Chapter 5

BIOFOULING MONITORING DEVICE COMPARISON IN A COOLING WATER SYSTEM

ABSTRACT

Although different biofouling monitors are currently in use in South Africa, few studies, to determine which devices most accurately reflect biofouling conditions, have been conducted under standard operating conditions. A study was initiated to compare different biofouling monitoring devices under the same system parameters. Four monitoring devices, a Robbins Device, a modified Robbins Device, a Pedersen Device and a Barry's Device were evaluated in the main, open recirculating cooling water system at a power station. The devices were evaluated for four week periods, under three different dosing regimes, namely when no biofouling control chemicals were added to the system, when a biodispersant was slug dosed weekly at 15 ppm and when the biodispersant was continuously dosed at 10 ppm. Total aerobic, anaerobic, and hydrogen sulphide producing bacteria were quantified on the monitoring devices and in the bulk water. Scanning electron microscopy was carried out on the sampling surfaces of the monitoring devices. Experiments using the Pedersen Device were discontinued after one week, as actual system water velocities could not be achieved through the device. No correlation between the numbers of planktonic and sessile bacteria was found during any of the three dosing regimes. No statistically significant differences were found between the numbers of total aerobic bacteria recovered from the remaining three devices, when no biodispersant was added to the system and in the H_2S producing bacteria when biodispersant was slug dosed. Statistically significant differences were found between the numbers of all the other bacteria during the three dosing regimes. However, numbers of all bacteria recovered from the modified Robbins Device were consistently higher than those from the other devices. No visible differences in the biofilms on the sampling surfaces of the different biofouling monitors, could be distinguished by scanning electron microscopy. No statistically significant differences, in all the numbers of sessile bacteria recovered from the monitoring devices, were found between the three dosing regimes. The modified Robbins Device and the Barry's Device were found to be the most suitable biofouling monitors.

INTRODUCTION

It has been common practice to monitor the extent of microbiological contamination in industrial water systems by the enumeration of microorganisms in the bulk water (Wolfaardt *et al.*, 1991). However, microorganisms in aqueous environments exist in both sessile and planktonic phases (Costerton *et al.*, 1981; Characklis *et al.*, 1982). Microorganisms in the sessile phase form biofilms that cause deleterious effects in cooling water systems, such as decreased heat transfer, decreased flow rates and microbiologically influenced corrosion (MIC) and should therefore be monitored (Colturi and Kozelski, 1984).

Techniques that have been developed to monitor sessile microbial populations in water systems can be indirect, where the effects of these microbial populations are determined, or direct, where microbial numbers, or biomass, are quantified (Characklis *et al.*, 1982; Costerton and Lashen, 1983). Corrosion monitors or devices that measure decreases in heat transfer as a result of microbial activity, are classified as indirect techniques (Characklis *et al.*, 1982). Indirect techniques do not require trained personnel to carry out microbiological analyses and an instantaneous reading can usually be obtained (Mansfeld and Little, 1990; Tullmin *et al.*, 1992). The Robbins Device is commonly utilised as a direct technique to monitor biofilm development in water systems worldwide (Bondonno *et al.*, 1989). Other direct techniques commonly used in South Africa are the Pedersen Device (Pedersen, 1982) and the Barry's Device. The latter is a jointed pipe, 25mm in diameter, constructed from an inert material such as polyvinyl chloride (B. Luddick pers. comm.)*. The major advantages of direct techniques are that more comprehensive information on the microbiological and chemical composition of a biofilm can be obtained. The numbers of sessile bacteria per square centimetre, or the bacterial species present can for example be determined (Pedersen, 1982). Although there are many devices for the monitoring of biofilms in water systems, limited information is available on the comparison of these devices.

If direct monitoring techniques are used to quantify sessile microorganisms, it is essential to be able to enumerate, as accurately as possible, the microorganisms removed from the sampling surface of the monitoring device. Commonly, bacteria removed from such sampling surfaces have been quantified on standard nutrient media, such as Nutrient Agar. However, Brözel (1990) demonstrated that low nutrient media yielded the highest number of planktonic bacteria isolated from cooling water systems. In keeping with these results, half strength

*B. Luddick, ISKOR, P.O. Box 2, Vanderbijlpark, South Africa

Nutrient Agar was found to be the most appropriate for the analysis of cooling water from Eskom power stations (M. Santa, pers. comm.)*. Historically, sulphate reducing bacteria have been identified as the major contributors to MIC in cooling water systems (Poulton and Nixon, 1990). It has however been suggested, that H_2S may play a role in anaerobic corrosion by directly attacking metal surfaces (McCoy, 1980). Numerous researchers have implicated H_2S producing bacteria in contributing to MIC (Pope *et al.*, 1982; Puckorius, 1983; Stoecker, 1984; Pope and Zintel, 1988). Mara and Williams (1970) demonstrated that Iron Sulphite Agar (ISA) could be utilised to quantify sulphate reducing bacteria (SRB), as well as other H_2S producing bacteria. Bacteria removed from the surfaces of biofouling monitoring devices in cooling water systems should therefore be quantified on low nutrient media and H_2S producing bacteria should be quantified and not only SRB.

The aims of this study were therefore, to evaluate a number of direct technique biofouling monitors, under standard operating conditions and to identify suitable monitors for use in South African cooling water systems. The devices were also evaluated when a biodispersant was added to the circulating water to determine their suitability for the assessment of the efficacy of biodispersants as biofouling treatment programmes.

MATERIALS AND METHODS

Experimental cooling water system parameters

Four biofouling monitoring devices were installed at Lethabo Power Station. This power station is operated at elevated cycles of concentration with make up water of poor microbiological and chemical quality. Visual inspections of the cooling water system were carried out over the last five years. Biofouling and MIC were identified in the system, even in those areas where the mild steel pipework was protected with an epoxy based coating (Figure 5.1). To date, the microbiological condition of the system was determined by microbiological analysis of the bulk water, in conjunction with a Robbins Device biofouling monitor. This type of on-line monitor was considered suitable for this particular cooling water system, as a resident microbiologist was available to sample and analyse for sessile bacteria. System parameters and chemical composition of the cooling water at Lethabo Power Station are detailed in Table 5.1.

*M. Santa, ESKOM, TRI, Private Bag 40175, Cleveland, 2022.

Table 5.1 : System parameters and chemical composition of the open recirculating cooling water at Lethabo Power Station.

System volume	128 megalitres
Cycles of concentration	15 - 20 cycles
pH at 25°C	8.5
Conductivity at 25°C	2700 $\mu S.cm^{-1}$
Total alkalinity	113 $mg.l^{-1}$ $CaCO_3$
Chloride	246 $mg.l^{-1}$
Sulphate	905 $mg.l^{-1}$
Total hardness	318 $mg.l^{-1}$ $CaCO_3$

Biodispersant dosing regimes

Biofouling monitors were evaluated under three different dosing regimes, each of four weeks duration, namely :

no biofouling control chemicals utilised.

slug dosing of an anionic biodispersant once a week at 15 ppm.

continuous dosing of an anionic biodispersant at 10 ppm.

