepted as inevitable; the Industry has now diminished corrosion costs by a significant factor. Cost savings are difficult to evaluate but a fair estimate would be 30%. Incidentally the number of corrosion engineers employed (as recorded by membership of relevant societies) doubled over 1970 to early 1980s. Having resolved the simpler, more conventional corrosion problems, the Industry has directed its attention to the lower priority corrosion mechanisms. Corrosion in the UK costs some 3.5% Gross Domestic Product (GDP) equivalent (Hoare, 1971), whereas biofouling is estimated at 0.5% GDP (Pritchard, 1981). It is likely that these values relate, at least in order of magnitude, to the Oil Industry.

Biofilms

Characklis (1985) reports a neat classification of fouling mechanisms:

1. **Biological fouling**: the attachment and metabolism of macro-organisms (macrobiological fouling) and/or micro-organisms (microbial fouling).

2. **Chemical reaction fouling**: deposits formed by chemical reaction in which the surface material (e.g. condenser tube) is not a reactant. Polymerisation of petroleum refinery feedstocks is an important example of this type of fouling.

3. **Corrosion fouling**: the surface material itself reacts with compounds in the liquid phase to produce a deposit or degrade the surface material.

4. **Freezing fouling**: solidification of a liquid or some of its higher melting point constituents on a cooled surface.
5. **Particulate fouling**: accumulation on the equipment surface of finely divided solids suspended in the process fluid. *Sedimentation fouling* is an appropriate term if gravity is the primary mechanism for deposition.

6. **Precipitation fouling**: precipitation of dissolved substances on the equipment surface. This process is termed *scaling* if the dissolved substances have inverse temperature solubility characteristics (e.g., $\text{CaCO}_3$) and the precipitation occurs on a superheated surface.

In general, several of these occur at the same time: usually (1), (3), (5) and (6) in oil production sectors. To the scientists interested in mechanisms, it is important to separate them. The engineer is much less interested. He requires to know whether his plant is:

(a) **operating efficiently**: good thermal efficiency, low pumping costs, low filter maintenance, etc.;

(b) **operating safely**: no corrosion, cracking, minimum maintenance, health hazards, etc.

He must also work together with his colleagues upstream and downstream.

It would be interesting to contemplate the fate of the offshore topside facilities engineer who, to keep his equipment clear of biofilms, passed his problem on to the Petroleum Engineers by plugging the reservoir. The engineer demands ever simpler procedures to alert him to initiate corrective action. Corrosion has been resolved by such monitoring techniques as weight-loss, electrical resistance, linear polarisation, dynamic polarisation, zero resistance ammetry and increasingly for the more intractable processes ac impedance and the signal analysis techniques: electrochemical potential and current noise.

At present for biofilms there is only the equivalent of weight-loss coupons. These have all the disadvantages for biofilm evaluation as they did for corrosion evaluation: limited number hence limited data points, indicating an integration of past history, with no real time information. Are the techniques listed above any better?
**Electrical Resistance**

Here a wire or thin tube or plate is exposed. As it corrodes, it becomes thinner and thus the electrical resistance increases. By measuring the change in resistance, the corrosion is measured. This is a widely used technique but has a drawback in that increased sensitivity means a thinner metal section and shorter life. Since biofilms usually cause pitting, the wires fail quickly and must be replaced. Equally, any cause of thinning is measured, not only the biological aspect.

These probes have been used (Oganowski, 1985) to study the growth of sulphate-reducing bacteria (SRB) under marine fouling at different levels of cathodic protection (the probe can be polarised as an electrode). Such techniques are also being used for studying biological corrosion mechanisms at Harvard (Ford, 1986).

**Linear Polarisation**

Here, two metal electrodes are exposed and the potential (E) between them is fluctuated by up to 20 mV; the current response (i) is measured. At such low potential oscillations the relationship between E and i is linear, so a corrosion rate can be calculated. Such techniques have been widely used for microbiological studies where the corrosion rates with and without a biofilm/biological effect have been measured.

