POLYSACCHARIDES IN BIOFILMS AND IN FREE EPS OF D. DESULFURICANS IN THE PRESENCE OF MILD AND STAINLESS STEEL AND THEIR ROLE IN CORROSION

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The objective of this research was to analyse qualitatively and quantitatively free and biofilm-bound extracellular polymeric substances (EPS) produced by a member of the group of sulphate reducing bacteria (SRB) in the presence of mild steel (MS) and stainless steel (SS) surfaces and to correlate the production of polysaccharide component of EPS with corrosion occurring on surfaces exposed to this bacterium.

SRB are the most significant anaerobes found in microbial corrosion processes. The corrosion of iron and ferrous metal alloys by SRB causes severe economic losses by the destruction of industrial and process equipment and devaluation of industrial products such as oil and natural gas. Various models are proposed to explain SRB induced corrosion, the most popular being the cathodic depolarisation theory and the involvement of ferrous sulphides in breakdown of metal passivity. SRB are also known to be capable of exopolymer production. However no attempt has been made to elucidate the possible involvement of their EPS in corrosion.

The involvement of bacterial exopolymer components in the biocorrosion of metals by other species has been demonstrated by several workers (1,2).

One of the main mechanisms thought to be important in the corrosion of metals by exopolymers is the polymer's metal binding ability. Bacterial exopolymers are not only able to bind metal ions (3) but polymers from different bacteria exhibit dfferent binding capacity for the various metal ions (4).

Only few publications refer to exopolymers produced by SRB. The most recent report is that of Ochynski and Postgate published in 1965 (5). They detected mannose in EPS precipitated from the culture media of a Desulfovibrio species. They used paper chromatography to identify this carbohydrate and since it was the only sugar detected they referred to Desulfovibrio EPS as mucopolymannoside. To our knowledge for the past 25 years no attempt has been made to analyse carbohydrate composition of SRB exopolymers. Neither are we aware of any work describing EPS production in biofilms formed by SRB on steel surfaces. In most studies involved in analyses of bacterial exopolymers no distinction is made between free (released into liquid phase) exopolymer and biofilm-bound exopolymer and it is generally accepted that there is no difference in carbohydrate composition between the two.

Our aims in this work were to establish:

1. The influence of the surface on EPS production and composition.

2. The existence of any difference in carbohydrate composition between free and biofilm-bound EPS.

An attempt has also been made to assess the involvement of these exopolymers in corrosion of MS and SS surfaces.

Free exopolymers were recovered by precipitating with isopropanol from the supernatant obtained from Postgate C (6) inoculated with Desulfovibrio desulfuricans subsp. desulfuricans New Jersey (NCIMB 8313) which was originally isolated from a corroded heat exhanger. Cultures were incubated for 7 and 28 days with and without mild steel (EN1A;BS970) and stainless steel (BSO21134) coupons. Biofilms were harvested from freeze-dried coupons by scraping the surfaces with a razor blade. Portions of crude biofilms were treated to remove metal oxides, sulphides and bacterial cells.

Free and biofilm-bound exopolymers were analysed for their carbohydrate and protein content by colorimetric methods and by SDS-gel electrophoresis. Exopolysaccharides detected colorimetrically in recovered exopolymers were reduced, hydrolysed and derivatised prior to quantitative and qualitative analyses by GC-MS and GC-FID.

SRB biofilms formed on steel coupons were examined under SEM. The corrosion of MS and SS coupons was assesed by SEM observations, EDAX analyses and by potentiostatic measurements.

SEM micrographs of the biofilms formed on MS coupons after 7 and 28 days of incubation revealed the presence of thick and fairly uniform biofilm with abundant EPS visible as fibres extending from the cells. Although no great difference in morphology between 7 and 28 day old biofilms was observed the latter seemed to contain greater amounts of corrosion products, mainly ferrous sulphides. In comparison with mild steel, biofilms formed on SS surfaces were very scanty and only few EPS fibres were visible. SEM observations are supported by quantitative evaluations. Dry weight measurements of lyophilised biofilms showed that a significantly greater amount of biofilm was present on MS coupons than on SS surfaces and that on both surfaces there was a significant increase in biofilm bulk with prolonged time of incubation (Table 1).

Significantly less protein was present in biofilms recovered from SS coupons compared to the amount detected in biofilms obtained from MS coupons. However with extended time of incubation the protein level in biofilms on both types of surfaces increased significantly.

