9 International Congress on Marine Corrosion and Fouling

PROGRAMME AND BOOK OF ABSTRACTS

17 - 21 July 1995

UNIVERSITY OF PORTSMOUTH
ENGLAND

John A Lewis

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Organising Committee:

Professor E B Gareth Jones
Dr Iwona Beech
Dr Sheelagh Campbell
Dr Robert Fletcher

Scientific Committee:

Dr S Pyne	Denmark
Professor G Relini	Italy
Dr S Jackson	Norway
Professor Th Skoulikidis	Greece
Professor F Laborda	Spain
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Sponsored by:

Comité International Permanent pour la Recherche sur la Préservation
des Matériaux en Milieu Marin

Biodeterioration Society

Royal Society of Chemistry (Industrial Physical Chemistry Group)

His Worship the Lord Mayor of Portsmouth,
Councillor Malcolm Chewter

Rohm and Haas, USA
for hosting the Mary Rose Reception

Hempel Marine Paints A/S, Denmark

Jotun, Norway

Sigma Coatings, The Netherlands

Topometrix Ltd

W J Leigh & Co

University of Portsmouth

The Mary Rose Trust

Conference Office, Portsmouth City Council

We are grateful to the Office of Naval Research, USA, European Office for their generous award of grant NOOO 14-95-1-80104 towards the cost of this conference

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University of Portsmouth: 17 - 21 July 1995

9 INTERNATIONAL CONGRESS ON MARINE CORROSION AND FOULING

PROGRAMME

Sunday 16 July

1400-1800	Registration School of Biological Sciences, King Henry I Street, Portsmouth

Monday 17 July

0830-0930	Late registration	
0900-0930	**Session I: OPENING CEREMONY**	
Chairperson:	Professor E B Gareth Jones	
	Dr M Bateman Acting Vice-Chancellor University of Portsmouth	Welcome to the University and Portsmouth
	Professor F Laborda	Welcome on behalf of the Biodeterioration Society
	Dr S Campbell	Welcome on behalf of the Royal Society of Chemistry
0930-1015	**Dr D C White,** & A A Arrage, D E Nivens, P Angell, R Palmer, J F Rice & G S Sayler	Bunker Memorial Lecture: Biofilm ecology: on-line methods bring new insights into MIC and microbial biofouling
1015-1045	Coffee/tea	
1045-1115	**Dr J J E Williams**	Financing of marine science engineering and technology

1115-1145	**Dr P Williamson** & L V Evans	NERC marine biofouling initiative
1145-1200	General discussion Domestic arrangements	
1200-1400	Lunch	
1400-1800	**Session II: Biodeterioration of wood and other materials**	
1900-2100	Reception at The Mary Rose Museum	

Tuesday 18 July

0830-1230	**Session III: Biofilms - attachment mechanisms**
1330-1700	**Session IV: Biofilms and surface interactions**
1715-1800	COIPM meeting

Wednesday 19 July

0830-1230	**Session V: Evaluation of biocidal compounds**	
	Parallel A (Theatre 0-11)	Parallel B (Theatre 0-10)
1330-1800	**Session VI:** **Environmental consequences of biocide use**	**Session VII:** **Corrosion offered papers**
1900-2000	Reception at The Guildhall, Portsmouth	

Thursday 20 July

	Parallel A (Theatre 0-11)	Parallel B (Theatre 0-10)
0830-1230	**Session VIII:** **Surface techniques in corrosion**	**Session IX:** **Macrofouling: new developments**
1330-1800	**Session X:** **Surface techniques in corrosion - cont**	**Session XI:** **Biological offered papers**

1930 for 2000	Banquet at L'Escargot Restaurant

Friday 21 July

	Parallel A (Theatre 0-11)	Parallel B (Theatre 0-10)
0830-1230	**Session XII: Microbial corrosion**	**Session XIII: Biological offered papers**
1330-1600	**Session XIV: Corrosion**	

ORGANISED SYMPOSIA

MONDAY, 17 JULY

SESSION II: **BIODETERIORATION OF WOOD AND OTHER MATERIALS**

Chairperson: E B G Jones

1400	**S M Cragg**	Developments in the control of marine crustacean borers
1430	**R A Eaton**	Novel techniques for wood preservation in the marine environment
1500	P A Wagner & **B J Little**	Biodegradation of composite materials under mechanical stress
1530	Coffee/tea	
1600	**H P Molitoris**	Decomposition of plastics in the sea
1630 -1700	A M Jones, **S B Pointing** & E B G Jones	Structure and chemistry of waterlogged archaeological wood recovered from the marine environment

TUESDAY, 18 JULY

SESSION III: BIOFILMS - ATTACHMENT MECHANISMS

Chairperson: M E Callow

0830	**C Scott**, E B G Jones & R L Fletcher	Attachment of marine microorganisms
0900	**D W T Au**, S T Moss & E B G Jones	Attachment of fungal spores and immunogold studies
0930	**P S Handley**	Advantages and disadvantages of the use of electron microscopy to study microbial attachment
1000	Coffee/tea	
1030	**J W T Wimpenny**	Sequence in biofilm formation
1100	**G G Geesey**, P Suci, B Frolund, R Weiner & E Quintero	Exopolymers in biofilms
1130	**K E Cooksey** & B Wigglesworth-Cooksey	Signalling leading to adhesive release in diatoms
1200-1330	Lunch	

SESSION IV: BIOFILMS AND SURFACE INTERACTIONS

Chairperson: K E Cooksey

1330	**A Tunlid**, S Rosén & B Nordbring-Hertz	Lectins and their role in fungal attachment
1400	**I B Beech**, S C W Cheung & P Watkins	Comparative studies of bacterial biofilms and their role in corrosion using techniques of atomic force microscopy and electron microscopy
1430	**Z Lewandowski** & J W Costerton	Structural heterogeneity of biofilms and its consequences
1500-1530	Coffee/tea	
1530	J Schmitt & **H-C Flemming**	FTIR-spectroscopy in biofilm research
1600	**C W Keevil**, J T Walker & J Rogers	Advanced light microscopy for biofilm investigation
1630 -1700	**O Wanner**	Modelling of biofilms
1715 -1800	**COIPM meeting**	

WEDNESDAY, 19 JULY

SESSION V: EVALUATION OF BIOCIDAL COMPOUNDS

Chairperson: S M Jackson

0830	**G W Swain** & M Schultz	Testing and evaluation of non-toxic antifouling coatings
0900	**P Watkins**, S Cheung, S Badger, S Barker & I B Beech	The use of different techniques for studying the efficacy of biocides against marine sulphate reducing bacteria in biofilms formed on mild steel
0930	**E G Haslbeck**, C J Kavanagh, H W Shin, W C Banta, P Song & G I Loeb	Determination of minimum effective release rates of antifoulants
1000	Coffee/tea	
1030	**R L Fletcher**	Bioassay tests for antifouling coatings/toxins: a review
1050	**J A Lewis**	Biofouling control: the Australian experience
1115	**P D Steinberg**, R de Nys, J Jennings & S A Dworjanyn	Chemical ecology of antifouling in the marine algae *Delisea pulchra* and *Ecklonia radiata*
1140	**R de Nys**, S A Dworjanyn, T Leya & P D Steinberg	Natural antifoulants from marine algae
1200	B Wigglesworth-Cooksey & **K E Cooksey**	A computer-based image analysis system for biocide screening
1230-1330	Lunch	

Parallel session A: Lecture Theatre 0-11

SESSION VI: ENVIRONMENTAL CONSEQUENCES OF BIOCIDE USE

Chairperson: G W Swain

1330	**C D Anderson** & J E Hunter	Leaching rate: a fundamental requirement for the regulation of antifouling paints
1400	**J Chadwick**	Triorganotins and the environment: the current UK position
1430	**S M Evans**	Monitoring populations of dogwhelk for imposex
1500	Coffee/tea	
1530	**P Schultze,** H Schweinfurth & R Länge	Worldwide monitoring of TBT concentrations in seawater
1600	**R Länge**	Risk evaluation of current levels of TBT in seawater in the context of recent ecotoxicity data
1630 -1700	**R M Turner**	The proposed EC biocides directive

Parallel session B: **Lecture Theatre 0-10**

SESSION VII: CORROSION OFFERED PAPERS

Chairperson: B J Little

1330	**A A Novokova**	Water corrosion of mild steel promoted by sulfate reducing bacteria
1350	**T S Whitham** & S Huizinga	Application of electrochemical noise analysis to the study of microbiologically influenced corrosion
1410	**D K Yfantis** & A D Yfantis	Evaluation of sacrificial aluminium anodes
1430	**P Angell** & D C White	Concentric electrodes in microbial corrosion studies of 304 stainless steel
1450	**P L Bonora,** S Rossi, L Benedetti & M Draghetti	Improved sacrificial anode for the protection of off-shore structures- Note 2
1510	Coffee/tea	
1540	S Clavell, E Arias, R Boss & **R George**	Non-toxic easy fouling release ship hull coatings
1600	**C Cambon**	New generation of antifouling paints for the French Navy: high performance tin and copper free paints, anti-adhesive coatings, and a new accelerated test for comparative evaluation of erosion rates of self-polishing coatings
1620	S M Beloglazov, **G S Beloglazov** & I V Katayeva	Deactivation treatment of water deteriorated by *Thiobacilli thiooxidans* and *ferrooxidans* with *Desulfovibrio desulfuricans*
1640 -1700	**G S Beloglazov** & S M Beloglazov	Quantum chemical study of nitrogen and sulfur containing substances as inhibitors of corrosion and hydrogenation of steel

THURSDAY, 20 JULY

Parallel session A: Lecture Theatre 0-11

SESSION VIII: SURFACE TECHNIQUES IN CORROSION

Chairperson: S A Campbell

0830	**J Robinson**	Synchrotron radiation based X-ray absorption studies of corrosion and passivation
0900	M P Ryan, M C Simmonds, G E Thompson & **R C Newman**	Atomically resolved STM of passive oxide films on Fe and Fe-Cr
0930	**S Turgoose**	The application of impedance spectroscopy for corroding systems
1000	Coffee/tea	
1030	**K R Trethewey**	Fractal models for corroding surfaces
1100	**G Denuault**	On the use of the scanning electrochemical microscope (SECM) to the study of localised corrosion (Lecture sponsored by the Industrial Physical Chemistry Group of the Royal Society of Chemistry)
1145	Discussion	
1200-1330	Lunch	

Parallel session B: **Lecture Theatre 0-10**

SESSION IX: **MACRO-FOULING: NEW DEVELOPMENTS**

Chairperson: D R Houghton

0830	**J C Thomason,** J Davenport & D P Knight	The structure and antifouling mechanisms of the dogfish eggcase
0900	**R L Fletcher**	Epiphytism and fouling in seaweed cultivation: an overview
0930	**G Relini,** F Tixi, M Relini & G Torchia	The macrofouling on offshore platforms at Ravenna
1000	Coffee/tea	
1030	**A S Clare**	Barnacle settlement 'revisited': recent progress with *Balanus amphitrite*
1100	**H S Rai** & A Sasekumar	Marine fouling organisms on experimental wooden panels in tropical waters
1130	**J D McKenzie** & I V Grigolava	How do marine invertebrates keep their surfaces free from microfouling?
1200	**D P Edwards,** A Davis, T G Nevell & R A Pullin	The effects of medium term exposure in sea-water trials on the surface properties of silicone elastomers
1230-1330	Lunch	

Parallel session A: Lecture Theatre 0-11

SESSION X: SURFACE TECHNIQUES IN CORROSION - Continued

Chairperson: Th Skoulikidis

1330	**D E Williams**	*In situ* electrochemical imaging of corrosion processes
1400	J F Halsall, **M Kalaji** & L K Warden Owen	The use of *in situ* FTIR spectroscopy for the study of corrosion processes
1430	J D Crossen, J M Sykes, D Knauss, **G A D Briggs** & J P Lomas	Acoustic microscopy of coating adhesion
1500	Coffee/tea	
1530	G J W Radford, F C Walsh & **S A Campbell**	AFM studies of the corrosion resistance of high strength cupro-nickel alloys
1600	**I T E Fonseca** & V L Rainha	Electrochemical techniques in MIC studies
1630	**D Wagner**, H Siedlarek, W Tschuschke, H Peinemann, O von Franqué & U Patalla	Evaluation of the influence of chloride ions on the corrosion performance of DHP-copper and 90/10 copper-nickel-iron
1700	Discussion	

Parallel session B: Lecture Theatre 0-10

SESSION XI: BIOLOGICAL OFFERED PAPERS

Chairperson: G Relini

1330	**C J Bass**, J Webb P F Sanders & H M Lappin-Scott	Influence of surfaces on hydrogen sulphide producing bacteria
1400	**M E Callow** & G L Willingham	Degradation of antifouling biocides
1430	**S L Hodson**, T E Lewis & C M Burke	Mechanical cleaning of salmon cages: removal and regrowth of fouling
1450	B Pietrangeli, M Camilli, R Gianna & **G Ventura**	Marine primary biofilm on stainless steel - quantitative and qualitative methods
1520	Coffee/tea	
1550	**J C Petrille**, M W Werner & W K Whitekettle	Effectiveness of an quaternary ammonium-based biocide to control marine macrofouling in industrial cooling water systems
1610	**K Becker**	Detachment studies on microfouling in natural biofilms on substrata with different surface tensions
1630	**J T Walker**, M Moralis & C W Keevil	Development of a continuous culture biofilm model to evaluate microbial biocides
1650	**R L Fletcher**	The occurrence of a major new fouling alga in the British Isles
1710 -1730	V Manickam & C Kalaiyappan	Influence of factors in microbial biofilm formation on metals in seawater

FRIDAY, 21 JULY

Parallel session A: Lecture Theatre 0-11

SESSION XII: MICROBIAL CORROSION

Chairpersons: I B Beech and W A Hamilton

0830	**B J Little &** P A Wagner	Surface analytical techniques applied to microbiologically influenced corrosion
0900	**D Feron &** I Dupont	Marine biofilms on stainless steels: effects on the corrosion behaviour
0930	**S R de Sánchez**	Chemotaxis and corrosion in copper alloys and other materials
1000	Coffee/tea	
1030	**H A Videla**	Biocorrosion problems in the marine environment: new perspectives
1100	T B Burnell, **J A Cella,** J Stein & G W Swain	Correlation of properties with performance of silicone foul release coatings
1130	**M J Feio,** J J G Moura, I B Beech & A R Lino	The relationship between SRB outer membrane protein profiles and their corrosion causing abilities: a comparative study
1230-1330	Lunch	

Parallel session B: Lecture Theatre 0-10

SESSION XIII: BIOLOGICAL OFFERED PAPERS

Chairperson: R L Fletcher

0830	**G Relini**, F Tixi, M Relini & G Torchia	Settlement on concrete blocks immersed in the Ligurian Sea (N-W Mediterranean)
0900	**C Holmström**, S James, S Egan & S Kjelleberg	Inhibition of invertebrate larvae, algae and diatoms by a surface colonising bacteria
0930	H S Elbro, **E B Kjær,** M Warnez & S Arias	Release of biocides from antifouling coatings
1000	Coffee/tea	
1030	A A Arrage & **D C White**	On-line fluorescence measurements as a bacterial biomass measure of prospective antifouling paints
1100	**T Ooba**, S Saito, M Kuwa & T Kitamura	Method for preventing aquatic organisms in active dissolving by the anodic electrolysis of metal
1130	**H Kang**, J Maddock, V Zinkevich, R Tapper, G A Mills & I B Beech	The use of pyrolysis-mass spectrometry for the analysis of sulphate-reducing bacteria
1200	**A Gab-Alla** & S Farghaly	Fouling communities on seagrasses in the Gulf of Aqaba (Red Sea)
1230-1330	Lunch	

SESSION XIV: CORROSION

Chairperson: F C Walsh

1330	**Th N Skoulikidis**, P Vassiliou & S Vlachos	New anticorrosive paints; use of n-semiconductor pigments
1400	**P Vassiliou**, G Papadimitrioui & N J Amadi	A study of the corrosion behaviour of laser treated cast iron alloys
1430	**P L Bonora**	Electrochemical evaluation of coatings for marine corrosion control
1500	Coffee/tea	
1530	G Batis & **N Kouloumbi**	Influence of inhibitors on the performance of reinforced light mortar in marine environment
1600	**N Kouloumbi** & G M Tsangaris	Metal-filled composites as protective coatings against marine corrosion
1630	**A Neville** & T Hodgkiess	Corrosion of high grade alloys in marine conditions containing the sulphate reducing bacteria
1700	**J H Cleland**	Corrosion risks in the IMO pathogen guidelines
1730	S M Beloglazov, G S Beloglazov & A A Myamina	Corrosion and hydrogen absorption inhibitors with biocyde action against sulphate reducing bacteria

SESSION XV: POSTERS Studio on 6th Floor

1.	J C Petrille & M W Werner	Efficacy of non-oxidizing antimicrobial agents to control hydrozoans
2.	G G Bausch & J S Tonge	Evaluation of silicone technology for fouling release coating systems
3.	J Rogers, D I Norkett & C W Keevil	The use of viability and immunolabelling techniques to determine the presence of pathogenic bacteria, cyanobacteria and parasites in biofilms
4.	R M Moate & M B Jones	Mechanisms of substrate attachment and settlement in *Tubularia larynx* (Ellis and Solander)
5.	H P Molitoris, S Moss, S Neumeier, K Rathberger, E Straubinger & B Zaunstöck	Decomposition of thermoplasts in the sea
6.	R Maximilien, R de Nys, C Holmström, S Kjelleberg & P D Steinberg	The effect of *Delisea pulchra* secondary metabolites on the attachment, survival and growth of marine bacteria
7.	V Bellini, M Le Guyader & R L Fletcher	A computerised annotated bibliography of marine fouling algae
8.	M Carepo, A S Pereira, R Franco, M J Feio, J Lopes, M A Reis, A R Lino & J J G Moura	Enzymic characterization of sulfate reducing bacteria involved in biocorrosion
9.	R Pereira, J F Batista, M Carepo, C V Pinto, J J G Moura & M A Reis	Influence of micronutrients concentration on growth and activity of sulfate reducing bacteria
10.	L Bartole & G Bressan	Biological aspects on marine microfouling treatments inside simulated industrial pipes

11.	C S Wu, R L Fletcher & E B G Jones	A biological study of the important marine fouling green alga *Ulothrix flacca* (Dillw.) Thur. in Le Jol
12.	H M Lappin-Scott	Formation and control of biofilms
13.	D K Yfantis	Protection of aluminized steel tubes by chromating (a case study)
14.	J B Sigurdsson, S L M Teo & C S C Lee	Screening for bioactive compounds from marine organisms: potential antifouling compounds in soft corals from Singapore reef flats

GENERAL INFORMATION

1. SYMPOSIUM LECTURE THEATRE

Lecture theatre 0-10, School of Biological Sciences, will be the venue for the majority of the symposia.