In the laboratory this technique is cheap, rapid and reliable. It has been used to study SRB action on cast irons, copper-nickels, C-Mn steels, and stainless steels. In the field, where highly controlled conditions do not exist, such a technique does not give a characteristic signal related to biofilming alone.

**Dynamic Polarisation**

This is essentially the linear polarisation method's "big brother". When the potential (E) on an electrode exceeds ±20 mV from its natural potential, then the current-potential relationship is not linear but becomes logarithmic. The potential changes used are usually from ±500 mV to ±1000 mV. The technique does show the effects of biofilms (Gilbert, 1987) but it also disturbs them or even destroys them such that a long period must elapse before the electrodes are relimed as before.
Zero-resistance Ammetry (ZRA)

Here two "identical" electrodes are exposed, wired together through an electronic ammeter (ZRA) such that the measuring current is electronically compensated for and the electrodes are effectively short-circuited together. This technique was used in the earliest corrosion studies (done manually using null-detectors) which has blossomed again since the late 1970s with the advent of reliable microchip amplifiers. It has begun to be used for biofilm work (Little, 1987). The latest findings (Gilbert, 1987, and Dexter, 1985) that biofilms cause major interfacial alterations and potential differences on surfaces may be an indicator that such a technique, coupled to potential measurement, may be one of the most suitable methods of biofilm evaluation.

Ac Impedance/Electrochemical Impedance

Here the potential of one electrode with respect to another (or reference electrode) is oscillated sinusoidally (ac) but at an ever changing frequency from kHz to mHz. The current response is measured and its lead/lag calculated. The results can be presented as Bode plots (impedance vs frequency) or as Nyquist plots (imaginary vs real impedance). The shape and location on the axes of these plots gives valuable information as to the condition of the surface and hence the condition of the biofilm.

The technique requires expensive and complex equipment and is used at present only in the laboratory for mechanistic studies (Merrique, 1978; Moosavi, 1987; Gilbert, 1987; Danko, 1988). Simpler forms of equipment are evolving and thus impedance techniques may be practicably applicable widely in the future.

Signal Analysis Techniques

Here a sensitive recording voltmeter is used to monitor the natural fluctuations in the potential of an exposed electrode or in the current flowing through a ZRA system. A given pattern of fluctuations in the potential is characteristic of a surface process, e.g. film formation and breakdown. This technique was used by Iverson (1968, 1985) to determine SRB attack on buried pipelines. More recently, the signals have been captured on microcomputer and analysed by amplitude/frequency modelling. It appears that particular distribution patterns are related to particular
events. To date, it is possible to determine SRB activity but only by a combination of noise signals and potential shift.

Combined Technique

The signals received from the surface of our electrodes reflect the processes occurring thereon: corrosion, biofilm development, scaling, precipitation. To determine the extent of a particular process, e.g. biofilming, it is necessary to extract the relevant part of the signal. This clearly is not possible except under defined conditions. However, the engineer rarely needs absolute information. For example, if he knows he has a biofilm problem then he requires to monitor this until it becomes unacceptable, at which point he will initiate a biocide treating programme; he will now require information on the efficiency of his treatment programme. This is now possible using a set of electrochemical monitoring techniques but not by one monitoring procedure alone. The most useful combination appears at present to be potential plus potential noise and current noise through ZRA systems, or at least for SRB-laden biofilms. "Simpler" biofilms may have a clearer fingerprint. Also worth considering is the option of using active (corrodible) and inert electrodes so that the biofilm component can be more easily identified from the combined signal.

RECENT CAPCIS MONITORING OF MICROBIOLOGICAL SYSTEMS

Background

The aim of the study was to identify a suitable electrochemical monitoring technique that could be related to a microbiological activity affecting the metal substrate and the influence of biocidal agents on that activity.