Biofouling monitors

Four biofouling monitors were compared, the Robbins Device, a modified version of the Robbins Device, the Pedersen Device and the Barry's Device (Table 5.2). The modifications to the Robbins Device involved an increased stud surface area (factor of 10) and the counter sinking of the stud into the holder (Figure 5.2). These modifications were intended to minimise errors incurred during sampling, since tearing of the biofilm would occur on the holder and not on the stud surface. The modified stud could therefore be removed without disturbing the biofilm. In addition, more representative samples of patchy or uneven biofilms would be obtained due to the larger surface area.

The four biofouling monitors were installed in series, on a by-pass line on the "hot" side of the main cooling water system, i.e. after passage of the cooling water through the condensers (Figure 5.3). The devices were exposed to the circulating water for three weeks before the commencement of each dosing regime. During these three weeks, no biofouling control chemicals were added to the system, to allow a biofilm to develop. A water velocity of 1.2 $m.s^{-1}$ was maintained through all the devices with the exception of the Pedersen Device where the maximum velocity was limited to 0.2 $m.s^{-1}$, due to the design.

Table 5.2 : Biofouling monitors evaluated in the open recirculating cooling water system at Lethabo Power Station.

BIOFOULING MONITOR	MATERIALS OF CONSTRUCTION		SAMPLING SURFACE AREA (cm^2)	SUPPLIER
	SAMPLING SURFACE	HOUSING		
Robbins Device	Nylon	Mild steel	0.5	Hydralube, P.O.Box 8725, Edenglen, South Africa
Modified Robbins Device	Nylon	PVC[a]	4.9	Eskom, TRI, Private Bag 40175, Cleveland, South Africa
Pedersen Device	Nylon	PVC	41.6	Chemserve Systems, P.O. Box 12055, Chloorkop, South Africa
Barry's Device	PVC	-	34.7	Iscor, P.O. Box 2, Vanderbijlpark, South Africa

[a] PVC = polyvinyl chloride

The sampling surfaces of the four monitoring devices are illustrated in Figure 5.4.

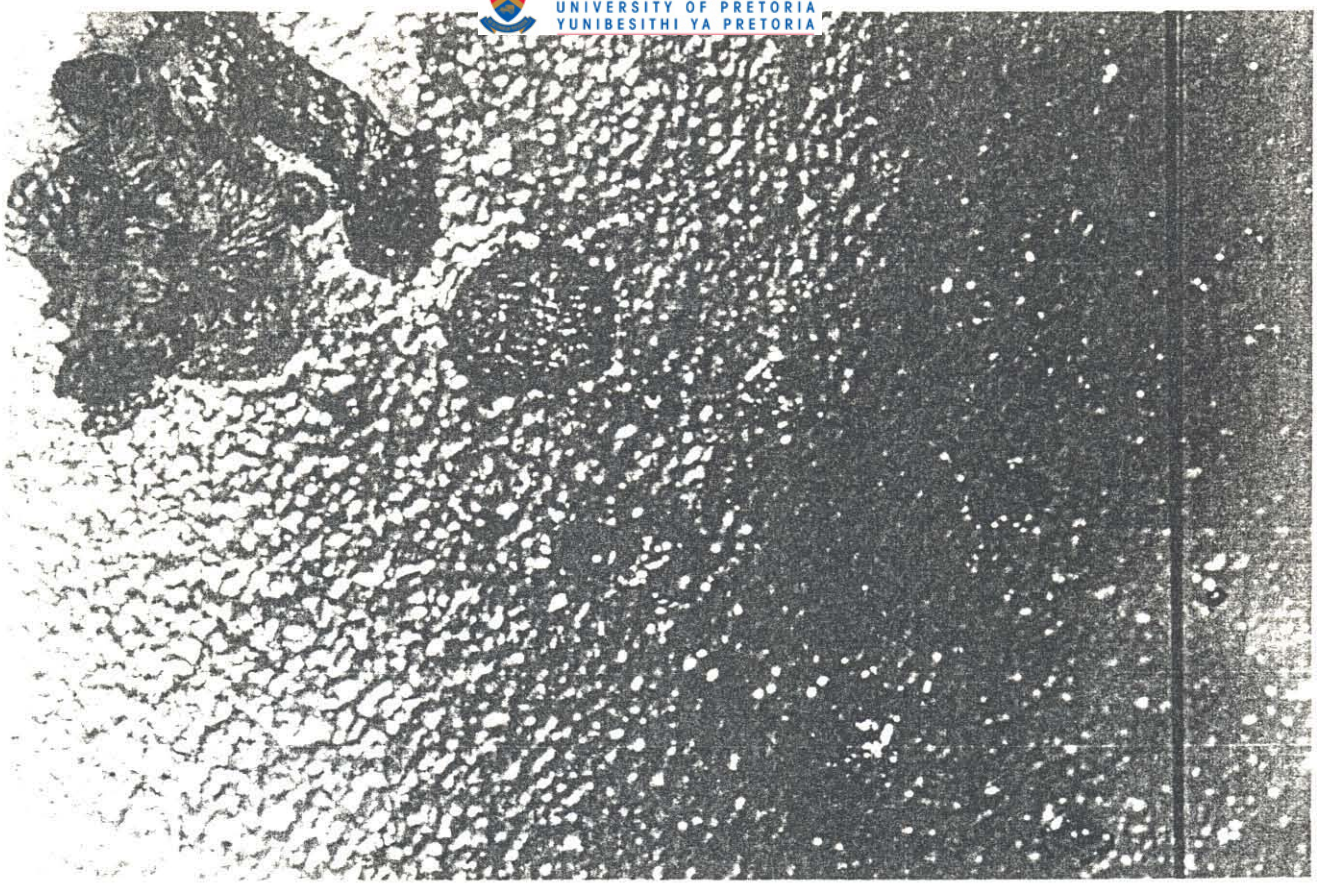

Figure 5.1 : Blistering of the epoxy coating and subsequent MIC in a condenser at Lethabo Power Station.

Figure 5.2 : Stud from a modified Robbins Device illustrating larger surface area of 4.9 cm^2 (A) countersunk into the stud holder (B).