Analysis of variance showed that biofilms of D.desulfuricans grown on MS surfaces contained significantly more neutral carbohydrates compared with biofilms formed on SS coupons regardless of biofilm age (Table 2). The change in the amount of neutral sugars present in biofilms with time was however insignificant.

Desulfovibrio biofilms formed on MS coupons after 7 days of incubation when analysed chromatographically for their neutral carbohydrate composition revealed (in order of prevalence)

glucose, mannose and galactose as the major sugar components with their respective molar ratios 1 : 0.7 : 0.6. Other neutral carbohydrates detected were Rhamnose, Xylose and Allose jointly contributing 23% of the total amount of sugar. Small amounts of uronic acid (0.5% of biofilm weight) were found in the biofilms established on MS coupons but could not be characterised due to the low yield.

Likewise, carbohydrates in SS biofilms were not characterised due to their low recovery.

The amount of free EPS harvested from bacterial cultures incubated with and without steel coupons is shown in Table 3.

Analysis of variance showed that there was no significant difference in the amount of polymer recovered from the bacterial cultures regardless of cultural conditions.

Proteins and neutral hexoses contributed on average 55% w/w and 30% w/w respectively to the total dry weight of EPS.

Neutral sugar composition of free EPS varied with growth conditions (Table 4).

Mannose and glucose were identified as the major carbohydrate components in all types of free bacterial EPS with mannose contributing on average 85% to the total neutral sugar content. Molar ratios of main sugars are given in Table 5.

Analysis of variance showed that the presence of steel surfaces stimulated the production of exopolysaccharides in Desulfovibrio cultures (Table 6).

Significantly more neutral sugar was detected in EPS recovered from cultures incubated with MS or SS coupons compared with the level measured in coupon-free cultures. No significant difference was found between the amount of free exopolymer harvested from SS and MS coupon containing cultures. Prolonged time of incubation had no influence on the amount of neutral sugars detected in free EPS.

Colorimetric assay showed the absence of uronic acids in free EPS of D.desulfuricans under all cultural conditions.

No correlation was found between the level of exopolysaccharides in free EPS of D.desulfuricans and corrosion of steel. SEM observations and electrochemical measurements showed extensive pitting corrosion on MS coupons. Although no such damage was noticed on SS surfaces there was no significant difference in the amount of polysaccharide produced by SRB between EPS recovered from cultures grown with MS and SS coupons.

Although the amount of polysaccharides present in biofilms formed on MS coupons was significantly greater than that found in biofilms developed on SS surfaces this factor alone can not be responsible for the degree of corrosion occuring on mild steel. Clearly other mechanisms must be involved in the observed deterioration. EDAX analysis and SEM studies showed the abundance of ferrous sulphides on MS coupons compared to SS surfaces. We therefore are

7 day old biofilm		28 day old biofilm	
on MS	on SS	on MS	on SS
118.7 + 20	10.1 + 1	251.8 + 19	32.2 + 2

TABLE 1. Dry weight of D. desulfuricans biofilms (mg+SD) recovered from MS and SS coupons.

7 day old biofilm		28 day old biofilm	
on MS	on SS	on MS	on SS
75 + 14.9	29.9 + 1.65	63.4 + 14.4	36.6 + 7.6

TABLE 2. Colorimetric estimation of neutral sugar level (ug/mg + SD) in biofilms recovered from steel coupons.

incubation	7 days of incubation	28 days of
MS	66.5 + 14.4	72.9 + 23.9
SS	68 + 26.11	70.2 + 12.35
Coupon-free	48.6 + 7.06	52.65 + 9.26

TABLE 3. Dry weights of EPS (mg + SD) recovered from culture media.

Type of sugar	MS	SS	coupon-free
Glucose	+	+	+
Mannose	+	+	+
Galactose	+	+	+
Rhamnose	+	+	+
Xylose	-	-	+
Altrose	-	+	-

TABLE 4. Neutral sugars detected in free EPS of D. desulfuricans.

	Glucose	Mannose	Galactose
Cultures grown with MS coupons	1	12	0.2
Cultures grown with SS coupons	1	5	0.2
Cultures grown with no coupons	1	11.5	0.4

TABLE 5. Molar ratios of main neutral sugars present in free EPS of D. desulfuricans.

incubation	7 days of incubation	28 days of
MS	19.45 + 1.33	22.05 + 1.04
SS	19.18 + 1.63	20.35 + 1.73
Coupon-free	12.89 + 0.57	13.85 + 1.53

TABLE 6. Total amount of netural hexose detected in free EPS (mg + SD).