Parallel sessions will be held in 0-11.

2. SYMPOSIUM SECRETARIAT

This will be situated in room 4-15.

3. REGISTRATION AND GENERAL INFORMATION DESK

The registration and general information desk will be open in the foyer on the ground floor of the School of Biological Sciences at the following times:

Sunday, 16 July	1400-1800
Monday, 17 July	0730-1200
Tuesday, 18 July	0830-1200
Wednesday, 19 July	0830-0930
Thursday, 20 July	0830-0930
Friday, 21 July	0830-0930

4. REFRESHMENTS

Morning and afternoon coffee/teas will be served at the times indicated in the daily programme on the 6th floor studio of the School of Biological Sciences.

5. POSTER DISPLAYS

All posters will be displayed throughout the symposium in the 6th floor studio of the School of Biological Sciences. Please have your posters mounted by Monday, 17 July.

6. TRADE EXHIBITS

Trade exhibits will be on the 6th Floor in 6-02.

7. BULLETIN BOARDS

Changes to the programme with urgent announcements from the Secretariat will be posted on the bulletin board in the foyer on the ground floor of the building.

8. MESSAGE BOARD

Mail received and messages for delegates during the Symposium will be put on the message board on floor 6, near to the refreshment area.

9. TOILETS

Ladies:	floors 1, 3, 5.
Gentlemen:	floors 2, 4, 6.

10. PRE-SESSION PROJECTOR FACILITIES

A Kodak Carousel Projector and screen will be available in Room 0-03 throughout the Symposium for those speakers who wish to check their slides. Mr David Maund and Mr Ali Gab-Alla are in charge of the projection equipment.

11. PROJECTION FACILITIES

Speakers are requested to give their slides to the projectionist at least 30 minutes before the start of the session in which they are to present their paper. Slides must be given to the projectionists in a Kodak Carousel slide tray which will be available before each session. Please put your name on your slide tray. Speakers should collect their slides at the end of each session.

12. PROCEEDINGS

The proceedings will be published in 3 volumes and authors will be informed during the conference of deadlines for submission of manuscripts.

13. USEFUL TELEPHONE NUMBERS

School of Biological Sciences (general office)	01705 842036
Gareth Jones (local organizer) University number	01705 842032
Gareth Jones (local organizer) home number	01705 613090 (emergencies)
British Rail	01705 825771
Conference Fax number	01705 842070

14. RESIDENTIAL ACCOMMODATION

Accommodation for delegates booked through the Secretariat is in:

Burrell House
Hambrook Street
Southsea PO5 3BS

Telephone number:	01705 828384	(first floor)
	01705 829168	(third floor)

Meals (breakfast and dinner) will be taken at Eldon Building, Winston Churchill Avenue.

Minibuses will leave Burrell House every 10 minutes from 0730 for breakfast. You can walk from Eldon Building to King Henry Building.

Dinner will be served in Eldon Building from 1800 until 1900. Please note that there will be no dinners served in Eldon Building on 17, 19 or 20 July.

15. MEAL TICKETS

Tickets for meals booked (breakfast and dinner) will be in an envelope in your Symposium bag. You must present these at Eldon Hall for your meal.

16. SOCIAL EVENTS

For all events listed, dress informal but smart.

Monday, 17 July

1900-2100	Reception at The Mary Rose Museum Short talk by Dr Margaret Rule

A minibus will leave every 10 minutes from Burrell House from 1830. A minibus will deliver you back to Burrell House after the reception.

Wednesday, 19 July

1900-2000	Reception by the Worshipful Lord Mayor of Portsmouth, Councillor Malcolm Chewter Banqueting Hall, The Guildhall, Portsmouth

Tickets will be in your information pack.

Thursday, 20 July

1930 for 2000	Official Conference Dinner L'Escargot Restaurant, Stanley Street, Southsea

Minibus will leave every 10 minutes from 1900 and will deliver you back after the Dinner.

Admission is strictly by ticket only which is included in your Symposium pack.

We regret that the Dinner is fully booked and therefore no late bookings can be taken. However, should cancellations occur during the conference, an announcement will be made.

17. BANKING

Barclays Bank is a two minute walk from the Biology Building and is situated at the end of King Henry I Street adjacent to the Guildhall Square. Banking times are 0915 to 1530 Monday to Friday. Other major banks may be found in the City Centre which is reached by crossing Guildhall Square and continuing past the railway station for about 200 metres.

18. EATING OUT

Portsmouth and Southsea are well provided with restaurants and bars for all appetites and prices. Near King Henry Building there are several pubs suitable for a snack lunch and there is also the University's Nuffield Centre nearby for lunches at a reasonable price. There are several restaurants near the Hall of Residence for those not taking conference dinners. Any of the staff on the registration desk will be pleased to help point you in the right direction and there is information in your Symposium pack.

19. LOCAL SHOPPING

There is a large shopping precinct 5 minutes walk from King Henry Building with supermarkets, pharmacy and drug stores. A similar smaller precinct is situated ten mintues walk from Burrell House at Palmerston Road, Southsea. See your information pack.

20. POST OFFICE

Proceed to Guildhall Square, turn left across the square and continue past the railway station. At the first traffic lights turn right. The Post Office is across the car park on your left.

21. IN AND AROUND PORTSMOUTH

Portsmouth City centre is a few minutes walk from King Henry Building, and Southsea shopping area and the seafront are 10-15 minutes walk from the City centre. Portsmouth City centre, Old Portsmouth and Southsea constitute an area rich in maritime heritage, reflecting the City's long association with the Navy. These include the seventeenth- and eighteenth-centrury town defences of Old Portsmouth, which are unique in England, the unusual system of land and sea forts built by Lord Palmerston in the nineteenth century. Perhaps the most unique naval heritage is the Maritime Museum with its collection of ships and artefacts. This museum complex is situated in the Naval Base just north of Portsmouth Harbour Station. Within this area, open to the public from 1030 to 1700 daily, are Nelson's Flagship - HMS Victory, HMS Warrior - the world's first iron-clad warship built in 1860, and The Mary Rose - King Henry VIII's battleship which sank in The Solent in 1445 and was eventually raised in the autumn of 1982, with a comprehensive museum showing the artefacts found within the vessel. See your information pack.

22. DEPARTURE AND TRANSPORT

Delegates departing from Heathrow and Gatwick can be taken by taxi to the airport. The cost of this service is approximately £30.00 one way, irrespective of numbers of passengers. If you wish to avail yourself of this service, we will book for you provided the following information is given:

- your full name and address for pick up
- flight departure time
- flight number
- flight carrier
- name of airport and terminal number.

You may also make your own reservations through Airway Cars on 01705 788988.

You will be expected to pay the driver on arrival at your destination.

There is a direct rail link to Gatwick airport and a rail/coach link to Heathrow. Details and timetables for these will be available at the registration desk.

There is also a coach service to Heathrow which runs at intervals during the day. The timetable for this service will also be available at the desk.

Flights can be confirmed for you by the registration staff if all the correct information is supplied to them in good time.

23. GENERAL HELP AND INFORMATION

If you have any queries or problems at times other than those when the registration desk is staffed (see 3 above), any of the local organizers or their helpers will be pleased to assist. Amongst them are speakers of French, Italian, Arabic, Malay, Greek, Thai, Mandarin, Cantonese, German, Polish, Russian and Welsh! Local helpers will have yellow badges.

24. GROUP PHOTOGRAPH

Tuesday, 18 July at 1230.

WE HOPE YOU ENJOY BOTH THE CONFERENCE AND YOUR STAY IN PORTSMOUTH

ABSTRACTS

As received from authors

SESSION I

Opening Ceremony

WHITE, D. C., A. A. ARRAGE, D. E. NIVENS, P. ANGELL, R. PALMER, J. F. RICE and G. S. SAYLER

Center for Environmental Biotechnology, University of Tennessee/Oak Ridge National Laboratory & Department of Microbiology, 10515 Research Drive, Suite 300, Knoxville, TN 37932, USA.

Biofilm ecology : On-line methods bring new insights into MIC and microbial biofouling

Microbial biofilms were formed on coupons with defined coatings in once-through laminar flow fields of controlled bulk-phase composition and shear. The biofilms were formed with pulses of inocula from continuous culture vessels. Dilute media was utilized to select for biofilm growth. The formation, succession, and stability of the biofilms were monitored with non-destructive on-line methods (fluorescence, bioluminescence, attenuated total reflectance Fourier transforming infrared spectrometry [ATR-FTIR] and electrochemical impedance spectroscopy) and by high resolution destructive analysis (viable & direct counts and phospholipid fatty acid signature methods) at the termination of the experiments. We demonstrated that biofilms of reproducible composition could be formed and that the order of inoculation of multi-component biofilms affected the composition at harvest. The corrosion rates of mild steel depended on the biofilm composition and composition but not the attached biomass. Examination of biofilms with the scanning vibrating electrode in a microscope field showed effects of heterogeneity in biofilm structure which promoted localized anodic activity. *Pseudomonas* strains were engineered to contain the *lux* gene cassette as a "reporter" and the formation of the exopolymer alginate was shown not to promote attachment of the strain or secondary colonization by *Vibrio*. Examination of mutants forming different alginate structures showed differential attachment and biofilm structure. Studies of mutants of lipopolysaccharide structure showed differential attachment to substrata. Specific antifouling and fouling-release coatings showed a wide range of attachment and release properties as well as sublethal toxicity.

WILLIAMS, J.J.E.

Marinetech South Ltd., Tizard Building (ISVR), University of Southampton, Southampton
SO17 1BJ, UK

Financing of marine science, engineering and technology

The offshore and marine industry represents an important element of the UK's economy, and is particularly important to the Solent region. The modern industry is highly dependent on skilled application of technology for its success in international markets: a 'much-tech' industry. But the industry is also fragmented, with a large number of relatively small suppliers, as well as a much smaller number of multinational companies.

The Marine Technology Directorate Ltd (MTD) has been charged with the mission of promoting research and engineering programmes which can generate technological advantage for industry, drawing on UK strengths in marine science and technology. In practice, MTD achieves its objectives through a range of activities, in the categories of:

- Research, via individual projects or programmes, and including funding of studentships;
- Technology transfer, including 'club' projects, training and publications.

As a private company with industrial members, whilst also being responsible for allocation of £5M/year of Research Council funds, MTD is a unique organisation. The success of MTD suggests that its principles could merit replication, in other industries and in other countries.

The keys to MTD's success are its use of Managed Research Programmes and its involvement of both industry and higher education in strategic planning. By talking with its members, MTD can develop an understanding of strategic technology needs within the industry. It can also define the research needed to underpin those technologies through consultation with the many universities and institutes it serves.

This knowledge provides the basis for initiating and managing a portfolio of activities targeting critical areas of technology. High quality research on its own is not enough to ensure technology development and innovation by firms. By including the commercialisation process as an integral part of programme management, MTD motivates the essential partnerships between firms and university research teams.

MTD's eight regional marine technology centres have an important role to play in achieving this high level of presence within industry, especially with smaller firms. Marinetech South Ltd is a new type of centre for MTD, being a joint venture company of several leading research bodies. With access to research expertise across all its shareholders, Marinetech South can initiate multi-disciplinary projects which relate to the immediate technological needs of firms. It can then incorporate these specific activities within a broader, synergistic programme of strategic research and technology development.

MTD and Marinetech South can draw on a variety of funding mechanisms recently introduced by Government. Of particular mention is the LINK initiative, in which concerted actions are set up in selected areas of research. Companies and universities submit joint proposals for projects to be funded, with the company committing 50% of the cost. A steering group selects the projects in order to achieve a coherence of the overall programme, and to maximise the opportunities for cross-fertilisation. At present, there is no LINK programme specifically relevant to the marine industries, but MTD hopes to correct this in the near future.

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NERC marine biofouling initiative

The marine biofouling initiative of the UK Natural Environment Research Council (NERC) is a new 3-year coordinated research programme, allocated a provisional budget of £1.4m and scheduled to start in early 1996. Its overall aims are to improve our understanding of the fundamental processes involved in surface selection and attachment, and the dynamics of the interactions between different taxonomic groups. Special emphasis will be given to the development of an interdisciplinary approach, by promoting collaborations between laboratories with different areas of expertise. Work will be supported at both the macro- and micro-scale, and it is expected that particular attention will be directed at:

- the physico-chemical characteristics of surfaces and conditioning films
- the molecular basis of signal reception and the discrimination of other settlement cues
- natural antifouling mechanisms
- experimental and modelling studies of 'succession' processes.

Such studies should assist in the development of non-toxic antifouling treatments, that might be developed further through industrial support. Progress will be reported regarding the establishment of an administrative framework for this initiative, as a NERC Special Topic programme.

SESSION II

Biodeterioration of wood and other materials

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Developments in the control of marine crustacean borers

The most economically-important wood boring Crustacea belong to the isopod families Sphaeromatidae and Limnoriidae, both of which have been recently revised and a number of new species have been recognised. Other wood boring crustaceans have now been recognised from tropical mangrove sites. Approaches to borer control which have proved effective against teredinids (use of naturally durable timber, copper-chrome-arsenic or creosote treatment, surface coatings) have been found under certain circumstances to be ineffective against *Limnoria* and *Sphaeroma*. The question of crustacean borer nutrition may hold the key to problems of their control. Sphaeromatid borers are capable of filter-feeding and thus may never ingest the treatments applied to wood. Limnoriids do ingest wood, but the role of wood degrading tunnelling bacteria, and soft-rotting ascomycete and deuteromycete fungi occurring in the wood they digest remains to be fully elucidated. The source or sources of wood-degrading enzymes which permit digestion of wood particles requires further investigation. Further insights into the problems posed by these borers may be obtained with a better understanding of their ecology. A better testing protocol for preservatives has been developed as a result of knowledge of the natural vertical distribution of *Sphaeroma*. The microecology of borer burrows has an important bearing on the availability of nitrogen for borers.