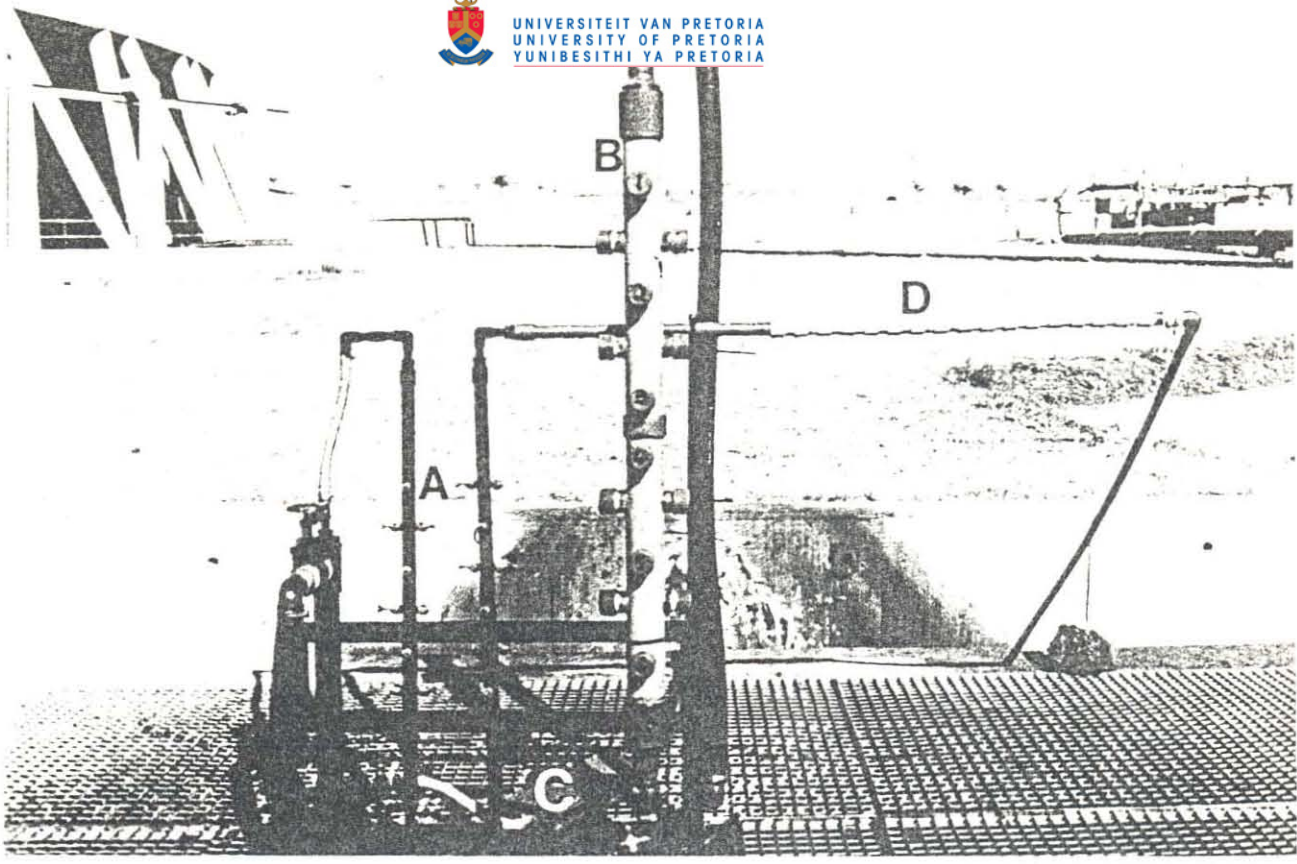

Figure 5.3 : Robbins Device (A), modified Robbins Device (B), Pedersen Device (C) and Barry's Device (D) evaluated in the open recirculating cooling water system Lethabo Power Station.

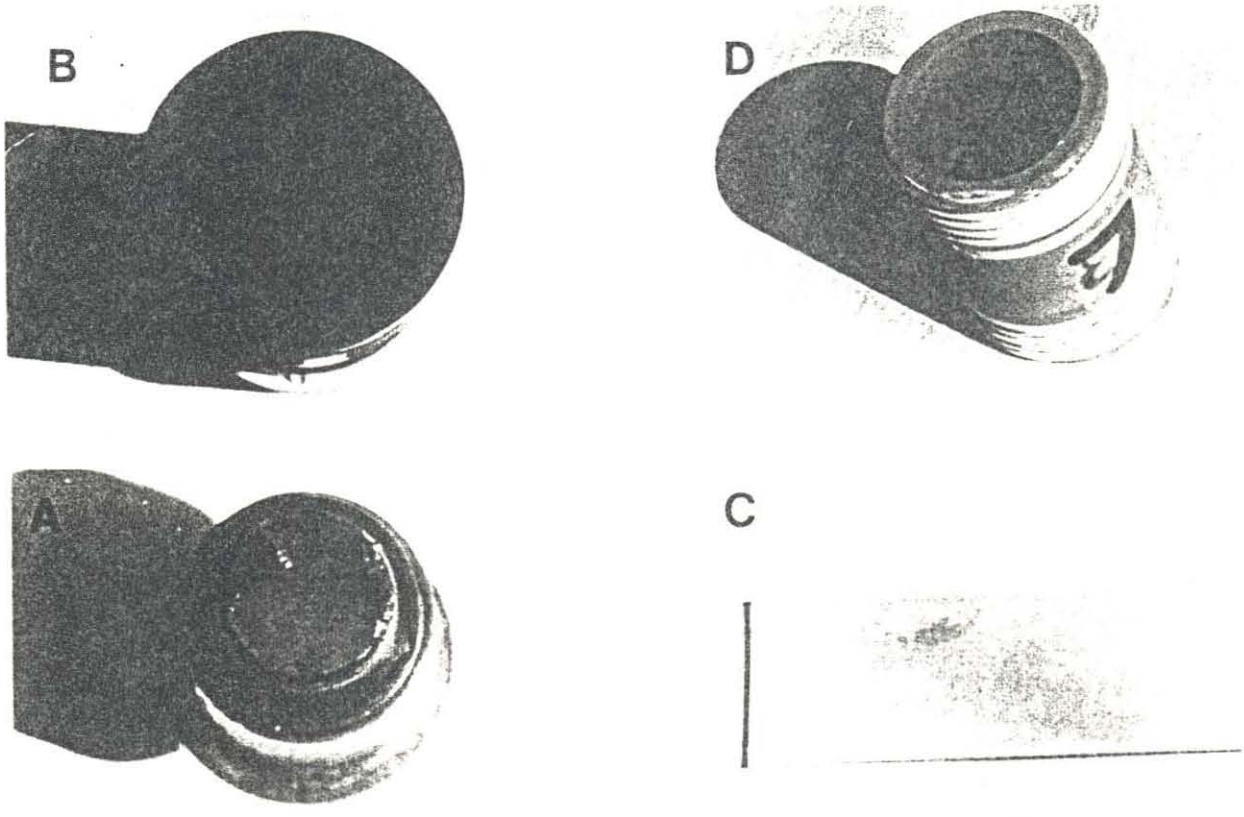

Figure 5.4 : Sampling surfaces of Robbins Device (A), modified Robbins Device (B) Pedersen Device (C) and Barry's Device (D).

Sampling procedure

Numbers of sessile bacteria were not determined during the three week period when no treatment chemicals were added. This allowed the development of a biofilm. Sampling surfaces were, however, removed from each of the devices before the start of each evaluation and thereafter, twice a week for four weeks. Two samples were removed from each device, at each sampling interval.