A large recirculatory flow rig containing a population of SRB in fresh water medium and an electrochemical bioprobe assembly were designed. High corrosion rates were not required as this was considered to be an undue complication of the interpretation of the electrochemical signals. Signal changes from mild steel in non-saline conditions have been shown to suffer from less background noise than saline environments. Fresh water environments therefore provide a more reliable base-line on which to investigate microbiological activity.
Biocide selection was restricted to a non-film breaking non-surfactant type. Over the period of the test, it was anticipated that filming of the loop walls would take place. Any film breaking biocides would have the effect of lifting biofilm from the electrode surface exposing active metal sites. Film lifting would result in a dual signal from the monitoring system, first a gross change in corrosion behaviour and secondly in the biological signal. It was decided to avoid this complexity in the present tests and to attempt to evaluate the more subtle changes on the corrosion kinetics that would be brought about by the action of non-slime breaking biocides. The biocide selected for the study was 25% glutaraldehyde solution.

**MATERIALS AND METHODS**

The biofilm flow loop (Figure 1) was a simple closed circuit incorporating a bypass for flow control. Flow was provided by a flexible vane self-priming pump which is capable of producing velocities of up to 0.5 m/s in the 50 mm internal diameter pipework. The main loop contained the biofilm probe, comprising four mild steel cylinder electrodes 40 mm I.D. by 60 mm length, each electrode separated from the adjacent by a 5 mm PTFE packing gland. Slower flow through the bioprobe assembly (0-0.5 m/s) was maintained by a fixed choke just downstream from the assembly. Each electrode was tagged for electrical connection and the arrangement allowed for continuous monitoring by Zero Resistance Ammetry (ZRA), Electrochemical Current Noise (ECN), Electrochemical Potential Noise (EPN) and D.C. potential. Disconnection of the electrical system further allowed AC impedance measurements to be made on a regular basis. The loop was maintained at 30 ± 1°C in the temperature controlled bath.

Monitoring of the conditions in the flow loop covered four phases:

1. Sterile system, viz. water plus 500 ppm glutaraldehyde.

2. System containing medium plus freshwater SRB inoculum.

3. Steady state condition of medium supporting active growth of microorganisms.

4. As for 3, with dosing with glutaraldehyde at consecutive levels of 25, 200 and 500 ppm.
FIGURE 1
BIOFILM FLOW RIG FOR USE WITH ELECTROCHEMICAL MONITORING TECHNIQUES

Biofilm Spool piece

Pump

Bypass loop valve

Temperature Controlled Bath

Zero Resistance Ammetry
Electrochemical Noise
A.C. Impedance

reservoir
The first phase determined the signals from the probes in the absence of microbiological activity. Once the stable condition had been established, the loop was emptied and filled with thirty litres of freshwater Postgate medium B plus an SRB containing culture isolated from the Manchester Ship Canal. This second phase was monitored until growth became apparent due to medium blackening, at which time the third phase was entered and monitoring continued until the system again stabilised. Once the system had reached its new stable condition, over the subsequent period of two days, 25 ppm, 200 ppm and 500 ppm of glutaraldehyde were injected. Finally, a dose of 200 ppm 1M HCl was injected to stimulate H₂S release. SRB numbers were monitored before and during biocide treatment. Throughout the study AC impedance runs were made on a daily basis.

RESULTS

The signals from ZRA, EPN, ECN and electrode potential are given schematically in Figure 2.

Prior to inoculation of SRB, all signals were very stable over the two days of the first phase. The freshwater medium plus SRB also gave stable signals which were maintained for approximately ten days. At this stage, day twelve of the run, phase three was entered and signal changes became highly significant. EPN and ZRA began to move prior to visual changes due to black iron sulphide, the ZRA responding 30 h prior to the darkening. The ECN increased from the time of the visual observation of the darkening and corrosion potential responded last of all, indicating an increase in anodic behaviour of the mild steel. Over the next three days, from days fifteen to eighteen, the signals stabilised, however the ZRA demonstrated a number of polarity reversals, the first of which was reflected by the EPN signal changes. Over this period of rapid microbial activity the AC impedance detected an increase in solution resistance (Figure 3). From days eighteen to twenty-one, following the last ZRA polarity reversal, the ZRA signal began to fluctuate. Concurrent fluctuations were noted in the EPN but fluctuations were not observed in the ECN until day nineteen. The fluctuations in all the signals became steady and consistent and this condition was considered to be suitable to commence phase four, the biocide dosing. SRB levels at this time were > 10⁶/ml.