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Novel techniques for wood preservation in the marine environment

The main agents of destruction in maritime timbers are marine wood-boring molluscs and crustaceans which can cause severe internal tunnelling and/or superficial damage to the wood. Softening of the wood surface of submerged structures by a lignolytic microflora composed of soft rot fungi (members of the Ascomycotina and Deuteromycotina) and bacteria (primarily tunnelling bacteria) is less important in the overall deterioration process, although the significance of microbial damage in the establishment of boring animals on the surface is as yet unresolved. Damage to the submerged surfaces of wooden boats can be attributed to the same biodeteriogens, but wooden harbour constructions maintained permanently above the waterline are susceptible to decay by basidiomycete fungi and attack by insects. Currently recognised procedures for the protection of wooden constructions in seawater depend on the use of naturally durable timber or species with low durability treated effectively with appropriate wood preservative chemicals. In situations when marine borer activity is severe, the most widely accepted and effective treatment of non-durable species is dual pressure-treatment with copper-chrome-arsenic (CCA) formulations followed by creosote. In geographical locations where the site hazard is less severe, single treatment with CCA or creosote to recommended retentions has been the traditional method of protection. Recent studies have focused on the use of alternative biocidal compounds in combination with traditional preservatives and the effectiveness of physical barriers in the form of plastic sheathing to protect the wood from borer infestation. The novel chemical treatments have given variable results in field exposure trials whilst the efficacy of plastic sheathing has been encouraging. These results will be discussed in the context of safer and more environmentally acceptable methods of protection of maritime timbers.

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Biodegradation of composite materials under mechanical stress

Possible mechanisms for microbial degradation of polymeric composites have been investigated, including direct attack of the resin by acids or enzymes, blistering due to gas evolution, and polymer destabilization by concentrated chlorides and sulfides. Current research was performed to add additional parameters to biodegradation studies: (1) environmental stress applied during exposure, (2) environmental exposure after mechanical impact, and (3) evaluation using acoustic emission (AE).

Carbon fiber reinforced epoxy coupons were assembled in 3 and 4-point bend stainless steel loading fixtures at two applied strain levels and exposed for 7 months to a mixed marine microbial culture known to contain sulfate-reducing bacteria. Examination after exposures indicated no evidence of galvanic activity. Bacteria and sulfide crystals were preferentially concentrated along fiber/resin interfaces and in superficial surface scratches. Fiber disruption was observed at a point of greatest strain and bacteria preferentially colonized the anomaly, but the disruption could not be attributed to their presence.

AE testing was performed with load levels continued until coupon fracture. As load is applied, discrete bursts of acoustic energy characteristic of the physical nature of each composite component and its adhesion to other components are produced. Stressed, microbially exposed coupons required a lower load to cause a rapidly increased number of hits than controls. Final fiber fracture was visibly random in all samples at conclusion of loadings. AE data indicate that the mixed marine microbial consortium may cause loss of mechanical strength in stressed graphite reinforced epoxy composites, but cannot be considered conclusive because of the small sample size, basic specimen variation, and the lack of an identifiable failure mechanism.

Microbial degradation of impacted laminated composites could not be demonstrated. Bacteria preferentially colonized broken fibers and other disruptions, but the gross effects of the mechanical trauma masked any subtle microbial influences.

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Decomposition of plastics in the sea

Thermoplasts, mostly derived from fossile resources, today are unrenouncable materials because of their recalcitrance, multiple applicability and relatively low price. Increasingly, however, they are causing problems due to exhaustion and price increases of the fossile resources, pollution due to their missing biological degradability and partially by their toxic endproducts. Biologically degradable, biosynthetic plastics with no toxic endproducts would be the solution. A number of such biosynthetic materials such as the bacterial Poly-hydroxyalkanoates (PHA, e.g. poly-hydroxybutyrate = PHB, poly-hydroxyvalerate = PHV, poly-hydroxyoctanoate = PHO), fungal Poly-L-Malate (PMA) or even chemosynthetic plastics such as Poly--caprolactone (PCL) could fulfill these postulates. Some of these materials (PHB, BIOPOL, PCL) are already commercially produced and used. Their biodegradability by bacteria has been shown in most cases, but biodegradation by fungi, the other large group of degrading microorganisms, is still an open question. Since our oceans are increasingly misused as wastedumps, in this study the degradation of plastics is studied especially for marine fungi and in seawater media. Since preferentially oceanic dump grounds belong to the deep sea area, it would hence be very important to know whether these materials could be degraded also under deep sea conditions. Therefore, for the first time, degradation studies of thermoplasts with marine fungi have been performed also under simulated deep sea conditions using recently developed apparatus and methods.

Under surface conditions (atmospheric pressure = 0.1 MPa) degradation studies were conducted on seawater and freshwater media using more than 360 marine and terrestrial yeasts and filamentous fungi of different ecological and systematic groups. Highest degradation rates were found for the chemosynthetic thermoplast PCL. It is used as carbon source and degraded by over 85% of the more than 230 fungi tested. No significant differences in PCL degradation appeared between seawater and freshwater medium and among the different groups of fungi tested. Second in biodegradability were the biosynthetic PHAs (PHB, BIOPOL, PHV, PHO) which are used as carbon source and degraded by terrestrial fungi on freshwater media up to over 50%, by marine fungi, however, by only over 5% of the strains tested. No significant differences in PHA degradation appeared between seawater and freshwater medium and among the different groups of fungi tested. Preliminary tests with the new fungal material PMA showed that only 6% of the strains degraded it on freshwater media, degradation on seawater media is expected not to exceed this figure.

Under simulated deep sea conditions (pressure of 10 MPa = 1000 m, 27°C) the degradation of PHB powder (enzymatic clearing test on agar plates) and PHB sheets (weight loss and enzymatic clearing test) in freshwater media by three highly active terrestrial strains was tested. With all fungi except one, PHB degradation occurred also under deep sea conditions, however, considerably slower and to a lesser extent. Experiments on seawater media, using marine strains, are under way.

From the experiments it is evident that thermoplasts are degraded by fungi also under marine conditions, however, the degree of degradation is strain-specific, is less pronounced among marine strains and decreases with increasing pressure.

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Structure and chemistry of waterlogged archaeological wood recovered from the marine environment

Although all cell walls of timbers comprise primarily polysaccharides and lignin, the amount of these complex organic polymers vary taxonomically. The mechanisms and extent of degradation of these various wood types are dependent on environmental conditions and the microbial flora that they favour. A wide variety of methods is available for characterising the state of preservation of waterlogged archaeological wood, including electron microscopy (scanning and transmission) and solid state nuclear magnetic resonance (NMR). In general, timbers show two or more distinct zones of progressive degradation. Electron microscopy supported by chemical analysis of these various zones, demonstrate a microbial degradation process advancing from the wood surface inwards. The microbial degradation of wood is fundamentally a chemical process that leads to selective alteration of wood cell wall structure and chemistry. Thus to understand and conserve ancient wet wood, it is useful to have a basic understanding of the structure and chemistry of this complex material.

SESSION III

Biofilms - attachment mechanisms

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Attachment of marine microorganisms

Structures immersed in the sea are colonised by a wide range of micro- and macroorganisms, unlike many systems where only bacteria give rise to biofilms, e.g. teeth, food processing systems. This wide range of organisms are problematic in the design of efficient and effective antifouling coatings, requiring broad spectrum biocides.

In Part 1 of the talk we shall review the diverse marine microbial community that develops on non-toxic substrata, paying particular attention to their mode of attachment and the chemical composition of the adhesives produced. These organisms will include bacteria, fungi, protozoa, diatoms and settling spores of macroalgae.

The role of marine blue-green algae in biofilm development has been little studied. Part 2 will be devoted to the genera and species colonising toxic and non-toxic surfaces, aspects of their vertical and seasonal distribution, and succession.

Finally, we shall consider the mode of attachment of blue-green algae in the colonisation of surfaces.

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Attachment of fungal spores and immunogold studies

Initial conidial attachment of freshwater hyphomycete species is achieved by the secretion of a mucilage from the area of contact. Subsequent attachment is mediated by the production of germ tubes and hyphae covered with mucilage. In some species adhesion is further facilitated by the formation of appressoria. Attachment of the tetraradiate conidium of *Lemonniera aquatica* was the most tenacious among all the ten species of aquatic Hyphomycetes studied (Read, 1990). It secreted mucilage shortly after settlement and exhibited a fast germination response with subsequent appressorium formation. *Mycocentrospora filiformis* was the most adherent among the species with sigmoid conidia. The difference in attachment strategies between *L. aquatica* and *M. filiformis* will be compared with respect to mucilage secretion, conidium morphology, germination and appressorium production. The strength of attachment of conidia to surfaces will also be discussed.

Further studies on chemical composition of mucilages were carried out by: 1) an indirect enzymatic method using proteolytic and carbohydrate digestive enzymes; 2) a lectin-gold labelling technique using Concanavalin A, wheat germ agglutinin and *Limax flavus* agglutinin. Results indicate that the mucilage is mainly acidic polysaccharide, comprising N-acetyl-D-glucosamine (GluNAc), N-acetylneuraminic acid (NANA), β-1,3-glucan, β-glucuoronide, α-D-glucose and α-mannosyl residues. Variability of mucilage composition exists: a) between the two fungi studied, e.g. abundance of β-1,3-glucans in *L. aquatica* > *M. filiformis*; b) among different structures of the same conidium, e.g. *L. aquatica* where GluNAc and NANA were more abundant on germ tubes, while α-D-mannose/α-D-glucose were abundant on appressorium; c) on different regions of the same structure, e.g. germ tube of *L. aquatica* where NANA was restricted only to the sub-apical region. Such complex mucilage systems at different stages of fungal germination and attachment imply that mucilage production is a dynamic process. The changes in the nature, amount and distribution of mucilage components may be associated with specific biological functions, environmental or other factors.

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Advantages and disadvantages of the use of electron microscopy to study microbial attachment

Both transmission electron microscopy (TEM) and Scanning electron microscopy (SEM) have been used extensively to study microbial attachment and biofilm formation. This paper will review the usefulness of the EM techniques that are currently available. Conventional fixation methods have generated information on cell morphology, cell orientation and cell spacing in biofilms, while ruthenium red staining has revealed polyanionic polymers between cells. All sectioning methods are subject to shrinkage artefacts. SEM methods include critical point drying, freeze-drying and low temperature SEM.

SEM gives details of cell distribution, on the substratum surface; organisation of cells into groups, chains and microcolonies and in some methods, dehydrated strands of extracelluar polymers (EPS) are visible. Two methods which have been recently used to study biofilm structure - low temperature SEM and use of the environmental SEM (ESEM) have revealed a canopy of EPS over the top of cells in a biofilm, which may be continuous or patchy. Freeze-substitution followed by sectioning reveals a dense fibrous matrix of polymer strands between cells in a biofilm and shows the detailed fine structre of the EPS matrix, although this method is underexploited.

Images generated using a variety of EM methods will be used to illustrate adhesion and biofilm formation of streptococci and staphylococci.

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Sequence in biofilm formation

Biofilm is ubiquitous forming on almost any surface provided that sufficient water and nutrients are available. Generally a clean surface is rapidly coated with a conditioning film that is composed of organic molecules. After this bacteria attach to this, first reversibly by electrostatic or Van der Waals forces, later more firmly via fimbriae or using excreted biopolymers. The bound cells reproduce and generate microcolonies. These expand, often within a blanket of extracellular polymer. They may form a confluent mass or the structure may be patchy and penetrated by water filled pores. After a period of maturation during which other bacteria and organic or inorganic debris may be incorporated, parts of the biofilm can slough off and the cycle started again.

The microbial component of natural biofilm is often a complex community of different species. For instance in dental plaque the earliest colonisers are generally streptococci and the more aerobic species such as *Neisseria*. As the plaque matures so more anaerobic organisms are incorporated amongst which are species of *Fusobacterium*, *Porphyromonas* and *Veillonella*. Corrosion biofilms also show a colonisation sequence starting with aerobic species. These, as they thicken, become anoxic at their base and allow the growth of anaerobic sulphate reducing bacteria.

Laboratory growth systems capable of following successional changes in biofilms include the constant depth film fermenter (CDFF) developed in Cardiff. Design criteria for this system will be described and a number of experiments designed to demonstrate its value presented.

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Exopolymers in biofilms

Cells of a marine film-forming *Hyphomonas* sp. elaborate an extracellular polysaccharide around the mother cell which appears to mediate attachment of the cells to solid surfaces in seawater. The purified polysaccharide absorbs readily to uncoated germanium surfaces and less readily to germanium surfaces coated with proteinaceous conditioning films in a seawater environment. A mutant strain has been isolated that produces much less extracellular polysaccharide than the wild-type *Hyphomonas* sp. The reproductive stalk from which the daughter cell evolves contains no detectable adhesive extracellular polysaccharide in wild-type *Hyphomonas* sp. Lipopolysaccharide is exposed on the surface of the stalk and daughter cell but not on the surface of the mother cell. Studies are currently in progress to compare the relative stickiness of the lipopolysaccharide and extracellular polysaccharide to various surfaces and to determine the types of interactions that occur between these cell surface polymers and the substratum with which they associate.

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Signalling leading to adhesive release in diatoms

Common fouling diatoms are positively or negatively chemotactic to small organic molecules. Chemotaxis requires that cells are associated with a surface in the presence of an extracellular signal. This leads to the generation of an intracellular signal which can then be transduced as a consequence of receptor occupancy. The transduction process can be expected to involve changes in intracellular calcium concentration. The chemotactic process can be explained in terms of the Berridge two-pool model for calcium-induced calcium-release. Furthermore, in order to show directed motility, receptor occupancy involves polarization of the cell, i.e. there is a front and a rear. Motility/adhesive polymer is secreted from the raphe in an asymmetrical manner, or cells could never change direction as a reaction to a signal. Thus response to an external signal leads to controlled adhesive release in diatoms.

Signals associated with surfaces in the sea are not likely be small molecules. It is more reasonable to suggest that they are macromolecules such as glycoproteins with side chains containing glucose or glucose-like sugars at the termination. A second possibility is that the diatoms themselves provide the surface- associated signal. If a diatom cell secretes a small amount of adhesive polymer continuously, it will diffuse away from the cell as long as it is in suspension. When the cell encounters a surface, diffusion will be restricted and the adhesive polymer concentration in the immediate vicinity of the cell membrane will increase. This may be the trigger that indicates the presence of a surface to the cell. Increased secretion as a result of this signal could then anchor the diatom to the surface. If the above model proves to be correct, diatom antifoulant molecules should be those that inhibit calcium transmembrane flux, inhibit polymer synthesis or antagonize chemosensory receptor binding. Calcium channel blockers and inhibitors of glycoprotein synthesis act as diatom antifoulants.

SESSION IV

Biofilms and surface interactions

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Lectins and their role in fungal attachment

One of the first examples indicating a lectin mediated interaction in a fungal-host system was in the nematode trapping fungus *Arthrobotrys oligospora*. This fungus captures nematodes by using adhesive polymers present on special hyphae called traps. Results from inhibition experiments using various soluble carbohydrates, indicated that the adhesion was initiated by a N-acetylgalactosamine (GalNAc) specific lectin in the fungus binding to a carbohydrate receptor present on the nematode surface. Later, similar experiments have indicated that lectins could be involved in the adhesion to host surfaces in a number of other fungi. Even though lectins have been isolated from several of these fungi, details of their structure, binding properties, and localization which are important for understanding their function at a molecular level, are not well known.

We have recently cloned and analyzed a gene encoding a lectin isolated from *A. oligospora*. The lectin (designated AOL) was isolated on a mucin-Sepharose column and has a similar molecular weight and antigenicity as a previously isolated GalNac specific lectin isolated from *A. oligospora*. AOL binds weakly to a GalNac-Sepharose gel, and closer examination of the binding specificities using various overlay assays, has shown that the preferred carbohydrate ligands of AOL are sulfated glycoconjugates, a binding specificity previously not reported for a fungal lectin. The primary sequence of AOL, deduced from the DNA sequence of the cloned gene, showed no significant homology to other fungal, plant or animal lectins. However, computer predictions of the secondary structure of AOL, using data from the sequence and circular dichroism, indicated that AOL had a high content of β-sheets. These analyses, as well as the hydropathy profile and the lack of a typical signal peptide in the N-terminus, indicate that AOL has a structural similarity to the water soluble and low molecular weight S-lectins (galectins) present in animals. These lectins comprise a family of water soluble, low molecular weight, and β-galactoside binding proteins present both intra- and extracellularly. AOL has been reported to be an extracellular protein, but recent experiments using the immunogold technique and a monospecific polyclonal antibody against AOL, demonstrated that the lectin is also present in the cytoplasm and nucleoplasm of the fungus.