Sampling surfaces were removed from each device using sterile forceps and rinsed in sterile, quarter strength Ringer's solution. A sterile scalpel was run around the outer edge of the sampling surface in the modified Robbins Device, before it was removed from the holder. The sampling surfaces were placed in bottles containing 100ml of sterile, quarter strength Ringer's solution, 20 ppm of a proprietary biodispersant and glass beads, to maximise biofilm removal. The one end of the section of PVC pipe removed from the Barry's device was sealed using a sterile end cap. The section of pipe was then filled with the beads, biodispersant and Ringer's solution and another end cap used to seal the remaining open section of pipe (Figure 5.5). The bottles and sealed sections of pipe were agitated on a shaker for a period of 30 min, after which the resultant bacterial suspensions were analysed (Table 5.3).

A single 500ml bulk water sample for microbiological analysis, was collected in a sterile Whirl Pak bag (Nasco, USA) from the by-pass line, each time sampling surfaces were removed from the biofouling monitors.

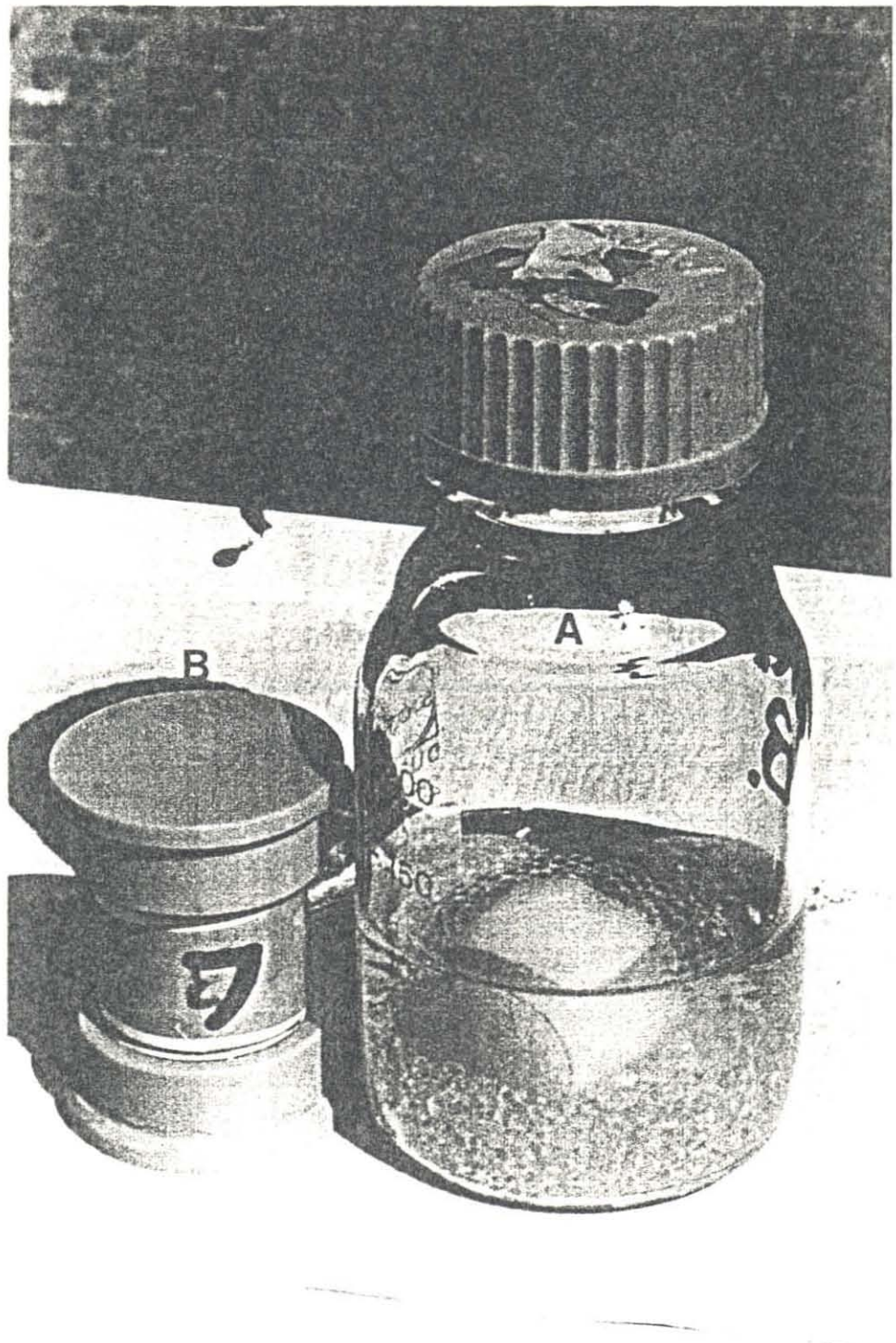

Figure 5.5 : Sampling procedure used for the Robbins Device, modified Robbins Device and Pedersen Device (A) and for the Barry's Device (B).

Microbiological analysis

Samples were diluted in sterile, quarter strength Ringer's solution and subjected to duplicate plate counts. All incubation was at 37°C, as this temperature was the approximate system temperature. All the samples were analysed within two h of sampling and stored at 4°C until they were analysed. Anaerobic incubation took place in a nitrogen atmosphere, in an anaerobic jar.

Half strength Nutrient Agar was prepared by using 50% of the amount of Nutrient Agar specified by the manufacturers and adding purified Agar (Biolab) to yield the specified amount of agar per litre as in full-strength Nutrient Agar. The techniques used for bacteriological analysis are detailed in Table 5.3. Plates containing between 30 and 300 colonies were counted.

Table 5.3 : Techniques used to quantify bacteria detached from the sampling surfaces of four biofouling monitors and in the bulk water of the open recirculating cooling water system at Lethabo Power Station.

BACTERIAL TYPE	TECHNIQUE	INCUBATION TIME (d)	ATMOSPHERE	GROWTH MEDIUM
Total aerobic bacteria	Pour plate	2	Aerobic	Half strength Nutrient Agar (Biolab)
Total anaerobic bacteria	Pour plate	3	Anaerobic	Half strength Nutrient Agar (Biolab)
H_2S producing bacteria	Agar tubes	5	Anaerobic	Iron Sulphite Agar (Oxoid)

Scanning electron microscopy

Sampling surfaces removed from each of the biofouling monitors were viewed using a scanning electron microscope (SEM) before the start and at the end of each of the four week dosing regimes.

Surfaces removed from the biofouling monitors were fixed in 2.5% gluteraldehyde for four h at room temperature, dehydrated for 10 min in each of a series of 10, 20, 30, 40 ,50, 60, 70, 80,

90, 95 and 100% ethanol, critical point dried, sputter coated with gold using an Edwards 150B sputter coater and viewed using a Phillips 5020 scanning electron microscope.

Statistical analysis

Numbers of sessile microorganisms on the sampling surfaces of the four monitoring devices were analysed using the f-test procedure for the analysis of variance at a 95% confidence interval. Variances between the four monitoring devices and the three treatments were determined (Brown and Hollander, 1977).