At 25 ppm glutaraldehyde, there was an immediate though small effect on the ZRA. There was also an immediate change in ECN. No effect was noted on EPN or corrosion potential. SRB numbers at this time remained
FIGURE 2

ELECTROCHEMICAL SIGNALS FROM MILD STEEL RING ELECTRODES IN POSTGATE 'B' MEDIUM DURING GROWTH AND DEATH OF SRB

- Darkening of medium: medium black
- Glutaraldehyde: 25 ppm, 200 ppm, 500 ppm
- Electrochemical current noise: 200 ppm HCl
- Electrochemical potential noise
- Zero resistance asymmetry
- Corrosion potential

Time: Days 12, 15, 18, 21, 22
FIGURE 3  IMPEDANCE RESPONSE OF MILD STEEL RING ELECTRODE IN POSTGATE 'B' FRESH WATER MEDIUM DURING GROWTH AND DEATH OF SRB.

IMAGINARY (ohm cm$^{-2}$)

DAY
- 2
- 3
- 5
- 12
- 15
- 20

blackening

REAL (ohm cm$^{-2}$)
At 200 ppm glutaraldehyde, ZRA again responded. ECN became quiet, but EPN now responded—although no effect was detected by corrosion potential. SRB numbers had now fallen to around $10^6$/ml.

The final dose of 500 ppm glutaraldehyde was also picked up by ZRA; neither ECN, EPN nor corrosion potential showed any change. SRB numbers at this stage had fallen to $< 10$/ml. Addition of 200 ppm HCl caused no reversion of the monitoring signals to any patterns seen during the pre-biocide phase. On clean down of the loop much fine black filming was evident, consisting primarily of iron sulphide. The wet weight of the biofilm slime on the steel monitoring surfaces was approximately 10 mg/cm$^2$.

DISCUSSION AND CONCLUSIONS

It appears that the ZRA detected the growth of SRB and the glutaraldehyde changes within the system. The initial effect of glutaraldehyde significantly upset the ECN signal, bringing it down to a very quiet level. Subsequent changes in glutaraldehyde had a pronounced effect on EPN bringing this signal also to a quiet state. Overdosing at 500 ppm was not detected by the noise technique. Throughout the entire period of the test the $E_{corr}$ had risen slowly and became more anodic. This was in keeping with expected mild steel potential changes in SRB infested systems. AC impedance appeared to have shown no changes over the period of biocide dosing, but did reflect a change in the system moving from no-growth to growth by demonstrating an overall rise in the solution resistance. However, this rise was not in a progressive and smooth manner. With time, the solution resistance first dropped, then rose rapidly as SRB blackening developed, then dropped and again rose. This may reflect changes in the bacterial population within the system as different organisms have been reported to alter the conductivity of their growth medium (Brown, 1985). The impedance plots indicated the kinetics of the metal-environment interactions were under diffusion control.

The scavenging effect of glutaraldehyde on $H_2S$ was considered a possible cause for signal changes as $H_2S$ was removed from the system. However, addition of 200 ppm HCl to regenerate $H_2S$ did not succeed in restoring any of the system’s signals to a level noted prior to biocide addition.

In summary, microbiological growth of SRB in freshwater medium has been shown to be detectable by electrochemical corrosion monitoring techniques.
Biocide additions can be detected by the system and can tentatively be associated with bacterial inactivity rather than from the scavenging effect of glutaraldehyde additions on the \( \text{H}_2\text{S} \) level.