Lectins with biochemical properties similar to those of AOL including solubility, molecular weight, amino acid composition and binding specificities have been reported in many fungi, which indicate that AOL could be a member of a family of related lectins present in fungi, including both parasitic and saprophytic species. The functions of these lectins are not known, but the finding of a structural similarity between AOL and the well characterized galectins suggests that they may be multifunctional proteins involved in cell-cell and cell-matrix interactions, growth regulation, immune and defence responses.

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Comparative studies of bacterial biofilms and their role in corrosion using techniques of atomic force microscopy and electron microscopy

In this communication techniques of electron microscopy (EM), including environmental scanning electron microscopy (ESEM), and atomic force microscopy (AFM) have been compared as tools for the non-invasive observation of bacterial biofilms developed on metal surfaces and for studying biodeterioration of such surfaces. Atomic Force Microscopy (AFM) is a relatively new surface analysis technique which has not yet been exploited to its full potential in the area of biological imaging. Its greatest advantage, apart from its resolving power at the atomic level, is the fact that the samples need no pre-treatment prior to viewing as in conventional techniques like scanning electron microscopy (SEM) and transmission electron microscopy (TEM). Furthermore, the data stored as a digital information allows manipulation of the image to its best advantage. Studies of biofilms by SEM or TEM techniques provide useful information regarding the distribution of cells within biofilms. However, the required preparation of a sample causes its considerable shrinkage and distortion. Environmental scanning electron microscopy allows observation of fully hydrated biofilms. Although the biofilm matrix is preserved during ESEM viewing the resolving power of this instrument is lower than that of the AFM. Unlike the AFM, ESEM images of biofilms do not provide information regarding the morphology of the extracellular matrix, nor do they offer details of bacterial surface topography. The advantage of the AFM technique over SEM and ESEM methods, in biofilm studies, its potential for in-vivo monitoring of the biofilm dynamics and its usefulness for investigation of biocorrosion, are discussed.

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Structural heterogeneity of biofilms and its consequences

Aerobic biofilms were found to have a complex structure consisting of microbial cell clusters (discrete aggregates of densely packed cells) and interstitial voids. We used the Confocal Scanning Laser (CSL) microscope in conjunction with dissolved oxygen microelectrodes to examine the structural and chemical heterogeneity of fully hydrated, living biofilms in real time. Heterogeneous biofilms seem to form three dimensional porous structures with a network of interstitial voids filled with water. This may necessitate a revision of our concepts of the mass transport near and inside biofilms and may influence the microbially influenced corrosion (MIC). The oxygen distribution within the biofilm was strongly correlated with these structures.

Nuclear Magnetic Resonance Imaging (NMRI) was used to show how fluid velocity varies around biofilms. The combined profiles of flow velocity and biofilm density revealed that there is motion of the bulk solution at a level which is occupied by the biofilm. This implied the existence of convective mixing inside biofilms - a process which may have profound consequences for mass transport in biofilm systems. Particle Image Velocimetry confirmed that water can flow through biofilms.

Structural and chemical heterogeneity may induce microbially influenced corrosion (MIC) on metal surfaces where biofilms have accumulated. We have used the Scanning Vibrating Electrode SVE to spatially and temporally map ion currents in solution above MIC anodic and cathodic sites and to map the course of corrosion under an artificial biofilm - the biopolymer, calcium alginate.

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FTIR spectroscopy in biofilm research

FTIR spectroscopy in biofilm research gives chemical information about biological molecules. Using attenuated total reflexion (ATR), it is possible to gain this information from a 1-2 μm thick layer of an interface between a solid surface and a water phase. Thus, FTIR-ATR spectroscopy is a tool to investigate the adhesion of molecules and microorganisms to this surface non-destructively, *in situ* and on line. The formation of extracellular polymer substances (EPS) under various environmental conditions can be observed. In presence of 5 ppm toluene, a mono-species biofilm increased formation of EPS, and if 15 ppm were present, an increase of carboxylic groups occurred. This suggests that an organic pollutant can lead to an increase of the sorption capacity of a biofilm for cationic pollutants such as heavy metals. To monitor the development of thicker biofilms, a flow through chamber was constructed and FTIR spectroscopy was used in the transparent mode. The method allowed (i) to assess biofilm growth by the increase of absorbance of IR light by the biofilm and (ii) to detect characteristic spectra of biofilm bacteria *in situ*, such as nocardia or mycobacteria. For further characterization of bacteria, FTIR spectroscopy is suitable as well as it provides fingerprint spectra, allowing the rapid characterization of microbial strains. Related to other identification methods, it was possible to establish a spectra library which allowed a rapid characterization of the strains. The library presently holds some 150 strains and is growing continuously. The use of neuronal networks enhanced the accuracy and decreased computing time. Using diffuse reflexion FTIR spectroscopy (DRIFT) it was possible to scan surfaces of test surfaces for the distribution of polysaccharides and proteins in a dimension of centimeters and, thus, for biofilm growth and local distibution. Giving strictly chemical information, the method allows to discriminate between organic and inorganic, biotic and abiotic contaminants. This approach goes into a different direction than microscopical surface analysis methods: it aims to the macroscopical distribution of material adhering to a given surface.

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Advanced light microscopy for biofilm investigation

For many years a dogma has persisted that biofilms are confluent and present an intact physical barrier, refractory to penetration by disinfectants and antibiotics. This was based on observations of the dehydration artefacts generated by sample preparation of native biofilms for scanning electron microscopy (SEM). Only recently have the environmental SEM, atomic force microscopy and scanning confocal laser microscopy (SCLM) techniques been developed to view moist biofilms. All of these microscopy techniques require the purchase of very expensive equipment, are comparatively labour intensive and would not normally be advocated for routine observation of many specimens. With this in mind, we developed the episcopic differential interference contrast (DIC) biofilm microscope. This novel approach utilises DIC prisms above opaque specimens and transillumination, non-contact longworking distances lenses which can observe curved specimens without the use of coverslips or oil immersion and, with image analysis, can observe live, unprepared specimens at 4000x magnification. The technique is rapid and cheap, in comparison to its rivals mentioned above, and has been used with immunogold labelling techniques to observe the location of individual legionellae cells within the biofilm topography. The technique has been successfully combined with UV fluorescence microscopy, immunofluorescence probes, and vital chromophores and fluorochromes to observe the structure, thickness and vitality of biofilms, and the presence of specific species therein. Exopolysaccharides can be resolved using the PAS Schiff's stain. More recently, Hoffman Modulation Contrast (HMC) transmitted light microscopy has been utilised to visulise biofilm developed on transparent surfaces or following gentle transfer from opaque surfaces to glass slides. These studies have confirmed recent observations using SCLM and ESEM that initial surface colonisation on the acquired pellicle or conditioning film is followed by growth to form small microcolonies. After hours or days, depending the availability of nutrients, temperature, pH *etc.*, the microcolonies produce a "film" over the surfaces. In reality, many native biofilms when fully mature consist of a low background of microorganisms which has tall finger-like stacks or fronds of microorganisms rising from the surface. The basal background is approximately 5 μm in depth and the stacks reach up to 100 μm in height. When the biofilm is viewed unstained under low power stereo light microscopy it is evident that the micro-organisms exist in microcolonies visible due to their colour within the biofilm. Some of the stacks of microorganisms consist of closely associated layers of two or more species growing in bands possibly acting in some symbiosis. The mosaic structure, typical for biofilms on a range of metal and plastic surfaces, both *in vivo* and *in vitro*, can now be considered to be one of the open architecture structures which are typical of biofilms so far examined. The biofilm mosaic footprint is responsible for the unique pitting corrosion profile on copper tubing.

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Modelling of biofilms

Mathematical models are helpful tools for the analysis of systems which are as complex as biofilms. In the past years mathematical biofilm models of increasing complexity have been developed. Today a model for the analysis of mixed-culture multi-substrate biofilm systems is available. This model describes the spatial distribution and the development in time of dissolved components (nutrients, dissolved oxygen, electron donors and acceptors) and of particulate components (microbial species, organic and inorganic particulate material) in the biofilm as a function of transport and transformation processes. The most relevant transport processes in the biofilm are diffusion of dissolved components and advection of particulate components. The most important transformation processes are microbial growth, endogenous respiration, inactivation, and chemical reactions in the liquid phase of the biofilm. The mixed-culture biofilm model is able to predict the physico-chemical conditions at the interface between the biofilm and the solid surface, on which the biofilm grows, as a function of the conditions in the bulk fluid, the microbial composition of the biofilm, and the transport and transformation processes which take place in the biofilm. Since in this model no a priori assumptions are made with regard to microbial kinetics and chemical reactions, the model can be applied to almost any biofilm system.

AQUASIM is a new computer program for the identification and simulation of aquatic systems. The program has a window-type user interface, which is simple to use, and solves the equations of the mixed-culture biofilm model. Thus, the program considerably facilitates the work with this model. AQUASIM runs on various platforms (Workstations, Macintosh and DOS-PC) and includes routines for simulation, sensitivity analysis, automatic parameter estimation and data fitting. The mixed-culture biofilm model and AQUASIM represent very useful analytical tools for the study of complex biofilm systems.

The mixed-culture biofilm model has been developed and is used primarily in the field of waste water treatment. However, under certain conditions and with some additional simplifications this model can also be used for the analysis of corrosion problems. In a first step the problem to be solved has to be identified and the objective of modelling has to be determined. Then the compartments, processes and components which are relevant to the problem are defined. In a next step numerical values of the model parameters must be collected. Finally model calculations are performed and are compared to experimental observations and data. The various steps of this modelling procedure are outlined, and the possibilities and limitations of the application of the mixed-culture biofilm model and of AQUASIM to corrosion problems are discussed.

SESSION V

Evaluation of biocidal compounds

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Testing and evaluation of non-toxic antifouling coatings

Concerns with regard to the ecological impact of biocides used in antifouling paints and regulations restricting or curtailing their use has precipitated a dramatic change in the direction of research aimed at providing an answer to the biofouling problem. One topic of endevour is to engineer surfaces that reduce the tenacity of bioadhesion. To evaluate the effectiveness of such systems two test methods have been designed to quantify the attachment of marine organisms to surfaces subjected to static immersion. These methods are: the measurement of barnacle adhesion strength in shear, and the use of a calibrated water jet for the removal of other fouling types. Data are presented for several test surfaces, and the results are discussed with respect to coating characteristics.

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The use of different techniques for studying the efficacy of biocides against marine sulphate reducing bacteria in biofilms formed on mild steel

Bacteria have been associated with corrosion for many years. Garret (1891) proposed that the increased corrosion of lead cable was attributed to bacterial metabolites. However, it was not until 1934 that the corrosion of ferrous metal buried in anaerobic clay soil was attributed to the activity of obligate anaerobic bacteria known as sulphate reducers. Since then, the importance of sulphate-reducing bacteria (SRB) associated with corroded pipelines and other industrial installations has been recognised, and studies aimed to understand the mechanism of bacterial corrosion undertaken. One method of reducing the effects of microbial corrosion to industrial systems is by the addition of biocides. These are normally successful at reducing the planktonic population however biofilm populations have been found to be more resistant to their effects. Therefore this investigation was aimed at determining how effective biocides were in reducing the corrosion of mild steel due to the presence of SRB biofilms. The two biocides used were glutaraldehyde (GA) and isothiozolene (ITZ) and these were tested on two different marine isolates of SRB recovered from corrosion failures in Indonesia and the UK. The assessment of the biocide efficacy against the different SRB isolates was carried out using the dual cell, a two compartment electrochemical cell separated by an ion exchange membrane. This arrangement allowed one compartment to be kept sterile and the other to be inoculated with the SRB. The galvanic current that flows between the two sides was then monitored in the presence and in the absence of biocides using a Zero Resistance Ammeter (ZRA). Since in the presence of SRB pitting corrosion rather than general corrosion is thought to occur, the effects of the biocide on the pitting potential of the mild steel were also evaluated.

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Determination of minimum effective release rates of antifoulants

A membrane perfusion device for determining minimum effective release rates (MERR) of antifouling (AF) agents is described. The technique is applied to known biocidal agents and to natural antifouling agents. This method allows controlled and reproducible release rates to be achieved independent of water chemistry and flow near the immersed surface. Unlike constant concentration methods, the device acts similar to a painted surface, allowing establishment of diffusion gradients thereby allowing fouling organisms to exhibit natural chemotactic responses to the released toxin. The method was applied at four remote sites to natural populations of micro and macro foulers. The MERRs of known (TBTCl and cupric ion) and experimental AF agents (natural AF agent zosteric acid) were determined. The effective rates of copper and tin were consistent with panel data and field observation on ship hulls. The effective rate of the natural AF agent zosteric acid was determined at two test sites. The agent showed some activity against natural populations of hard foulers at relatively high fluxes. Such data provides coatings formulators with valuable information relating to the suitability of an agent for AF coating use and, when suitable, provides a target release rate for the formulator. The MERR device is a practical and versatile alternative for screening AF agents.

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Bioassay tests for antifouling coatings/toxins: a review

There is continuing demand for the development and application of new marine antifouling coatings and additives which will be effective against algae and slime. One important step in the screening process is an evaluation of the antifouling effectiveness of the potential compounds against suitable target organisms and, to this end, a number of laboratories have developed a range of *in vitro* test procedures. The present paper briefly reviews and assesses the range of toxicological test procedures and the micro- and macroalgae which have been used. Some consideration is also given to the use of marine macroalgae in the evaluation of novel, non-stick antifouling coatings.

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Biofouling control: the Australian experience

In Australia, as in other parts of the world, the contamination of inshore marine waters by the antifouling biocide tributyltin (TBT) has been widespread. In addition to the chemical detection of TBT in waters and sediment, TBT-related growth abnormalities have been observed in intertidal oysters in the major oyster growing areas of New South Wales and Tasmania, and scallop populations in Victoria are thought to have been affected by TBT in the surface microlayer. Imposex was also found to occur in gastropods in the four southern mainland states.

From 1988 onward, the various Australian States have progressively introduced measures to restrict the release of TBT into the sea. The form of the legislation in most States has been to ban the application of TBT-containing antifouling paints to vessels less than 25 m in length, with no exemptions. For larger vessels, only paints with a TBT release rate of less than 5 $\mu g/cm^2/day$ can be applied. Measures to minimise TBT release from slipways and vessel repair facilities have also been introduced. Such restrictions appear to have proven effective, as commercial shellfish populations no longer exhibit TBT-related problems. However, the use of TBT-containing antifouling paints remains under scrutiny.

Within Defence, a program is underway to assess levels of antifouling biocides in the waters around Australian Navy facilities. Surveys of TBT and copper levels in harbour waters and sediments have been undertaken at naval bases in Western Australia, north Queensland and the Northern Territory. Also, a long term monitoring program, using the marine mussel *Mytilus galloprovincialis*, was initiated at the fleet base in Western Australia in 1991.

Comprehensive studies are also in progress to evaluate the performance of tin-free antifouling coatings as alternatives to tin-based products for large vessels. Candidate coatings are initially evaluated by exposing test panels on a rotor apparatus and on rafts in temperate and tropical Australia. Selected materials are then applied as either patches or full scale applications on vessel hulls. These studies have demonstrated that tin-free coatings do not yet match the performance of the TBT-copolymer antifoulings, particularly in tropical harbour waters. Studies are now in progress in an attempt to determine the failure mechanism of the coatings in this environment. Non-toxic, low surface energy coatings are also being evaluated with promising results.

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Chemical ecology of antifouling in the marine algae *Delisea pulchra* and *Ecklonia radiata*

An appropriate starting point for the search for novel antifouling compounds from natural sources is an understanding of the role the metabolites play in the fouling ecology of the producing organisms. Two co-occurring species of macroalgae near Sydney, Australia both produce secondary metabolites which have been implicated as antifouling agents. One, the brown alga (kelp) *Ecklonia radiata,* produces large amounts of phlorotannins in the water column near kelp beds were 3-4 orders of magnitude lower than those which deterred settlement and development of the common epiphhyte *Ulva lactuca*. Moreover, variation in phlorotannins among different parts of the plants did not correlate with variation in epiphyte load - which was more easily explained by differences in the age, or position in the water column, of different parts of the thallus. In contrast to *Ecklonia*, spatial and temporal variation in epiphytes was strongly correlated to variation in levels of halogenated furanones in the red concentrations which inhibit *Ulva* in bioassays. To understand the ecological roles of natural antifouling agents produced by marine organisms, we must have a) realistic measurements of the concentrations of compounds that epibiota experience in the field, b) tests of these concentrations against ecologically relevant organisms, and c) an understanding of alternative factors affecting fouling biota.