RESULTS AND DISCUSSION

Suitability and visual observations of the four biofouling monitors

The experiments involving the Pedersen Device were discontinued one week after the start of the evaluation, due to the fact that a maximum water velocity of only 0.2 $m.s^{-1}$ was achieved. Sludge had deposited on the base of the device (Figure 5.6) and as this device did not simulate actual water velocities occurring in the system, it was decided to discontinue its use.

A visible biofilm was observed on the sampling surfaces of the other three biofouling monitors, particularly on the exposed surfaces of the Barry's Device. Visual examination of the nylon studs in the Robbins Device, after exposure to the recirculating cooling water for four weeks, revealed deposits of what appeared to be corrosion products (Figure 5.7).

Microbiological analysis of bulk water and biofouling monitor sampling surfaces

Numbers of attached bacteria recovered from the sampling surfaces of the Robbins Device, Modified Robbins Device and Barry's Device are shown in Figures 5.8 - 5.10. Numbers of planktonic bacteria in the bulk water are plotted on the same graphs. Since all the monitors were evaluated under the same operating conditions, it was suggested that the monitor from which the highest number of sessile bacteria were recovered, represented the most accurate simulation of system conditions. It was hypothesised that there would be significant differences between the numbers of sessile bacteria on the sampling surfaces of the different monitoring devices.

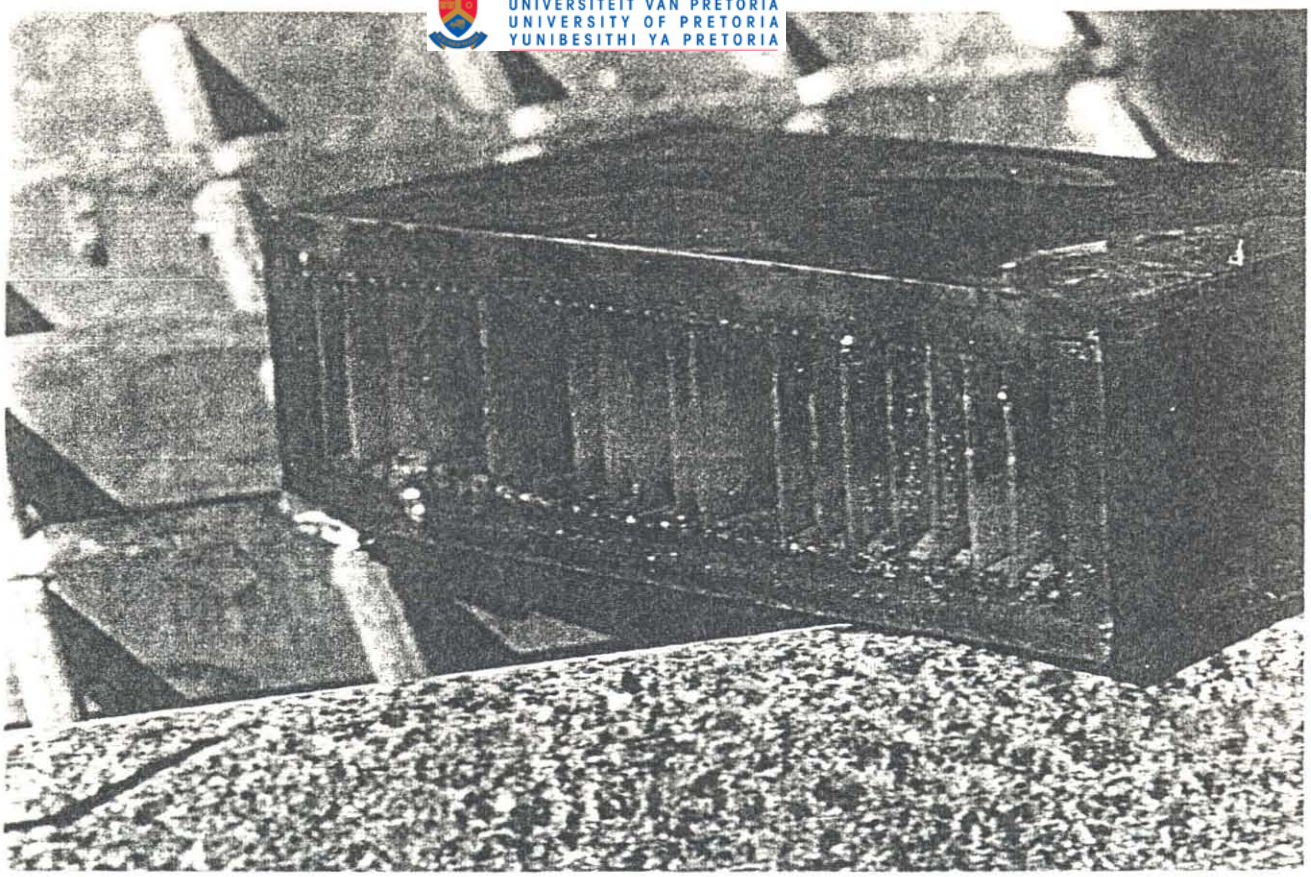

Figure 5.6 : Sludge deposition in the Pedersen Device one week after installation in the open recirculating cooling water system at Lethabo Power Station.

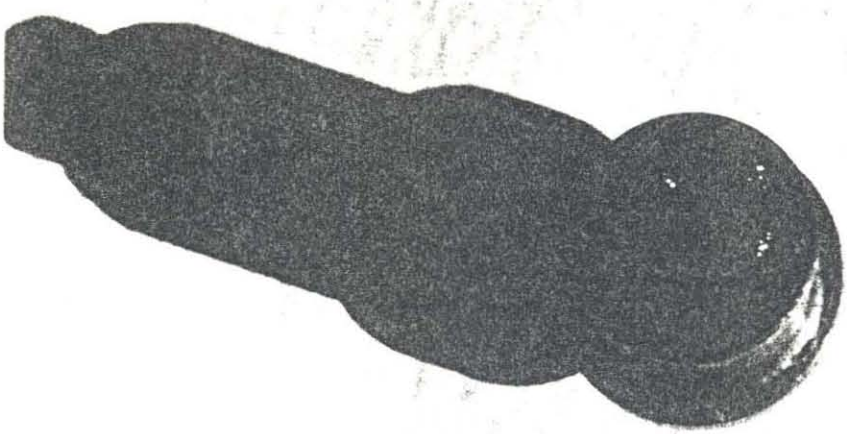

Figure 5.7 : Deposition of corrosion products on the surface of a Robbins Device stud after four weeks exposure to the recirculating cooling water at Lethabo Power Station.