Work is ongoing and it is hoped a further update on the suitability of the various electrochemical techniques to different microorganisms/metal combinations will be available later in the year.

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POSTER PRESENTATIONS
L'importance des bactéries sulfato-réductrices (BSR) dans l'accélération des phénomènes de corrosion a été largement démontrée. Mais leur développement sur des surfaces métalliques immergées en milieu naturel nécessite la présence de conditions favorables à leur fixation et à leur croissance (anaérobie, substrats organiques et inorganiques assimilables). Selon de nombreux auteurs, c'est la colonisation préalable par d'autres espèces de bactéries, aérobies et hétérotrophes, qui est responsable de l'établissement de ces conditions.

Cette expérimentation visait dans un premier temps à reproduire in vitro en aérobiose cette succession de métabolismes à l'aide d'un petit nombre de souches pures d'origine marine mises au contact d'échantillons d'acier inoxydable (type 316L). Deux méthodes de culture ont été adoptées: culture en batch de petit volume et culture en continu. L'étape suivante consistait à mettre en évidence les colonies de BSR sur l'échantillon lui-même et à l'intérieur du biofilm par observation directe (épifluorescence, immunofluorescence).

Les souches "colonisatrices" choisies étaient Vibrio natriegens et Vibrio anguillarum, les BSR Desulfovibrio vulgaris et Desulfobacter postgatei. La corrosion de l'acier inoxydable choisi pour cette expérimentation a été étudiée par suivi du potentiel d'abandon des échantillons et tracé de courbes de polarisation anodique et cathodique à différents stades de la colonisation.
Corrosion of stainless steel (316L) exposed to cultures of marine bacteria. (Translation of previous abstract)

The significance of sulphate-reducing bacteria (SRB) in the acceleration of corrosion processes has been widely demonstrated. Propitious conditions (anaerobiosis, presence of organic and inorganic substratum) are required in order for them to settle and grow on metallic surfaces immersed in the natural environment. Many authors suggest that these conditions may be established by previous settlement of other bacterial species, aerobic and heterotrophic.

The first aim of this experiment was to reproduce in vitro and aerobically this succession of metabolic types, using a small number of pure marine strains grown in the presence of stainless steel coupons (316L). For this, two methods of culture have been utilised: batch culture in small volumes and continuous culture. The second step consisted of showing the presence of SRB colonies on the metal coupon and inside the biofilm by direct observation techniques (epifluorescence and immunofluorescence).

The colonising strains chosen were Vibrio natriegens and Vibrio anguillarum, the SRB Desulfovibrio vulgaris and Desulfobacter postgatei. The corrosion of the stainless steel chosen for this experiment was studied by measuring regularly the potential of the samples and tracing anodic and cathodic polarisation curves at different stages of colonisation.
La corrosion bactérienne peut être définie comme la dégradation de matériaux résultant de la présence physique et de l'activité de microorganismes sur les surfaces soit par production de métabolites agressifs, soit encore en intervenant dans les processus électrochimiques se produisant à la surface de ces matériaux.

L'utilisation conjointe de méthodes biochimiques et microbiologiques permet d'accéder à une meilleure connaissance des microorganismes, de la structure des populations bactériennes présentes sur les échantillons analysés et ainsi à une meilleure connaissance des processus mis au jeu lors d'initiations de corrosions localisées. Ces méthodologies ont été utilisées lors de travaux visant à étudier la colonisation bactérienne et la corrosion localisée d'alliages métalliques exposées à une eau de mer circulante ou encore lors de travaux visant à étudier et optimiser la protection cathodique en milieu aérobie et anaérobie.
INFLUENCE DE LA POLARISATION CATHODIQUE SUR LA COLONISATION BACTERIENNE DES SURFACES METALLIQUES

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La protection cathodique est très utilisée en offshore et, de manière plus générale, dans les installations au contact de l'eau, pour lutter contre les phénomènes de corrosion. Ce traitement électrochimique de surfaces métalliques peut éventuellement perturber les forces physiques (forces de Van Der Waals et interactions électrostatiques) et chimiques (excrétion par les bactéries de polymères qui facilitent leur adhésion) qui régissent la fixation irréversible des bactéries aux surfaces.