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Natural antifoulants from marine algae

One of the most promising new solutions to the problem of marine biofouling is the use of marine natural products as antifouling agents. Of the many groups of organisms that live in the oceans, marine algae are perhaps the most susceptible to fouling. Many algae have no obvious mechanical or physical defence against epiphytes, yet remain largely epiphyte free. This strongly suggests chemical mechanisms for deterrence of epiphytes. Using ecological and chemical studies we have identified chemical deterrents against epiphytes from a number of Australasian algae. The most effective of these have been isolated from the red algae *Delisea pulchra* and *Laurencia rigida*. Each of these algae produces a series of chemically related secondary metabolites which strongly inhibit growth or settlement of representatives of the four major groups of fouling organisms bacteria (strain SW8), microalgae (*Ulva lactuca*), barnacles (*Balanus amphitrite*) and diatoms (*Amphora* sp.). The overall activity of these metabolites is comparable to that of heavy metals and biocides currently used in antifouling paints. However, these groups of related metabolites have very different modes of action. *Delisea* metabolites do not act as broad spectrum toxins. Rather, they have specific activities against one of more organisms depending on their structure and effectively deter the settlement and growth of some fouling organisms without killing them. The activity and mode of action of these natural antifouling compounds is compared with those of *Laurencia rigida* and biocides, and their potential as novel antifoulants discussed.

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A computer-based image analysis system for biocide screening

Biological contamination of man-made surfaces in aqueous milieu is inevitable. All we are able to do at the moment is to slow fouling or amelioraate its effects. Recently we have lost one of the major weapons in our armory since in many parts of the world trialkyl tin compounds are no longer considered to be environmentally acceptable components of antifouling coatings. Thus there is need to find new antifouling strategies which must be environmentally-friendly. There are several means to approach this. The first of these depends on reducing the physicochemical interaction of the cellular adhesive with the substratum [so-called fouling release coatings]; the second requires a slow release of a benign toxicant. This latter, seemingly oxymoronic, goal could be accomplished if the signalling mechanism that informs the organism that is in close proximity to a surface could be specifically confused. A molecule that could achieve this end may well be highly specific for eukaryotic fouling organisms. However, so far as has been published, means to screen for such compounds are not routinely employed in industry.

Classical surface antifouling assessment techniques are slow , labor intensive and often depend on the death of the organism as an end-point. They are also difficult to interpret unless the result is dramatic. It seemed to us that methodology that depended on behavioral changes rather than death of the oraganism might be a reasonable approach to screen for toxic compounds and surface treatments that promote fouling release.

The organism we have chosen as the model is the fouling diatom *Amphora coffeaeformis*. Its choice has relevance beyond diatom fouling. Diatoms are eukaryotes with most of the attributes of other organisms of the same type. For instance, diatom adhesion like that in other eukaryotes reqires metabolic energy, protein synthesis, glycoprotein synthesis, the presence of sufficient calcium and cytoskeletal activity. Cells of *Amphora* were attached to a microscope slide cover glass at a defined areal concentration. A diffusion chamber designed to expose cells to uniform concentrations or spatial gradients of test substances was assembled using the cover glass with attached cells. Dark field phase contrast microscope images were video-taped. After digitization of individual microcsope fields, images were analysed. The following parameters were measured: number of cells, number of cells moving greater than a predetermined speed, the paths of these cells, speed of moving cells together with their angular velocity, acceleration and compass bearing. The analyses show that motility is changed by chemotactic effectors. We have shown that the experimental system is capable of detecting small behavioral changes such as speed, and should therefore be of use as a rapid screen for compounds affecting behavior or treatments that change the hydrophobicity of the substratum.

SESSION VI

PARALLEL A

Environmental consequences of biocide use

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Leaching rate: a fundamental requirement for the regulation of antifouling paints

The current regulations concerning Antifouling paints in each region worldwide are shown. The importance of Leaching Rate to the regulatory process is discussed, and the methods for Leaching Rate measurements of Tributyltin (ASTM D5108-90) and copper (a modified version of D5108-90) are outlined. Examples are given of typical leaching rates from standard antifouling paints using these methods, which demonstrate their reproducibility and validity as indicators of environmental input. The effects on Leaching Rate of various external factors (pH, salinity, temperature, turbulence) are discussed.

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Triorganotins and the environment: the current UK position

Triorganotin compounds (TOT's) have been used in antifouling products since the 1960's. TOT's are compounds that are characterised by three tin carbon coavelent bonds present in the tetravalent state. Used in antifouling products they are extremely efficient at preventing freshwater and marine organisms fouling ship hulls. The use of TOT's substantially reduces the need for large vessels to visit dry docks for re-treatment, they also enhance the efficacy of ships in terms of fuel and speed and their service life is much longer than other available antifouling agents.

However, TOT's are known to induce physiological stress malformations and toxic effects in a wide range of marine and freshwater organisms at concentrations as low as 0.001 mgl-1. In particular they cause shell thickening in oysters and imposex in neogastropods. In the past this has resulted in loss of viability in sections of the shellfish industry and localised extinctions in dogwhelk populations. TOT's are lipophillic and are known to heavily accumulate in the tissue of molluscs. They have a low aqueous solubility and adsorb strongly to sediment. The half life in sediment can be measured in years in contaminated sites and decades where contamination is high. Because of the catastrophic effects on parts of the shellfish industry the UK and other EC states restricted the use of TOT's used in antifouling paints in the mid 1980's to vessels over 25m in overall length. Further measures were taken in 1991 to control remaining uses.

In general since the restrictions on the use of triorganotin antifouling paints to large vessels only (> 25m overall length) in 1987, the levels of TOT in the environment have declined.However, in key areas such as around dry docks, levels are still sufficiently high to prevent recolonisation of the dogwhelk, and possibly acute and chronic effects on several other aquatic organisms. These levels are certainly considerably higher than the EQS's set by the DOE. This situation is environmentally unacceptable.

This paper explores the ecological implications of the UK and international regulatory activity in considering further revokation against the considerable other benefits of these compounds.

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Monitoring populations of dogwhelks for imposex

There have been conflicting claims about the extent to which populations of dogwhelks *Nucella lapillus* (L.) have recovered from imposex. Since the introduction of regulations restricting the use of TBT-based antifoulants to vessels more than 25m in length. This paper will consider the results of monitoring programmes in the Clyde Sea, Irish Sea and North Sea. Although dogwhelks have become locally extinct in some areas of high boating activity, most surviving populations have shown significant recovery. Centres of commercial boating activity remain hot-spots of TBT-contamination but their impact is surprisingly local. Difficulties in using imposex as a bio-indicator of TBT pollution will be considered. These will include in particular : (i) non-specificity of imposex to organotoxins; (ii) the validity of using imposex scores to assess reproductive performance; and (iii) biased sampling programmes.

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Worldwide monitoring of TBT concentrations in seawater

Two major monitoring programmes were launched by organotin producers in the early 1990's to study the occurrence of tributyltin (TBT) compounds in the aquatic environment. The purpose was to monitor the effectiveness of control measures taken by European and U.S. governments and to provide information on the distribution of TBT related to ocean going vessels.

Within the European programme the distribution and trends of TBT compounds in coastal waters were studied from 1990 to 1993. Water samples were taken quarterly in three regions of the Southern North Sea and British coast (Milford Haven, Bremerhaven, Rotterdam) and one in the Mediterranean (Genova). Furthermore, a long-term monitoring programme has been conducted since 1992 in the USA by a Consortium of Tributyltin Manufacturers as required by the U.S. Environmental Protection Agency. It includes sampling in the regions Pacific Northwest, Gulf of Mexico, Atlantic Northwest and Great Lakes. Water, tissue and sediment samples have been analyzed.

The analytical method used was pentyl-derivatization/gas chromatography with flame photometric and mass spectroscopic detection, resp. The data will be discussed in the context of results from further monitoring programmes. Interpretation of data for environmental samples is often hampered since different approaches to determine analytical characteristics are being used as well as by background contamination problems.

Generally TBT concentrations found in water and tissue samples are relatively low in areas affected by traffic of larger vessels, but with little or no ship-building activities. Most coastal and offshore water samples show either no detectable TBT or concentrations below 10 ng/dm^3 TBT. The results of the monitoring programmes demonstrate that the control measures taken in Europe and the USA on the use of TBT paints for small boats were successful. Additionally, the progressive withdrawal of TBTO free association antifoulants and the consequent introduction of the self polishing TBT-copolymer technique lead to a strict control of constant but very low TBT-release from the hull of ships. On the other hand, dry-docks still can be a major source of TBT. Continuing improvements of paint application and removal procedures should lead to a further reduction of TBT in the aquatic environment.

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Risk evaluation of current levels of TBT in seawater in the context of recent ecotoxicity data

Tributyltin (TBT) compounds are widely used as antifouling agents in primarily for ocean-going vessels. This use results in elevated concentrations of TBT in areas affected by shipping activities.

Recent results of monitoring programmes are compared to results of long-term ecotoxicological studies, which were performed with marine organisms, including those known to be very sensitive to TBT contamination.

In a 2-year study over two generations the marine dog-whelk snail was exposed to TBT in concentrations of 2 to 128 ng/l. Abnormal development of male sexual organs in females (imposex) was oberved in the first generation in all treatment groups. In the second generation also the animals in the dilution water control (including ethanol as solvent) developed imposex. Reproduction was significantly affected at concentrations of 32 ng/l. The NOEC for parameters relevant for the sustainability of dog whelk populations was 8 ng/l as TBT.

Furthermore, the results of chronic toxicity studies in oysters are reviewed. The results of these studies we discussed regarding their relevance and a risk assessment for TBT is proposed.

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The proposed EC biocides directive

Antifouling products are included within the scope of the authorisation scheme proposed by the European Commission for Biocidal Products placed on the European market. This proposal is still under discussion between E.U. Member States. This paper will outline the main features of the proposal at the current stage of discussions, highlight parallels and differences with the current U.K. system and describe the risk assessment approach currently being pursued.

SESSION VII

PARALLEL B

Corrosion offered papers

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Water corrosion of mild steel promoted by sulfate reducing bacteria

The initial stages of corrosion layer forming on mild steel coupons exposed in different places of the cooling water system were investigated by means of Mossbauer spectroscopy, X-ray diffraction and thermomagnetic analysis. Microbiological analysis of the moist samples was performed also.

Our study has shown the presence of different kinds of compounds on the surface of the samples. Only $CaCO_3$ is in crystalline form. The other compounds are in fine dispersed form: α-FeOOH (with particle sizes 50-70 A), a-Fe_2O_3 (50 A). Pyrite (FeS_2) and monoclinic pyrrhotite (Fe_7S_8) are in X-ray amorphous form. It is evident that the presence of the latter two compounds in the corrosion scale may be explained by sulphate reducing bacterial activity only.

We analyzed the influence of temperature and pH of water medium on the activity of biofilms, created on the surface of mild steel coupons.

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Application of electrochemical noise analysis to the study of microbiologically influenced corrosion

Experiments were undertaken to determine whether electrochemical noise analysis could be used to follow the development of an SRB-containing microbial biofilm on the surface of mild steel test coupons.

In initial experiments, the test coupons were housed in a bench-top glass corrosion cell containing artificial sea water. Noise signals were recorded for non-biofouled coupons in an oxygen-free environment, in one where the water was amended with 50 ppb oxygen and another where the sea water was sparged with air. These data were compared to noise signals recorded on biofouled coupons exposed to the same gasing regime.

Clear differences were observed between some of the conditions and a further set of experiments were performed in bench-top biofouling housings, where the changes in noise signals were observed as biofilm developed on the surfaces of the electrodes. The data arising from these studies will be presented with a discussion of the potential of electrochemical noise analysis for monitoring the development of microbial biofilm and associated corrosion.

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Evaluation of sacrificial aluminium anodes

This paper describes the experience gained by the development and testing of Al-Zn-In alloys as sacrificial anodes. Various compositions were tested. Experimental data were collected by the variation of Zn and In content. The iron content in most of the experimental series was kept constant to 0.1 %. The results of various testing techniques are discussed. The main conclusions can be summarised under the following topics:

- Electrochemical tests

Capacity was determined by using two methods: impressed current technique and short circuit couple technique (free running). Close-circuit potential measurements were also performed during both types of electrochemical testing. The impressed current technique seems to be unable to distinguish quantitatively the effect of Zn and In addition, whereas the short-circuit technique exhibits much better results. The difference was attributed to the passivation which is not taken into account by using the impressed current technique. Indium addition beneficially affects the stability of close-circuit potential.

- Morphology of anode's dissolution

The morphology of the anode surfaces after having been used for the performance of both types of electrochemical testing, was studied. It was pointed out that increasing of In content does not always result in more uniform dissolution. However, low reproducibility was the main characteristic of this type of test. This fact leads to the conclusion that other parameters, except of chemical composition such as current density, agitation, initial surface condition, can substantially affect the morphology of anode's dissolution.

- Other tests

Other tests such as bond test (material/insert), visual examination for cracks and shrinkage cavities, were performed in semi-industrial scale. A correlation of these parameters and actual anode performance was attempted. However, more study and much more experimental data are needed in order to have quantitative results.

The whole study points once again to the need for well defined quality control specifications of aluminium sacrificial anodes. It is also pointed out how difficult it is to predict reliably the behaviour of the anodes by using short time laboratory tests. Many parameters should be taken into account and the quantitative limitations of each one are extremely difficult to determine. This clearly demonstrates that until now testing of anodes in actual environmental conditions remains a necessity.

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Concentric electrodes in microbial corrosion studies of 304 stainless steel

A system has been developed in which reproducible microbial influenced pitting of 304 stainless steel can be initiated. Application of a current of 11 μA cm^2 to concentric electrodes, generated an anode surrounded by a cathode. A galvanic current was maintained after release of the impressed current in the presence of a mixed community containing a *Vibrio* sp. and the sulphate-reducing bacteria (SRB), *Desulfovibrio vulgaris*. *Desulfovibrio desulfuricans* as the SRB in the biofilm did not maintain a strong galvanic current possibly related to the poor colonization by the bacteria. The cathodic and anodic process were different allowing some interesting observations to be made on the effects of the bacteria on the various processes. The anode showed a low charge transfer resistance (Rct) (<3 KΩ cm^2) only in the presence of a mixed biofilm. The cathode gave a high Rct (>100 KΩ cm^2) in the presence of the SRB whether in an axenic or mixed culture. Biofilms of *Shewanella putrefaciens,* chosen for its ability to utilize nitrate, thiosulphate, and iron as terminal electron acceptors in the absence of oxygen, was also examined as it cannot utilize sulphate, but generates sulphides from thiosulphate. Metabolic sulphide production was observed with large sulphide containing pits rapidly formed on both the anodes and cathodes in the presence of thiosulphate. When the level of thiosulphate was decreased to trace amounts, with nitrate present as the terminal electron acceptor, no such pits were detected but a galvanic current was maintained. In the absence of thiosulphate and nitrate only very low galvanic currents were maintained which may be due to the oxidation of the iron by the bacteria.

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Improved sacrificial anode for the protection of off-shore structures - Note 2

A new type of sacrificial anode was studied to be used for the cathodic protection of steel structures immersed in sea water, such as the steel jackets of off-shore platforms. The protection current which is needed to assure the immunity strongly depends on the marine parameters but mostly on the surface state of the off-shore structure to be protected, i.e. on the features of the calcareous coating which has to act as a dielectric coating in reducing the current requirements for the maintenance of protection. It follows therefore that even in the initial service life of the structure, when the deposits are not yet developed to a protecting stage, the need is evident for high protection current values, which are likely to become the lower, the better is the quality of the precipitated layers in the initial phase. The anode we will describe offers high current density in the early stage and assures protection to the structure during all service life.

This new anode has a composite structure in which the aluminium alloy forms the heart and the magnesium alloy is the external skin with a thickness of 1,5 cm.

After the microstructural investigation and preliminary electrochemical measures we monitored the new anodes during service life in North Adriatic Sea on two AGIP platforms. We measured both electrochemical potential and current output and also studied the calcareous deposits on the structures with E.D.X.S. microprobe, scanning electron microscope and X-ray diffraction.

We made parallel laboratory tests on a small scale suitable to simulate the actual service conditions. From the experimental results it was possible to conclude that the calcareous deposits developed on the cathodic areas in the presence of the composite anode were compact and protective; their structure was in form of uniform and well distributed aragonite crystals, instead of the usual calcite crystals found with the traditional aluminium anodes.

From both laboratory and field results one can predict good savings in weight, volume and costs of the cathodic protection of off-shore structures.