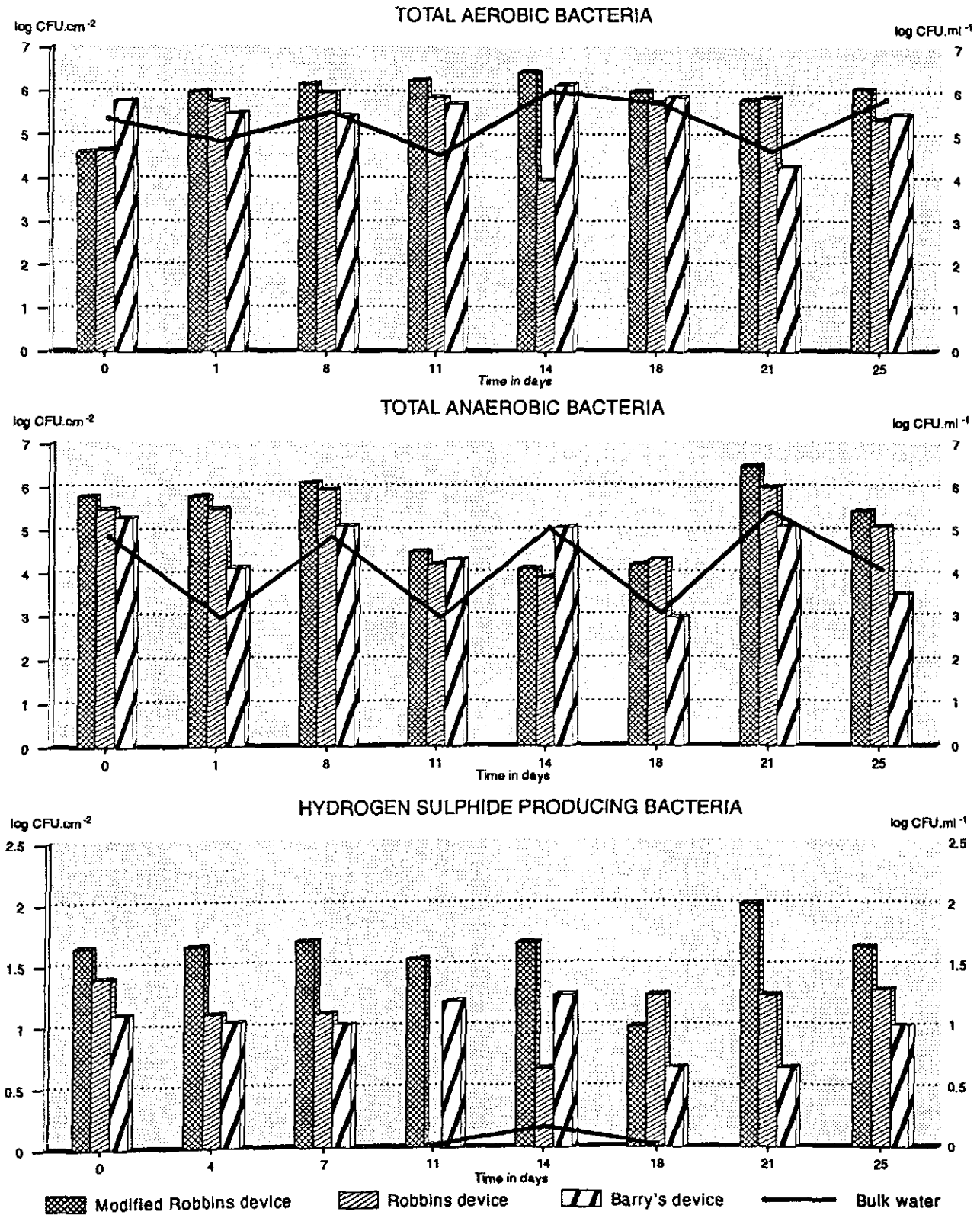

FIGURE 5.8 Comparison of attached bacteria on modified Robbins, Robbins and Barry's device biofouling monitors where no specific treatment chemicals were used.

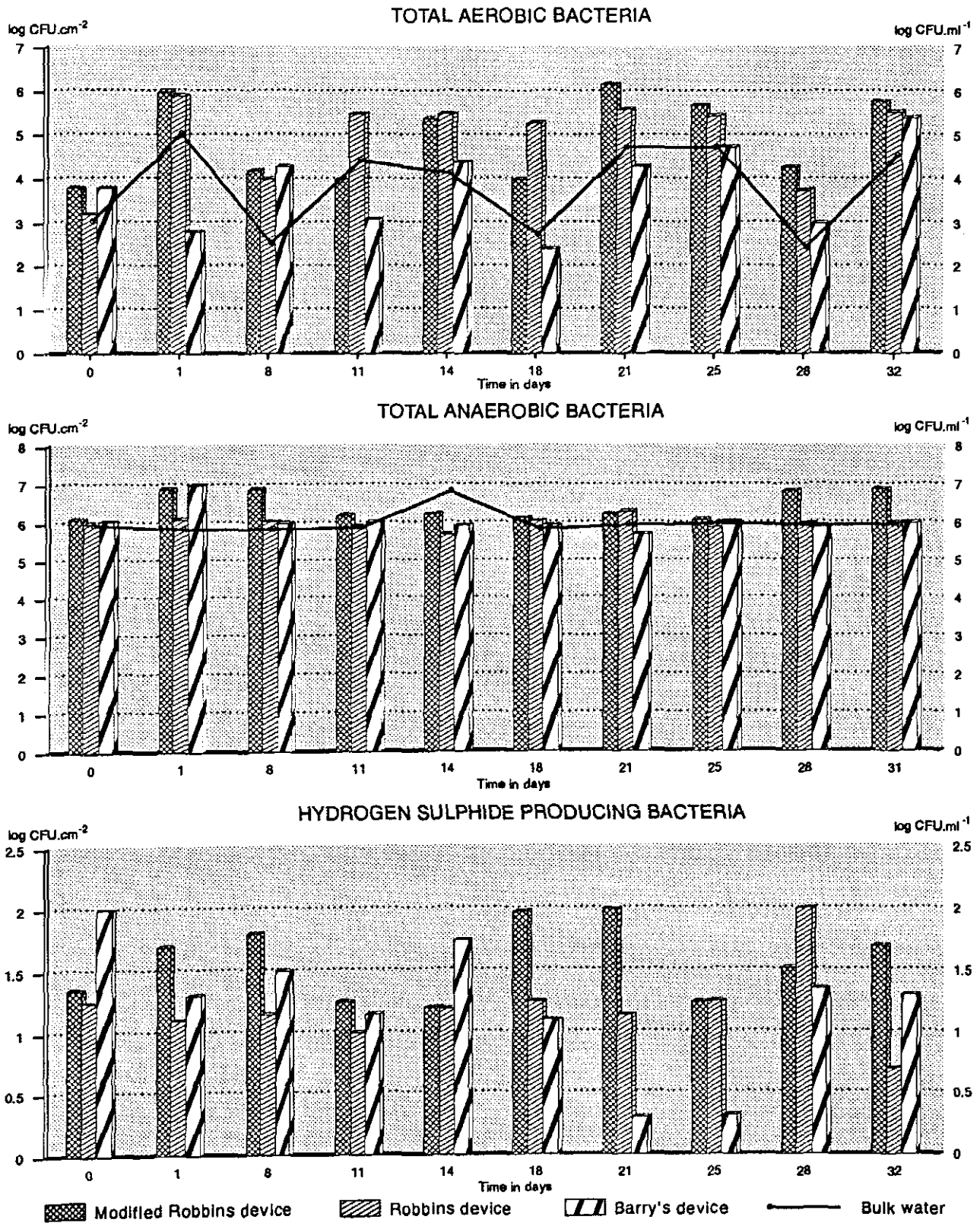

FIGURE 5.9 Comparison of attached bacteria on modified Robbins, Robbins and Barry's devicebiofouling monitors with continuous dosing of biodispersant at 10ppm.