L'imposition de différents potentiels cathodiques sur un acier inoxydable immergé dans des cultures de bactéries sulfatoredductrices hydrogénase positive ou négative, produisant ou ne produisant pas de sulfures, a montré une nette influence de la polarisation sur la colonisation bactérienne des surfaces métalliques. En fonction de l'intensité du traitement et en l'absence de sulfures, le nombre de bactéries absorbées sur les surfaces augmentent. Cependant, cette évolution est masquée par la présence de sulfures, qui par leur toxicité, entraînent, en fin de croissance, une baisse de la densité bactérienne sur les surfaces polarisées.

Le traitement cathodique des surfaces métalliques semblent également perturber les interactions existant entre les bactéries et le métal ce qui traduit par une baisse des effectifs bactériens sur les surfaces polarisées par rapport aux surfaces non polarisées.
EFFECT OF CONTINUOUS AND INTERMITTANT DOSING OF NaClO IN NATURAL SEAWATER ON THE CREVICE CORROSION PROPAGATION OF STAINLESS STEEL

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Taking into account that the slime settlement on the active-passive alloy surfaces exposed to natural seawater produces a catalysis of oxygen reduction and consequently a rapid propagation of localised corrosion, we studied the effect of a biocide such as NaClO as an inhibitor of corrosion.

For stainless steels, it was observed that a continuous NaClO addition of about 0.2ppm residual chlorine in sea water is able to maintain the corrosion rate about two orders of magnitude lower than that measured in untreated sea water.

Reductions of more than one order of magnitude in the course of one day were observed with intermittent NaClO additions equal to 0.2 - 0.6ppm residual chlorine.

In the case of intermittent additions, to be rapidly effective in reducing the corrosion rate the residual chlorine amount must increase from 0.2 to 0.6ppm as the slime grows older.
ETUDE DE L'INTERACTION DU BIOFILM BACTERIEN ET DE LA PROTECTION CATHODIQUE D'ACIER INOXYDABLE PLACE EN EAU DE NATURELLE CIRCULANTE

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La relation pouvant exister entre la protection cathodique d'acier inoxydable 316L placé en eau de mer circulante et l'attachement bactérien a été étudiée en laboratoire. Le renouvellement de l'eau de mer dans les cellules d'essai était de 12 litres/h et les essais menés sur des périodes de cinq jours. Les échantillons ont été polarisés à -800mV/ECS, -900mV/ECS ainsi qu'au potentiel de +100mV/E équilibre observé après immersion dans le milieu. Ces expérimentations ont également été réalisées sur des échantillons non polarisés.

Durant l'expérience l'évolution des potentiels des échantillons sans protection et des densités de courant pour les échantillons polarisés ont été enregistrées. A la fin de chaque essai des courbes de polarisation et des diagrammes d'impédance ont été tracés. Les aspects qualitatif et quantitatif de l'activité microbiologique sur les surfaces ont également été examinés: qualitativement par épifluorescence et/ou microscopie électronique à balayage, quantitativement par numérotations de bactéries fixées à la surface des échantillons. Le rapport calcium/magnésium a été déterminé sur les différentes surfaces polarisées.

Ces essais constituent une première partie de l'étude qui doit se poursuivre par des essais analogues en eau de mer circulante sur des périodes d'exposition plus importantes, mais également en eau de mer stérilisée.
In order to protect stainless steels against biologically induced corrosion in sea water, poison alloying elements were added to 316L stainless steel.

Electrochemical tests and surface analysis performed on different doped stainless steel samples immersed in synthetic sea water show that the behaviour of the metal depends on both the nature of the poison alloying elements and the presence or absence of bacteria.