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Deactivation treatment of water deteriorated by *Thiobacilli thiooxydans* and *ferrooxydans* with *Desulfovibrio desulfuricans*

Thiobacilli produce sulfuric acid and $Fe_2(SO_4)_3$ in aerobic conditions. *Thiobacilli thiooxydans* is able to produce so much H_2SO_4 that pH of e.g. coal mining water (CMW) changes from 7 to 2 or to 1.0. Neutralization of CMW is attended with the great difficulties (great quantity of neutralizer, ionic strength increasing etc.). The biological research technique of CMW deacidification was performed in laboratory and checked on the effectiveness in coal mines of Kizel. Our investigation revealed that *Desulfovibrrio desulfuricans* is suitable for deacidification. The most efficient technology has been found. The cost of biological technique of deacidification was evaluated to be 0.74 of the currently used technique with neutralizing agent CaO. *Desulfovibrio desulfuricans* acts effective in CMW with pH not more 2.8 and temperature not less then 279K. IntroductionAbout 5x106 m^3 coal mining water (CMW) was pumped in sump water, and about 10 per cent of CMW of Kizel coal basin is for 99.8 per cent and acid and can have Fe^{2+} + Fe^{3+} 300...1500 mg/l, SO_4^{2-} 600...4000 mg/l. CMW of old underground working (UW) can have Fe^{2+} + Fe^{3+} to 3000 mg/ l and SO_4^{2-} to 12000 mg/ l. And therefore Kizel coal basin is a convenient natural pattern for experimental study of biological treatment of CMW. In our research programme the developing SRB culture was studied in sour water and biological deacidification method was tested in laboratory and in UW. We have put more emphasis on factors that determine the deacidification process rate and investigated the influence of crude sewage, sawdust and others.

SRB were separated from sour CMW by repeated subculturing in Tauson medium including microquantities of Fe^{2+}, Zn^{2+}, Mn^{2+}, Co^{2+}, Cu^{2+}, Ni^{2+}. .Stockculture of SRB was being cultured in that medium at a temperature between 291 and 293 K. By pumping of CMW at 288 K after addition 0.1 volume per cent of stockculture (maintained 10 9 SRB cells in 1 ml) and 3 vol. per cent of crude sewage the deacidity process gaes 24 hours and pH is rased from 2.8 to 3.8, Eh goes down from +400 to +73 mV, Fe3+ is reduced completely, Fe^{2+} is reduced from 165 to 11 mg/ l and SO_4^{2-} from 2994 to 490 mg/ l. The deacidification at T=279 K is less effective: pH is raised from 2.9 to 3.2, SO_4^{2-} quantity is reduced in 57 per cent, Fe^{3+} in 58 per cent, Fe^{2+} in 57 per cent.

Semi-commercial biotank experiments were carried at 279 and 288 K in CMW of Kisel UW. Throughout the 72 hours the biomass increases from 1.0 to 6.5 g/l (dry weight). Cleaning grade for Fe^{2+} in the upper chamber of biotank throughout 24 hours was 60%, in the second chamber-70%, for Fe^{3+} 70% and 79%, for SO_4^{2-} 42% and 45%, respectively. Reduction occurred very effectively, and after 72 hours biotank was converted to flow run condition.

Using laboratory equipment and semi-commercial biotank with SRB, the CMW conditions have been optimised. The above results clearly show that the deacidification process rate is sufficient at temperatures from 279 to 288 K if the crude sewage together with sawdust addition is made. Deacidifed water has less values of the Fe^{2+} and SO_4^{2-} iones content and zero Fe^{3+} content, hydrogen sulphide is absent.

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Quantum chemical study of nitrogen and sulphur containing substances as inhibitors of corrosion and hydrogenation of steel

Different organic substances are used as steel corrosion inhibitors in acid and water-salt media, but conventionally their choice was only empirical. We have already made some attempts of using quantum chemical approach to a number of molecules belonging to several groups of N-containing organic compounds.

The experiments were performed with either artificial sea water or a solution of inorganic salts in water (standardised microbiological *Postgate B* medium). Within first 48 hours after inoculation the media with accumulation SRB culture the considerable growth of number of cells has been obtained. 72 hours after inoculation, the inhibitors at concentrations 0.1...10 mMol/l were added, which resulted in decrease of intact moving bacterial cells number. The quantity of hydrogen absorbed by the steel subsurface layers by corrosion V_H was less in the cases of medium with added organic substances than in the reference series of experiments. The greater corrosion inhibitor concentration, the more was suppressing of hydrogen absorption by the steel. The loss of steel plasticity measured on wire specimens was significantly decreased by adding some organic substances with inhibiting efficiency to the corrosion medium.

The best inhibitor of steel hydrogenation during corrosion in water-salt media in the presence of SRB proved to be that with R: p-Br C_6H_4, and this was also the best inhibitor of corrosion and the best biocide. The greater inhibiting efficiency of the addition of derivative with R= p-ClC_6H_4 in comparison with the one with R= p-$CH_3C_6H_4$ is in agreement with previous results in the study of hydrogenation of carbon steel under cathode polarisation in solutions of sulphuric acid.

It is clear that both biocyde and corrosion suppressing activity of organic N- and S-containing compounds are based on adsorption of their molecules on solid surface but in the first place the nature and composition of these ones is inequivalent, and, in the second place, the biological activity must be the function of ability of organic molecules to penetrate the cell membranes. For example, the industrial detergent OP10 with high surface activity is a very effective biocyde against sulphate reducing bacteria but least effective steel corrosion inhibitor.

Correlation analysis of our quantum chemical results together with our experimental data make evidence that different active centres of inhibiting molecules are responsible for the protective action against hydrogenation and sour corrosion depending also on the presence of stamp of sulphur reducing bacteria.

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Non-toxic, easy fouling release ship hull coatings

The overall goal of this project is to develop non-polluting, easy fouling release, ship hull coatings based on flexible, low-surface-energy polymers. Ship hull protection from marine fouling organisms is essential for efficient fleet operation and energy conservation. Presently, the Navy standard antifouling (AF) coating contains copper compounds as a toxicant, which may create an environmental hazard and potential for pollution. Low surface free energy coatings work by a physical property of the solid surface rather than toxicity of released antifouling agent. Since nothing is released into the environment, there is no risk of environmental pollution.

Such coating have a significant potential for reducing costs associated with dry-dock and in-water hull cleaning operations. They could also result in less degradation of operational performance due to fouling drag between scheduled hull cleanings.

Our approach takes advantage of the weak adhesion characteristics of materials that have low surface free energy. All marine fouling organisms use some sort of adhesive for attachment to solid surfaces. However, the lower the surface energy of the solid, the weaker is the adhesive bond. We have bound perfluorinated compounds into a polymeric backbone to create a comb type polymer with perfluorinated alkyl sidechains. Our polymer formulations include acrylate based copolymers polymerized from varying ratios of acrylate/methacrylate monomers. Critical technical issues which will be addressed include the surface orientation of the molecules and fluorinated side-chains and the degree of polymer flexibility which can be achieved without sacrificing toughness/durability. Emphasis will be placed on optimizing the polymerization process; minimizing variability in preparing and testing samples; and correlating surface and bulk characterization data with the coatings actual fouling-release performance. These correlation studies will allow the design of new polymer formulations with maximum fouling-release properties.

SESSION VIII

PARALLEL A

Surface techniques in corrosion

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Synchrotron radiation based X-ray absorption studies of corrosion and passivation

It is of course well recognised that thin surface films play a pivotal role in both corrosion and passivation. However, the typical nature of these films, e.g. their thinness and frequent lack of long range order, precludes their investigation by conventional structural techniques. Whilst considerable progress has undoubtedly been made through the application of ex situ techniques, such as electron diffraction and X-ray photoelectron spectroscopy, this approach necessarily requires the removal of the sample from its environment to ultra high vacuum. This transfer, with its consequent loss of potential control and removal of the aqueous part of the interface, may lead to structural changes and therefore it is always uncertain whether conclusions drawn from this type of approach truly relate to the film that was present in situ.

It is clear that a need exists for more probes capable of providing structural information relating to these thin surface films, and particularly probes that can operate in situ. With the ever increasing availability of dedicated synchrotron radiation sources attention has turned to the development and application of techniques that utilise these sources of intense tunable radiation, particularly in the X-ray part of the spectrum. One topic that has attracted considerable attention is the determination of interfacial and surface structure and electrochemists have been particularly active in this area, using both X-ray absorption spectroscopy (XAS) and X-ray scattering. For the purpose of probing the structure of surface films relevant to corrosion science XAS undoubtedly has the most to offer and it is the application of this approach that will be discussed here.

The X-ray absorption spectrum of a monatomic gaseous sample consists of sharp absorption edges followed by regions exhibiting monotonic decay of the absorbance. By contrast the spectrum of a solid or liquid exhibits much more structure. Structure in the region of the absorption edge, known as X-ray absorption near edge structure (XANES), arises from bound state transitions and multiple scattering of the photoelectron produced in the absorption process. It can provide information about the local co-ordination about the absorbing atom and about its chemical state. Some examples of how XANES can be used to study corrosion and passivation will be presented with particular emphasis on how this approach may be used to study changes in real time.

The structure above the absorption edge, extended X-ray absorption fine structure (EXAFS), arises from scattering of the photoelectron by nearest neighbouring atoms around the absorbing atom. From an analysis of the EXAFS information about bond lengths, co-ordination number and neighbouring atom types can be determined. Some examples of how an analysis of in situ EXAFS spectra may be used to elucidate the structure of passive films will be described.

The study of corrosion and passivation using XAS requires careful consideration of the experimental techniques to be used and of the electrochemical cell design. The approaches that make possible this type of investigation will be described.

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Atomically resolved STM of passive oxide films on Fe and Fe-Cr

Passivating oxide films are typically 2-3 nm thick and form at oxidizing potentials in aqueous solutions. Alloying elements, such as Cr in Fe, can modify the composition and structure of such films, leading to improved corrosion resistance. Various techniques including X-ray absorption spectroscopy have revealed controversial evidence for disordered atomic arrangements. The use of *in situ* STM has the potential to resolve this structural controversy, at least to the extent that the outer film surface is representative of the film structure as a whole. In the present work the surface structures of passive films on iron and iron-chromium alloys have been revealed by *in situ* electrochemical STM study [1-3].

The passive film formed on sputtered microcrystalline iron in borate buffer solution, pH 8.4, was studied shown to have a distorted triangular lattice with the angles between lattice rows varying from ca. 55 to 65°. The spacing between the corrugations was 3.0 ± 0.1 Å. In some areas this lattice was absent and an ordered ring structure consistent with the Fe^{3+} sites in γ-Fe_2O_3 was observed at a lower level. The outer part of the film is probably γ-Fe_2O_3. It is notable that the close-packed oxygen planes were approximately parallel to the surface, albeit somewhat distorted, on a range of low-index planes that are present on the sputtered iron surface. Extremely ordered surfaces were observed on specimens examined *ex situ* in air.

At low Cr contents such as 13%, Fe-Cr alloys showed very similar surface structures to that found on iron, but with a slightly larger spacing between the corrugations (3.2 Å). This film is probably crystalline Cr_2O_3. At higher Cr contents the film was disordered in accordance with a percolation or gelation model of its formation. Studies of these films carried out in air showed time-dependent crystallization, but this has not yet been observed in solution.

The present results support rather simple models of the passivation process and the role of the passive film in corrosion resistance. Disorder is observed at high Cr contents in Fe-Cr alloys, but since pure Fe and pure Cr both show crystalline film surfaces, the disorder seems to result from the particular film formation mechanism in an alloy experiencing selective dissolution of one element (Fe). In other words, disorder in the film results from disorder in the atomic arrangement in the metal and has no causal relation with corrosion resistance.

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The application of impedance spectroscopy for corroding systems

Impedance spectroscopy has been extensively used in recent years for corrosion studies, but there are still many systems where the usefulness of the technique is limited by interpretation of the data obtained. This paper considers examples of corroding metal systems, especially those with corrosion product or other surface films. It attempts to describe the types of information, both mechanistic and about corrosion rate, that are available from impedance spectroscopy, and the approaches by which this information can be obtained from the experimental data. The limitations of the method are also discussed and briefly compared to other electrochemical methods.

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Fractal models for corroding surfaces

'Fractal' is a term coined by Benoit Mandelbrot in the 1970s to describe complex patterns, shapes, curves or functions. Fractals have been shown to be particularly relevant to natural phenomena, whilst possessing their own seemingly magical qualities and beauty. During the twenty years since their first description, fractals have begun to permeate many corners of art, science and engineering. Localised attack is a well recognized and dangerous form of corrosion which has long resisted methods to monitor it and thence to make lifetime predictions. As a long term strategy, we have adopted the goal of developing improved techniques for the monitoring and quantitative measurement of localized corrosion and other surface degradation. A pivotal part of this approach is the correlation between the kinds of traces obtained from surface profile with time series analyses. Besides using well-established mathematical functions such as spectral density plots with fast Fourier transforms (FFT) or other algorithms such as the Maximum Entropy Method (MEM), we have adopted more modern techniques such as the Stochastic Pattern Detector technique or Rescale Range Analyses (R/S). Because we have found that fractal dimension of surfaces changes with the nature of the surface, and, indeed, may therefore change across a single surface because of the different mechanisms which may have been at work, it has been necessary to develop scanning analytical techniques which analyse data in segments and create maps of data from a given set. Thus, we have shown that corroding surfaces of pure copper can be modelled by fractal geometry and have determined the changes of fractal dimension which accompany the pitting of aluminium alloy. We have shown the inadequacies in Richardson plots for fractal dimension evaluation in this area and as a result have developed a unique scanning method for determining the fractal dimension of different parts of the time series and for using this to identify geometrical features of the localized corrosion. Finally, we have shown how the initiation of crevice corrosion of Type 304 stainless steel, as measured by scanning electrochemical probes correlates with a fractal indicator applied to the electrochemical noise spectrum.

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On the use of the scanning electrochemical microscope (SECM) to the study of localised corrosion

The scanning electrochemical microscope, (SECM), belongs to the family of scanning probe microscopies. It consists of a microelectrode, known as the tip, held precisely a few micrometers above a sample surface, known as the substrate, in an electrolytic medium. The principle of any SECM experiment is to carefully control the electrochemistry of the tip and monitor the tip response while scanning the tip above the substrate or while perturbing the substrate chemically (e.g. by modifying the pH of the solution) or electrochemically (e.g. by controlling the potential of the substrate). In the most common mode of operation, the feedback mode, a redox mediator is used by the tip to probe the electrochemical activity of the surface of the sample. Because the tip has micrometer dimensions, the technique achieves a very high spatial resolution, typically on the order of a few micrometers. It is therefore able to monitor highly localised electrochemical processes and it has been used to probe surface reactivity, in particular to image the distribution of active sites. Unlike other imaging techniques, e.g. the scanning reference electrode technique, the SECM probe is active and can be used to locally perturb the sample surface. In addition to qualitative two dimensional information, the SECM provides the means to obtain quantitative information about localised surface activity, e.g. local rate of electron transfer and rate of surface reactions. The presentation will review some of the fundamental aspects and discuss advantages and difficulties of the technique in relation to the study of localised corrosion. Experimental results will be shown to illustrate various modes of operation, approach curves, imaging and tip substrate voltammetry.

SESSION IX

PARALLEL B

Macrofouling: new developments

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The structure and antifouling mechanisms of the dogfish eggcase

The dogfish, *Scyliorhinus canicula*, unlike most fish produces few eggs. However these eggs are large and the embryos are well developed when they hatch. The development time for the embryo can be up to a year, during which time protection against fouling is required. This is provided by the eggcase, a sophisticated collagenous material with great strength and excellent antifouling ability. This paper will describe the structure of the eggcase and the possible mechanisms, such as rugosity, charge, hydrodynamics and chemistry, by which the eggcase achieves its antifouling capacity.

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Epiphytism and fouling in seaweed cultivation: an overview

Epiphytism and fouling are major, world-wide problems in seaweed cultivation, severely reducing the productivity and cost efficiency of a diverse range of open-water and on-shore farms; the problem is especially acute in tank cultivation systems. The present paper provides an overview of the literature on epiphytism and fouling in the seaweed mariculture industry. Topics considered include the identity of the main epiphytes and fouling organisms, aspects of their ecology and biology which contribute towards their success as contaminants, the detrimental effects which epiphytes exert on the host plants, and the range of physical, chemical and biological control methods which are currently available. Recent changes in the literature, with less descriptive information and more emphasis on experimental studies of host/epiphyte interactions, are welcomed as these offer hope for the development of improved control measures.