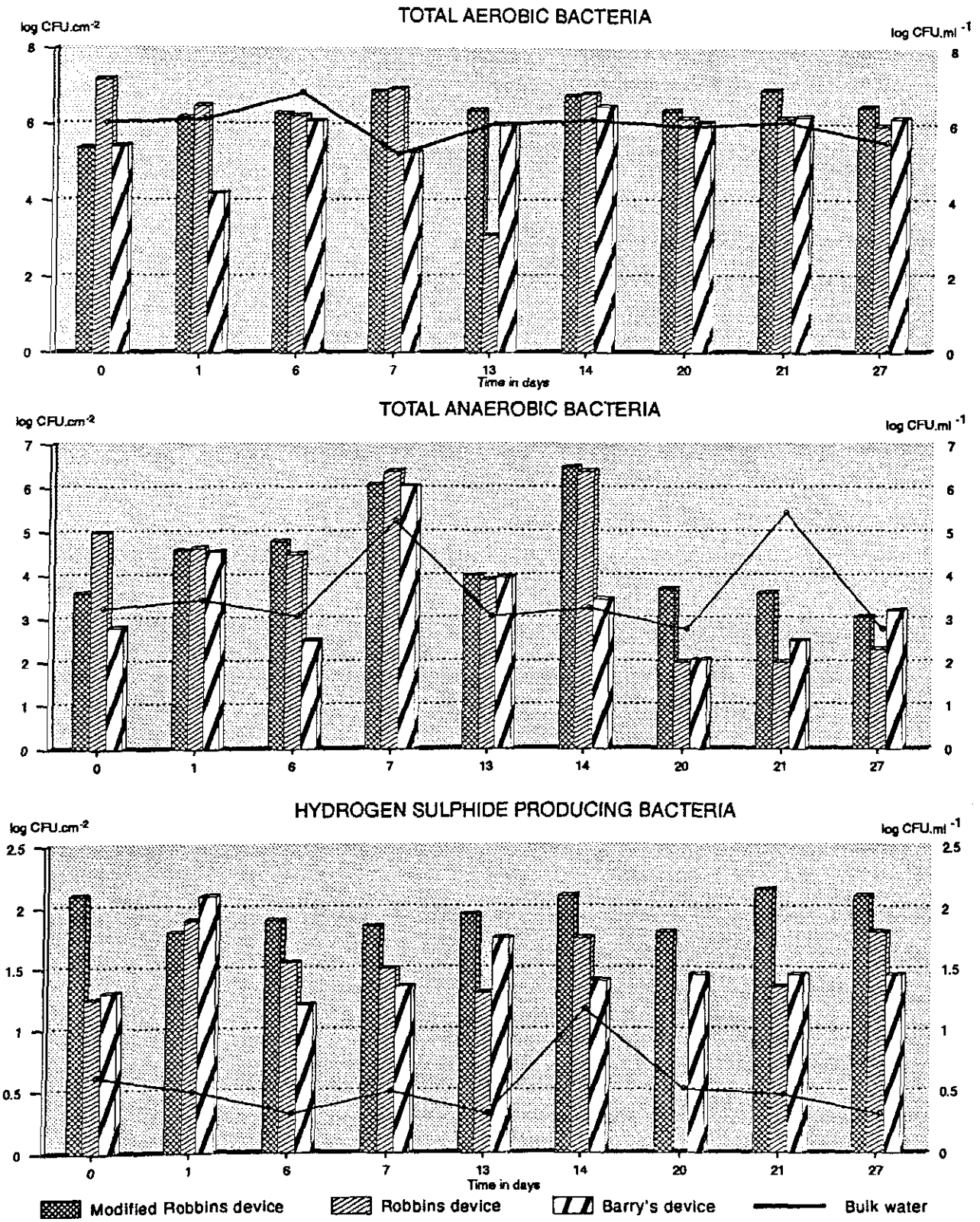

FIGURE 5.10 Comparison of attached bacteria on modified Robbins, Robbins and Barry's device biofouling monitors with slug dosing of biodispersant at 15 ppm once a week.

Changes in numbers of planktonic and sessile bacteria

In all four-week dosing regimes, changes in the numbers of attached bacteria measured by the monitoring devices, did not correspond with changes in the numbers of planktonic bacteria. For example, when no biodispersant was added to the system, the anaerobic bacteria in the bulk water decreased after one day and then increased after eight days, while the sessile anaerobic bacteria recovered from the Modified Robbins and Robbins Devices, gradually increased over the same time period (Figure 5.8). It has, however, been widely reported that no correlation can be made between numbers of planktonic and sessile bacteria, thus, these results were expected (Costerton *et al.*, 1985; Blenkinsopp and Costerton, 1991). A noticeable increase in the numbers of planktonic bacteria, after the biodispersant was added, either continuously or as a slug dose was, however, not recorded (Figures 5.9 and 5.10). Previous studies have shown that the addition of an effective biodispersant, will result in a characteristic increase in the numbers of aerobic and anaerobic planktonic bacteria (Poulton and Nixon, 1990; Lutey and Allison, 1991). These results indicated that the particular biodispersant used in this study, was not effective in dispersing sessile microorganisms.

During the four-week period, when no treatment chemicals were added, no overall increasing or decreasing trends in numbers of sessile or planktonic bacteria were noted on any of the devices. There were, however, variations in the numbers of sessile and planktonic bacteria (Figure 5.8). For example, the total aerobic bacteria recovered from the modified Robbins Device, varied from approximately $3.0x10^4$ CFU.cm^{-2} to $3.0x10^6$ CFU.cm^{-2}, the total anaerobic bacteria from $1.0x10^4$ CFU.cm^{-2} to $3.0x10^6$ CFU.cm^{-2} and the H_2S producing bacteria from $1.0x10^1$ CFU.cm^{-2} to $1.0x10^2$ CFU.cm^{-2} (Figure 5.8). These variations in sessile bacterial numbers could be due to natural phenomena such as periodic sloughing and thickening of biofilms (Characklis and Marshall, 1990). In addition, anaerobic bacteria could be more sensitive to slight changes in biofilm thickness than their aerobic counterparts. Changes in oxygen concentrations within a biofilm may range from supersaturation to total depletion within micrometer distances (Jorgensen and Revsbech, 1983; Ledandowski *et al.*, 1991). Thus, slight changes in biofilm thickness may result in anaerobic bacteria being exposed to lethal levels of oxygen and fluctuations in numbers of anaerobic bacteria.