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The macrofouling on offshore platforms at Ravenna*

The fouling settled on piles of gas platforms was studied using samples taken in 1993 on the PCWA and ANTARES platforms positioned, respectively, at 7 and 10.5 Km from the shore and on bottoms at 12 m and 14 m, and by means of photographs and video shots taken over the whole lenght of the chosen pile and also those nearby. For each platform a pile was chosen as representative of the macrofouling settled on the whole platform. In March and September 1993 samples were obtained by scraping an area of 600 cm^2 using three or four different exposures at the same depth. The levels considered were 0.5, 5.5 and 11 m on the PCWA and 0.5, 7 and 12 m on the ANTARES.

On the PCWA the macrofouling is characterized by a dominance of mussels up to a depth of around 9-10 m. In the midst of the mussels, or as their epibionts, one finds barnacles, other bivalves, serpulids, hydroids and sea-anemones. Non sessile fauna is represented by brittle stars, flat worms, ribbon worms, amphipods and decapods. Near the bottom mussels disappear and oysters dominate, in particular *Crassostrea gigas*, together with sea-anemones and zoanthid *Epizoanthus arenaceus* (Delle chiaje); the latter cover broad surfaces, at times even higer up, especially when the mussels are eliminated. Also present are: barnacles (*B. trigonus*), hydroids (*Obelia*), serpulids (*Pomatoceros, Hydroides, Serpula*), which settle both directly on the piles and on other organisms; bryozoans are extremely scarce.

The macrofouling on the ANTARES is very similar both quantitatively and qualitatively speaking. Up to approx. 10-11 m depth mussels dominate accompanied by most of the organisms described in reference to the PCWA. Near the bottom, and only more rarely higher up, one finds large colonies of bryozoan *Schizoporella errata* which contribute to differences in the fouling compared to that found on the PCWA. At this level there are also *Crassostrea gigas*, barnacles, hydroids, serpulids, but *Epizoanthus arenaceus* and sea-anemones are missing.

There are no large differences in the fouling or mussel weights between the two platforms and the two seasons, even though the highest values were registered in September on the PCWA (- 1 m) with 1558.6 g/dm^2, out of which 1553 g/dm^2 were due to mussels. One can draw the conclusion that over a period of 5-6 months it is possible to reach fouling weights of 155 kg/m^2, which thus exceed the 100 kg/m^2 found on the one-year panels during the experiment carried out in 1975-76. On the whole there are no substantial differences between the fouling found off Ravenna in 1975-76 and that found in 1993.

*Research supported by a contract between CEOM Palermo and Università di Genova

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Barnacle settlement revisited: recent progress with *Balanus amphitrite*

Traditionally, the boreoarctic barnacle, *Balanus balanoides (=Semibalanus balanoides)*, has been used in laboratory studies of cypris larval settlement. A perennial iteroparous breeder and requiring flowing water for settlement, this species does not lend itself to studies on the nature of chemical cues and the mechanisms for their perception. Within the past decade, the cypris larva of another species, the cosmopolitan barnacle, *Balanus amphitrite*, has gained prominence in such studies. *B. amphitrite* can readily be manipulated in the laboratory to breed throughout the year, and larval development to the cyprid is typically complete within five days under optimal conditions. In this presentation I will briefly review some of the bioassays that have been developed using this species and the progress that has been made with respect to the nature and perception of settlement cues. *B. amphitrite* is an important fouling organism and the settlement of its cyprids is emerging as an useful screen for potential antifoulants. The assays have largely been borrowed from fundamental studies of settlement and may not be optimal for their new role. The case for assay development and standardisation needs to be made.

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Marine fouling organisms on experimental wooden panels in tropical waters

Seventy three taxa of fouling organisms were recorded from experimental wooden panels at the subtidal and intertidal zones for a twelve month period. The diversity of the fouling organisms at the subtidal zone was twice that at the intertidal zone. The diversity of the fouling organisms was similar between the top and under surfaces of wooden panels at the intertidal zone, but there were differences in abundance for some species of foulers between the two surfaces. Sessile animals (Balanidae, Mytilidae, Ostreidae and Coelentrata) and the colony forming (colonial) organisms (hydroids, sponges and bryozoans) were the abundant fouling organisms at the study site. The former were dominant at the intertidal zone while the latter were dominant at the subtidal zone. Associated organisms (opportunistic species) like the brachyurans (Porcellanidae, Menippidae, Pilumnidae, Majidae, Ocypodidae and Grapsidae), brittle stars, polychaetes (Nereidae, Sabellidae, Polynoidae and Eunicidae), gastropods and flatworms were also recorded, but with varying abundance at the intertidal and subtidal zones. Vertical distribution of the fouling organisms was related to their tolerance to thermal stress (dessication and drying). Animals inhabiting the intertidal zone are those that are tolerant to dessication and exposure. At the subtidal zone space was limiting with wooden panels completely covered by both the sessile and colonial foulers. Morphological changes were observed among the Balanidae due to space limitation at the subtidal zone when compared to the intertidal zone. The succession of fouling organisms at the subtidal zone is also discussed.

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How do marine invertebrates keep their surfaces free from microfouling?

Clean surfaces placed in aquatic environments are quickly fouled by macro-molecules and bacteria, with algae colonising surfaces in shallow water. Many marine invertebrates rely on their surfaces for gas-exchange and sensation. Both of these functions are likely to be severely impaired by microfouling, resulting in strong selection pressure for marine invertebrates to develop effective antifouling defenses. As the ability to prevent unwanted organisms adhering to oneself is a fundamental requirement for living systems, such antifouling defenses are likely to have arisen early in evolution. While the original systems may have been embellished through further evolution, it is possible that a fundamental, archaic system of antifouling still exists and would be common to many organisms. Many soft-bodied marine invertebrates have a fuzzy coat of material that forms either the whole cuticle or its outermost part in more structured cuticles. This coat has been likened to the sugar-rich coats of eukaryotic cells, which are in turn reminiscent of the slime capsules found around many bacteria. We hypothesize that this fuzzy coat in invertebrates is a glycocalyx and its primary function is to mediate adhesive interactions between marine organisms and their surrounding environment. As this layer is the outermost part of these organisms, it would seem the logical site for an antifouling defence. Such a layer could prevent fouling in a number of ways that are not mutually exclusive and could be working in synergy. Firstly the coating could render the surface difficult to adhere to by merit of its physico-chemical characteristics such as hydrophilicity, charge or surface energy. Secondly the coat could be regularly shed, thus freeing the surface from adhering bacteria. Lastly, there could be a biocidal or bio-repellant capability intrinsic to the glycocalyx. We have been investigating the surface coats of echinoderms to elucidate the physical and chemical nature of the surface coats and determine their turnover rates. The cuticle in echinoderms consists of a outermost fibrillar coat consisting of long (>0.5μm) filaments, often in very regular array. This coating is difficult to preserve using conventional TEM fixatives. Below this are a further two layers that are probably structural. Our results confirm the glycoprotein-rich nature of echinoderm surfaces and provide information on the maximum turnover times for the surface coats.

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The effects of medium term exposure in sea-water trials on the surface properties of silicone elastomers

A series of one- and two-part, room temperature vulcanising, silicone elastomers and silicone elastomers with incorporated silicone oil has been tested for resistance to marine fouling and some surface properties of the materials have been examined.

Samples have been exposed for *ca* three years on rafts in Poole and Langstone Harbours. The samples have been monitored on a monthly basis over the spring and summer seasons: identification of species and estimation of the extent of fouling and resistance to removal have been carried out according to a standard protocol.

The exposure trials showed that one poly(dimethylsiloxane) and one poly(dimethyldiphenyl siloxane) had much greater resistance to the attachment of marine plants or animals, and were more easily cleaned. Addition of silicone oils achieved mixed results. Where blooming occurred, however, antifouling performance was enhanced and then maintained for the full period of the trial.

The surfaces of prepared materials were characterised by measurements of liquid-solid contact angles, using recently advanced drops of water, propane-1,2,3-triol, 1,2-ethanediol and diiodomethane and air bubbles under water. For exposed materials, these measurements were repeated as far as was practicable, according to the extent and ease of removal of fouling.

Drops of all liquids showed time-dependent behaviour when applied to the surfaces of silicone elastomers; this behaviour is not seen with other materials (*eg* ptfe). Advancing contact angles for water were initially 105 - 130 degrees and then decreased towards 95 - 110 degrees. For most elastomers, a rapid decrease effecting most of the overall change occurred over 2 - 5 minutes; the value declined much more slowly. For some of the most fouling-resistant surfaces, the contact angles were initially high and decreased *via* two or more distinct stages over 5 - 30 minutes; the value then declined slowly. This effect was eliminated, however, by the addition of silicone oil. These characteristics may be associated with processes such as the uptake of small amounts of solvent or structural rearrangement in the surface.

SESSION X

PARALLEL A

Surface techniques in corrosion - continued

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Photo-electrochemical imaging of corrosion processes

Interfaces are not uniformly reactive. The field of corrosion provides some spectacular examples of the consequences of such non-uniformity: for example, pitting corrosion, where isolated holes are drilled through an otherwise passive metal; it can be embarrassing when the liquid contents of a stainless steel tank leak out. The chemistry which precedes the formation of such pits is largely unknown. The question is what sort of heterogeneity is responsible, and why is the resulting attack so sparsely dispersed. Other examples of heterogeneity include effects at grain boundaries, again having consequences in corrosion. Heterogeneity of reaction rate affects the operation of batteries and fuel cells. *In-situ* imaging methods, which probe spatial variations in reactivity and which can be used to understand the origins of such variability, are needed. We have developed one method, using photo-electrochemistry, and are in the course of developing another, using infra-red spectroscopy.

The specimen is exposed to an electrolyte solution. In the photo-electrochemical imaging method, a focused light beam, scanned in a raster pattern over the surface, acts as a stimulus. The response is stored in a memory at a location corresponding to the position of the beam at that particular instant. The response is usually a change in electric current flowing around the circuit, across the interface between specimen and solution. It can occur because the temperature change induced locally by the focused spot gives an increase in reaction rate at the interface, or (when a metal specimen is carrying a thin insulating or semiconducting film formed by reaction with the solution, or when the specimen is a semiconductor) as a consequence of photoelectric effects. The photoinduced current is sensitively affected by changes in reactivity at the interface - electron/hole pairs are formed as a consequence of absorption of light, and separated by the electric field present in the specimen in the vicinity of the interface; the charge carriers may then either recombine, usually in states at the interface (giving a transient current flow) or react at the interface (giving a steady state current); the image contrast is therefore affected by the absorption coefficient of the specimen for the light, the electric field strength and the reaction kinetics at the interface.

A variety of electrochemical methods has been studied using this method.

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The use of in-situ FTIR spectroscopy for the study of corrosion processes

The marriage of in-situ infrared spectroscopy and electrochemistry has been very successful in elucidating a large number of reaction mechanisms occurring at electrode surfaces such as the electrooxidation of small organic molecules, the hydrogen evolution reaction, the redox switching of conducting polymers, electroless plating, the etching of silicon, etc.

Here we present a general overview of the different in-situ infrared techniques and new data for the corrosion of steel in neutral and acidic media. We conclude with a prediction of the tremendous potential these techniques have in providing a better understanding of the processes involved in corrosion.

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Acoustic microscopy of coating adhesion

Acoustic microscopy can be used to observe changes in adhesion between a metal substrate and a protective coating. In the acoustic microscope, a scanned focussed acoustic wave is used to obtain an image with resolution which can be submicron, and which can exploit the ability of acoustic waves to penetrate opaque materials and give contrast which depends on the elastic properties of the specimen. Water disbondment and wet adhesion of organic coatings on metals can be studied by acoustic microscopy. When an epoxy/polyamide lacquer on mild steel is exposed to water, there is a reduction in the adhesive strength which falls to a steady value after 24 hours. If the sample is allowed to dry, there is rapid recovery of adhesion, but to a maximum value which is significantly below that before exposure. Acoustic microscopy of a similar coating on polished stainless steel reveals that water reaches the interface rapidly at specific points in the coating. Disbonded areas, roughly circular in shape, spread rapidly from these points, but show little growth after 24 hours. Regions of good adhesion are still present even after 4 weeks of exposure. Recovery during drying leads to disappearance of the blisters, but on subsequent exposure blisters reappear in the same places as before. Using time-resolved acoustic microscopy it is possible to tell whether a blister is filled with water or air.

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AFM studies of the corrosion resistance of high strength cupro-nickel alloys

Copper and its alloys are used extensively in sea water. Their excellent corrosion resistance and anti-fouling properties result from naturally occurring, and protective corrosion products. In recent years there has been a rapid increase and development in their use as coatings and fasteners. For example, copper alloys, particularly cupro-nickels are used as cladding in antifouling applications and high strength, age hardening alloys such as MARINEL have been used extensively for off-shore bolting applications.

In this study, the formation and stability of passivating layers formed on MARINEL, have been investigated by atomic force microscopy. Film growth has been observed in both sea water and sulphide contaminated media. In the uncontaminated solution, film formation occurred within *ca.* 1 minute of immersion and growth was seen to increase with time. In the sulphide contaminated sea water, differences in the rate of formation as well as in the morphology of the layer were observed. For example, a 4 to 5 fold increase in particle diameter was found for film formed in the sulphide contaminated solution.

These studies, coupled with traditional electrochemical and corrosion monitoring experiments, have been used to correlate passive layer structure with corrosion rates.

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Electrochemical techniques in MIC studies

Microbiologically influenced corrosion (MIC) like many other corrosion processes is essentially due to electrochemical reaction. Therefore it is not surprising that contributions from electrochemical techniques will be expected. Microorganisms lead, quite often, to highly localized corrosion, i.e. in the form of pitting or crevice.

Classical electrochemical methods, such as open circuit potential (OCP), resistance polarization (R_p), split cells, polarization techniques as well as electrochemical impedance spectroscopy (EIS), electrochemical noise analysis (ENA), *etc.*, applied to MIC studies have been well reviewed by Mansfeld and Little 1 and also by Videla *et al.* 2.

A brief summary of those techniques will be given in this talk, with particular emphasis to their usefulness to monitor, evaluate and control MIC corrosion in laboratory and in field studies. Techniques particularly useful for pitting corrosion studies are open circuit potential (OCP), cyclic voltammetry (CV), potentiostatic transients, microcells (Microelectrode arrays), scanning vibrating electrode technique (SVET), electrochemical quartz crystal microbalance (EQCM), *in situ* imaging electrode surfaces, electrochemical impedance spectroscopy (EIS), among others.

Due to the lack of time of this presentation we will focus on CV, microelectrodes, OCP and potentiostatic transients. For each of these techniques a brief description will be given with at least one example of experimental data.

The simplicity, sophistication of the equipment, cost, expertness required to perform data acquisition and analysis as well as the capabilities of the technique for on-line monitoring are aspects to be discussed.

The theoretical description of chaotic processes and their possible application to MIC studies will also be considered.

Emphasis will be given to the fact electrochemical data should be complemented by microbiological methods (e.g. cell counting, microscopic examination, determination of bacterial activity, immunological detection) and other possible approaches for a better understanding of MIC mechanisms.

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Evaluation of the influence of chloride ions on the corrosion performance of dhp-copper and 90/10 copper-nickel-iron

DHP-copper and 90/10 copper-nickel-iron show a repassivating pitting attack in chloride ion containing electrolytes in concentrations relevant for potable water. With increasing chloride ion concentration a remarkable change in the corrosion mechanisms of both materials has been observed as indicated by a drastic increase of the mass loss during anodic polarization. These observations have been investigated in detail performing potentiostatic series and cyclo-voltammetric experiments accompanied by relevant analytical and spectroscopic techniques to evaluate quantitatively the change in the corrosion mechanisms. Special attention will be given to changes in composition and morphology of the reaction layers formed on both materials during anodic polarisation as a function of increasing chloride concentration. Possible corrosion mechanisms will be proposed.