Comparison of the four biofouling monitors

Numbers of sessile bacteria recovered from the three remaining biofouling monitoring devices were analysed statistically, to determine variances between the devices (Table 5.4).

Table 5.4 : Statistical analysis of variances between numbers of bacteria quantified on the biofouling monitoring devices, for each of the three different biodispersant dosing regimes.

	DEVICES		BACTERIAL TYPE	f VALUE	SIGNIFICANT DIFFERENCE ($p<0.05$)
NO TREATMENT	MRD[a]	: RD[b]	Total aerobic bacteria	3.43	No
	RD	: BD[c]		1.02	No
	MRD	: BD		3.48	No
	MRD	: RD	Total anaerobic bacteria	7.19	Yes
	RD	: BD		5.66	Yes
	MRD	: BD		40.72	Yes
	MRD	: RD	H_2S producing bacteria	8.06	Yes
	RD	: BD		2.85	No
	MRD	: BD		23.01	Yes
BIODISPERSANT DOSING SLUG	MRD	: RD	Total aerobic bacteria	3.43	No
	RD	: BD		25.42	Yes
	MRD	: BD		9.00	Yes
	MRD	: RD	Total anaerobic bacteria	2.82	No
	RD	: BD		10.91	Yes
	MRD	: BD		10.34	Yes
	MRD	: RD	H_2S producing bacteria	1.06	No
	RD	: BD		2.86	No
	MRD	: BD		1.68	No
BIODISPERSANT DOSING CONTINUOUS	MRD	: RD	Total aerobic bacteria	5.03	Yes
	RD	: BD		15.47	Yes
	MRD	: BD		77.81	Yes
	MRD	: RD	Total anaerobic bacteria	69.91	Yes
	RD	: BD		51.07	Yes
	MRD	: BD		1.37	No
	MRD	: RD	H_2S producing bacteria	9999.99	Yes
	RD	: BD		59.31	Yes
	MRD	: BD		9999.99	Yes

[a]MRD = Modified Robbins Device [b]RD = Robbins Device
[c]BD = Barry's Device

No statistically significant differences were found amongst the variances of the numbers of total aerobic bacteria recovered from the Robbins, Modified Robbins and Pedersen Devices when no biodispersant was added to the system (Table 5.4). This was also true for the numbers of H_2S producing bacteria when the biodispersant was slug dosed.

Consistently higher f values (1.37 - 9999.99), and thus significant variances between the numbers of sessile bacteria enumerated on the devices, when the biodispersant was continuously added to the system, were noted (Table 5.4).

Variances between the devices were lower (1.02 to 40.72), when no biodispersant was added and when the biodispersant was slug dosed (Table 5.4). However, the numbers of sessile bacteria on the modified Robbins Device were consistently higher for all groups of bacteria tested. Eight sampling surfaces were removed from each monitoring device during the period when no biodispersant was added to the cooling water. During this period, the total aerobic and anaerobic bacteria recovered from the sampling surfaces of the modified Robbins Device, were higher than on the other devices in 75% of the cases, while the H_2S producing bacteria were higher in 88% of the cases (Figure 5.8). This pattern was also observed when the biodispersant was added to the system (Figures 5.9 and 5.10). This may be due to the larger sampling surface area of the modified Robbins Device (4.9 cm^2), as compared to the standard version (0.5cm^2). The larger surface area may have allowed a more uniform biofilm attachment, or increased the sampling accuracy for patchy or unevenly distributed biofilms (Donlan *et al.*, 1990). Another possible explanation is the difference in the materials of construction. The modified Robbins Device was constructed entirely of PVC and nylon, whereas the Robbins Device consisted of nylon studs in brass holders in a mild steel pipe (Table 5.2). It was observed that deposition of corrosion products from the mild steel pipe occurred on the nylon studs of the Robbins Device (Figure 5.7). These corrosion products may have interfered with the quantification of sessile microbiological populations, as they were firmly attached to the stud surface and therefore difficult to disperse. Thus, a reduced number of bacteria may have been recovered from the sampling surface. Another contributory factor may be the changes in the design of the stud holder in the modified Robbins Device, which allowed removal of the studs without tearing the biofilm.

No visible differences on the sampling surfaces of the biofouling monitors could be distinguished when viewed by scanning electron microscopy. It was, however, noted, that large amounts of amorphous material were attached to the surface of the Barry's Device (Figure 5.11). The sampling surface area of the Barry's Device was larger than both the Robbins and modified Robbins Devices (Table 5.2). However, larger numbers of attached bacteria per

cm^2 were not recorded. Visual observations of thicker biofilms on the sampling surfaces of the Barry's Device also indicated that other, non-microbiological material was entrapped in the biofilm. This could be due to the different material of construction of the sampling surface of the Barry's Device which was PVC, as opposed to nylon in the other devices (Duddridge and Pritchard, 1983). Fletcher (1985) reported that the nature of a surface can affect the number and composition of sessile bacterial populations on it, as well as the activity of the attached bacteria. As the PVC surface was rougher than the nylon surfaces, it may have facilitated improved biofilm formation (Characklis and Marshall, 1990).

Figure 5.11 :Scanning electron micrograph of biofilm attached to a sampling surface removed from the Barry's Device after four weeks exposure to the recirculating cooling water at Lethabo Power Station when no biodispersant was added (arrow indicates amorphous material).

Comparison of dosing regimes

Statistical analysis of the variance detailed below, showed no significant differences between the three dosing regimes.

Null hypothesis (H_0) was that the means are equal
$F_{2.231,0.025} = 3.69$
$f = 3.25$
H_0 is accepted if $f \leq F_{v2, vl, \alpha}$
$f \leq 3.69$

Therefore, the null hypothesis that there is no significant difference between the three treatments in terms of bacterial types or monitoring devices was accepted.

These results indicated that slug doses or continuous dosing of the biodispersant were apparently ineffective in removing sessile bacteria. The fact that increases in the planktonic bacteria were not recorded after the addition of the biodispersants, confirmed the ineffectiveness of the biodispersant treatment (Figures 5.9 and 5.10).

CONCLUSIONS

The hypothesis that there would be significant differences between the numbers of sessile bacteria on the sampling surfaces of the different monitoring devices was found to be valid. The modified Robbins Device was found to be the most accurate monitoring device for the direct monitoring of sessile microbiological populations in the open recirculating cooling water system at Lethabo Power Station. It is anticipated that the modified Robbins Device will be utilised to directly quantify sessile microbiological populations, and the Barry's Device for indirect biomass determination. Thus, the realistic assessment of not only biological fouling but also possible organic deposition and will be determined. These two monitors will allow the optimisation and assessment of cooling water treatment programmes with a higher degree of accuracy. The biodispersant used in this study was found to be ineffective in removing sessile bacteria.

ACKNOWLEDGEMENTS

Chemserve Systems and Mr B. Luddick at Iskor are gratefully acknowledged for supplying biofouling monitors and the staff at Lethabo Power Station and TRI, particularly Mr C. Scholtz, for their assistance and co-operation.