SESSION XI

PARALLEL B

Biological offered papers

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Influence of surfaces on hydrogen sulphide producing bacteria

Two mixed cultures containing sulphide producing bacteria were isolated from two separate microbiological surveys of facilities sited on chalk formation oil fields in North Sea reservoirs. Both are thermophilic cultures, with optimum growth at 60°C, and have been denoted EX251 and EX258. Hydrogen sulphide producing bacteria in reservoirs are of great economic importance in terms of corrosion, fouling and souring. Recent research has involved investigation of starvation survival and growth characteristics of both cultures. The chalk formations from which the cultures were isolated are highly porous matrices, providing ideal conditions for bacterial attachment, survival and growth. This study included assessments of metabolic activity and total cell counts of planktonic bacterial cultures followed by comparison of sulphide production rates in the presence of a variety of surfaces of relevance to the oil industry. Metabolic activity was determined using intracellular reduction of *p*-iodonitrotetrazolium chloride (INT) to insoluble coloured INT-formazan, visualised and counted using computer-enhanced image analysis microscopy. Counter-staining with acridine orange allowed total cell counts from the same sample. Results obtained allowed accurate estimates of total and metabolic activity counts and provide a useful addition to the methodology for assessing activity of thermophilic hydrogen sulphide producers. In separate experiments, surfaces in a granular form were added in varying amounts to the planktonic cultures suspended in suitable media in a series of stoppered and crimp-topped vials. The rate of production of sulphide was measured by a micro-assay technique involving diamine mediated methylene blue release from acidified samples. Each culture vial was sampled only once (by hypodermic and syringe) to avoid the risk of sulphide loss through lack of integrity of the butyl rubber stopper. Statistical integrity was assured by sampling three vials at each time point selected. This sacrificial vial technique provided reliable and reproducible sulphide data. For both cultures it was found that the availability of a surface contributed significantly to the rate and extent of total soluble and insoluble sulphide generated. In another series of experiments, cultures were added to chalk rock chips in the presence of growth media containing different concentrations of nutrient and incubated for one year. Sulphide and cellular metabolic activity from the planktonic phase of the bottles was assayed, and after several months, additional nutrients were added to the system, which resulted in further sulphide generation from cultures in contact with rock chip surfaces, while there was none from cultures not containing a surface. The inference is that the thermophilic sulphide producing bacteria were preferentially attached to the rock chip surfaces (since very few cells were recovered from the planktonic phase of the system). Implications of these results are that in the reservoir situation, the bacteria are able to withstand long periods of nutrient deprivation while attached to the porous rock matrix and opportunistically utilise nutrients when they become available. This has implications for biocide control regimes employed by reservoir personnel to control the level of sulphide generating bacteria and therefore the souring of oil and subsequent corrosion of topside facilities.

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Degradation of antifouling biocides

The most important factors causing degradation of biocides are generally considered to be light, especially UV, and microorganisms. In the aquatic environment, the impact of light is minimal due to poor penetration through water. Thus, microbial activity is the major process by which biocides are degraded. The ecotoxicological profile of antifouling biocides is becoming increasingly important. Probably the most important factor in terms of environmental acceptability of a biocide is its ability to degrade quickly into non-hazardous products. Published methods for determining the biodegradability of a substance are generally unsuitable for compounds which are toxic or inhibitory to microorganisms unless sensitive analytical methods are available which detect the biocide at sub-inhibitory concentrations. In this paper, a simple method will be described which utilizes marine bacteria to degrade the test compound. The disappearance of the test compound is measured by bioassay using the ship-fouling diatom *Amphora*.

Degradation was studied in three media: (1) natural seawater (NSW), (2) NSW to which an aliquot of a mixed culture of marine bacteria was added, designated BSW, (3) sterile seawater (SSW). The use of BSW allows the concentration of viable bacteria to be increased when studying compounds inhibitory to bacteria. The test compound was added to NSW, BSW and SSW and the solutions were aged in the dark at 25° C for up to 8 weeks. After ageing, an aliquot of *Amphora* culture was added and growth after 96h measured. Bacterial numbers were monitored throughout the experiment by incorporating a dilution series into seawater agar.

Results will be presented on the biodegradability of a number of compounds in current use in antifouling paints viz. Sea-Nine 211 (4,5-dichloro-2-n-octyl-4-isothiazolin-3-one), Irgarol 1051 (2-methylthio-4-tert-butylamino-6-cyclopropylamino-s-triazine), diuron(3-(3,4-dichlorophenly)1-1-dimethylurea), chlorothalonil (tetrachloroisophthalonitrile) and TBTO (tribyutyltin oxide). The results show that biodegradability ranges from very readily biodegradable (Sea-Nine 211) to non-biodegradable (Irgarol 1051 and diuron). The results will be discussed in relation to published data on the degradation of antifouling biocides.

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Mechanical cleaning of salmon cages: removal and regrowth of fouling

The efficacy of an *in situ* mechanical net cleaner was evaluated during the summer of 1994/1995 in Tasmania, Australia. The development of fouling and the effect of mechanical cleaning were quantified non-destructively through close-up underwater photography and subsequent image analysis. The blockage of netting caused by fouling growth was recorded at 3 depths (20-70 cm, 100-150 cm and 180-230 cm) on the northern and southern sides of a 65 m circumference cage (96 ply, 22 mm bar netting) weekly for 5 weeks. The blockage at these depths was also recorded on the northern side of an adjacent 40 m cage (150 ply, 25 mm bar) weekly for 3 weeks. While the cleaner removed some fouling from the 65 m cage, insufficient contact between the cleaning brushes and netting prevented significant reduction in net blockage. The performance of the cleaner was further hampered by extensive growth of *Scytosiphon lomentaria* which was unaffected by the cleaning process. Modification of the cleaning process to give greater contact between the brushes and net, combined with weekly cleaning, prevented colonisation and growth of fouling over a 10 week period. A subsequent trial in the 40 m cage also demonstrated that a significantly greater mass of fouling was removed from an established community at 180-230 cm depth. The fouling was reduced from 5.9 _1.3 g/100 cm^2 to 3.3 _0.7 g/100 cm^2 using the original process and from 4.9 _2.1 g/100 cm^2 to 0.8 _0.5 g/100 cm^2 using the modified process. This research has enabled the development of a prototype *in situ* mechanical cleaner which can both prevent development of fouling and remove established fouling, so that blockage of fish-cage netting can be kept to a minimum.

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Marine primary biofilm on stainless steel - quantitative and qualitative methods

The adhesion of bacteria to solid surfaces and the subsequent development of bacterial biofilms occur in natural habitats, e.g. freshwater and marine environments and on man-made structures, e.g. pipelines, water treatment equipment, etc. The primary microbial film may prepare the surface for subsequent colonization by sessile superior organisms. In heat exchangers and cooling towers microbial biofilm can be an important technical problem because it can impair the transfer of the thermal energy.

Scanning Electron Microscopy (SEM) Image Analysis, Acridine Orange Direct Count (AODC) and Dehydrogenase Activity (DHA) were tested in order to quality the biofilm on stainless steel surfaces, to enumerate the attached bacteria and to assess their metabolic activity. Moreover, the bacterial genera involved in the primary biofilm were isolated and identified.

All the tested methods seem to be suitable to assess the primary biofilm development on surfaces, although each method offers certain advantages and disadvantages.

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Effectiveness of an quaternary ammonium-based biocide to control marine macrofouling in industrial cooling water systems

A macrofouling control strategy employing an quaternary ammonium-based biocide has gained wider acceptance as an alternative to chlorination in both freshwater and sea water cooling systems. Quaternary ammonium-based biocides are administered for 3 to 24 to eradicate recently settled fouling organisms. The biocide is periodically applied during the fouling season to prevent the growth and accumulation that would otherwise hinder operations of these cooling systems. This paper will discuss the effectiveness of quaternary ammonium-based biocide to control dominant macrofouling organisms including bivalve mollusks, *Mytilus edulis* and *Crassostera virginica*, barnacles, *Balanus sp.* and hydrozoans. In addition, recommendations for controlling these various fouling organisms will be presented. This presentation will also address the environmental impacts of treating cooling systems with this biocide.

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Detachment studies on microfouling in natural biofilms on substrata with different surface tensions

Attachment strength of bacteria, diatoms and five protozoan groups in natural biofilms was studied on artificial substrata by exposing them to laminar flow in a radial flow chamber. The protozoa which were considered were thecate choanoflagellates, *Corthunia*, *Vorticella*, *Zoothamnium* (Peritricha), and *Ephelota* (Suctoria). Seven different artificial materials (PTFE, FEP, PFA, ETFE, HC, PC, glass) with surface tensions between 19 and 64.5 mN m^{-1} were used. Test panels were immersed between 3 hours and 8 days in the sea. Attachment strength was studied by exposing them to 4 different laminar flow pressure intervals (between 3.9 and 16.9 N m^{-2}) in a radial flow chamber. The results showed that bacteria and diatoms improved attachment strength on all substrata within 8 days, particularly during the first 2 days. Both groups detached initially (3 h to 5 d) more easily from materials with surface tensions between 20 and 25 mN m^{-1} than from other substrates. However, significant differences between the substrata disappeared after 8 days. These results show that a minimum bioadhesive range between 20 and 25 mN m^{-1} exists for bacteria and diatoms during early colonisation periods. However, bacteria and diatoms possess compensation mechanisms to overcome weak attachment strength on these materials. Protozoa were studied after 5 and 8 days only because their densities were too low for evaluation at earlier intervals. Their attachment strength did not improve during that interval. Significant differences between the materials were observed for *Corthunia*, *Vorticella*, and *Ephelota*. Highest detachment rates were usually recorded on materials between 20 and 25 m Nm^{-1} after exposure to maximum flow pressure (16.9 N m^{-2}). *Corthunia* was also easily detached from glass (64.5 mNm^{-1}). However, even after exposure to a flow pressure of 16.9 Nm^{-2} an average of more than 50% of the investigated protozoan species remained on each material. The present results indicate that surface tension has a limited role in the colonisation of surfaces under natural conditions. Within a few days most investigated microfouling groups were able to resist considerably strong flow pressure on every material tested.

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Development of a continuous culture biofilm model to evaluate microbial biocides

A continuous culture laboratory model has been developed from chemostat vessels to simulate a flowing water pipe system such as those found in buildings and aboard ships.

A primary vessel was set up to grow an initial inoculum that would represent a water storage tank feeding the other secondary vessels downstream that simulated the water system. In this case filter-sterilised tap water was used as the growth medium in the continuous culture vessels with no exogenous carbon sources. In addition the microbial culture consisted of a mixed consortium obtained from a potable water system. Use of secondary vessels enabled the biofilms to be formed before challenging with appropriate biocides. Even when testing the efficacy of the different biocides the vessels were still being challenged with the microbial consortium which was constantly flowing from the primary reservoir vessel. This configuration ensured the reproducibility of the model when testing to destruction in the secondary vessels.

The flexibility of the system enabled different materials and biocides to be evaluated. These were examined in different vessels to prevent cross contamination using different types of water, such as used in potable water supply and/or for cooling water systems, at different temperatures and at different shear rates. The reproducibility of the model system was such that errors of less than 10% were observed for biofilm enumeration, either before or after biocide treatment.

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The occurrence of a major new fouling alga in the British Isles

An attached population of the large brown kelp *Undaria pinnatifida* (Harvey) Suringar, a native to the shores of Japan, has recently been discovered in the Solent region of the south coast of England. This represents the first report of this important fouling species in the British Isles. The plants are attached to floating pontoons of a marina and occur in dense stands at the water line region. Plants are also commonly epizoic on the fouling tunicate *Stylea clava*. Some of the larger plants measure up to 1 m in length and are clearly fertile with mature zoospore-releasing sporophylls. *Undaria* must now be considered as firmly established on our coasts and any attempts at its eradication would undoubtedly prove futile.

The introduction of *Undaria* into the British Isles was not unexpected given its adventive background. It was acccidentally introduced into the Mediterrean from the Pacific in 1971 and, more recently, populations have been discovered in New Zealand and Tasmania. Suggested vectors for these introductions include imported shellfish, hulls of boats and ballast waters. Boats are the most likely vector for the introduction of *Undaria* into the Solent, the plants originating from populations deliberately introduced from the Mediterranean into Brittany for commercial reasons.

The present paper examines the adventive background of *Undaria* which has lead to its arrival into the British Isles, describes its current and future role as a marine fouling organism and discusses aspects of its likely spread and consolidation in the North Atlantic.

SESSION XII

PARALLEL A

Microbial corrosion

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Surface analytical techniques applied to microbiologically influenced corrosion

The study of microbiologically influenced corrosion has progressed from phenomenological case histories to a mature interdisciplinary science in which surface analytical techniques are used to interpret the relationship between electrochemical and microbiological phenomena. Recent developments in image analysis systems, including electron, atomic and laser microscopy, make it possible to image biological materials on metal surfaces in the hydrated state. Environmental scanning electron microscopy (ESEM)/energy-dispersive x-ray spectroscopy has been used to demonstrate the spatial relationship between iron related bacteria and tubercles on stainless and galvanised steels. Three-dimensional images with surface contours and critical dimensions have been prepared of bacteria on metal surfaces within scratches, milling lines and grain boundaries using confocal laser scanning microscopy (CLSM). Atomic force microscopy images of copper exposed to bacteria demonstrated that bacterial cells were associated with pits.

CLSM and ESEM images of hydrated biofilms have shown that the bulk is extracellular organic material, not microorganisms. Furthermore, cells may be concentrated either within upper or lower regions in microcolonies. The concentration and speciation of metal ions bound within exopolymers has been determined using x-ray absorbtion near-edge structure.

Ion-selective and gas sensing microprobes with tip diameters less than 10 μm are being used for direct measurements of pH, dissolved oxygen and sulfide, as well as diffusion controlled reactions within biofilms and corrosion products. An iridium microelectrode was used to map the spatial distribution of pH over a mild steel surface on which a drop of agar had solidified. The solid polymer created a differential aeration cell and the area immediately under the polymer became anodic. Microelectrodes have also been used to measure surface chemistries associated with ennoblement of stainless steels in marine environments.

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Marine biofilms on stainless steels: effects on the corrosion behaviour

Stainless steels are widely used in marine environments, particularly in power plants and in off-shore structures. Their surfaces, exposed to natural seawater, are covered in time by a biological layer, the biofilm, which may affect the stainless steel susceptibility to localised corrosion. It has been shown by several laboratories that the open-circuit potential of stainless steel is shifted towards the noble direction for stainless steels exposed to natural seawater and may be displaced to values near the breakdown potentials for several of these materials. This effect is often referenced in the literature as "potential ennoblement".

This potential ennoblement has been extensively studied by experiments performed in the SIRIUS facility of the CEA/SCECF laboratory. Part of these results have been obtained in the framework of a European MAST II program called "biofilms on stainless steels" (MAS2 CT92 0011). The obtained results coupled with the literature data show that :

I. the potential increase due to the biofilm development is independent of the stainless steel grade : no difference in the corrosion potential versus time curves were found for the investigated alloys when they are in the passive state.

II. in SIRIUS conditions, the ennoblement up to 200 mV/SCE occurs before the irreversible adhesion of the micro-organisms on stainless steel surfaces, observed by Scanning Electron Microscopy. Reversible adhesion has been followed during the potential increase, by epifluorescence observations and then, bacteria were found on stainless steels before the potential ennoblement.

III. the potential ennoblement was observed at all seasonal exposures. But it takes more time to reach the maximum potential (about 300 mV/SCE) in winter than in summer.

IV. polarisation currents at 0 mV/SCE or at ± 20 mV/corrosion potential increase also with the biofilm settlement, but after the ennoblement of the corrosion potential due to the fact that very few current is needed to move the potential of stainless steel in the passive region.

V. with seawater thermally altered (seawater at 15°C heated up to 40°C), potential ennoblement was not observed while biofilm formation was occurring. This result clearly shows that the potential ennoblement in seawater is not directly connected to the biofilm development itself, but rather to the chemical or microbiological composition of the biofilm, or to its microbiological activity.

Crevice corrosion experiments show that in natural seawater, the susceptibility to the initiation of crevice corrosion is increased (corrosion potentials above the breakdown potentials) and also the rate of propagation of the crevice corrosion (increase of the reduction reaction).

All these results are in agreement with an increase of the reduction reaction induced by an anaerobic biofilm, although the exact mechanism(s) responsible for the potential ennoblement of passive materials is not elucidated and even if the presence of a biofilm alone is not sufficient as shown by the experiments at 40°C.

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Chemotaxis and corrosion in copper alloys and other materials

Bacterial interaction with metallic surfaces has attracted a great deal of interest due to its relationship with technical problems, for instance biofouling of marine installations or corrosion of metallic structures in the presence of bacteria.

In this work the effect of metal ion gradients on chemotaxis is investigated. Data about copper alloys and titanium are presented. Also a simple chemotaxis model based on the solution of non-linear diffusional equation is developed.

Related to the corrosion of materials mentioned above, results on oxygen reduction, enhancement by bacteria and the relationship with the presence of electroactive species in the supernatant are shown.

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Biocorrosion problems in the marine environment: new perspectives

Metal dissolution in the marine environment is conditioned by two different processes occurring at the metal/solution interface : corrosion products forming from the metal into the solution, and biofouling settlement from the bulk solution towards the metal surface. Thus, a new "biologically conditioned" interface is produced, and the consequent corrosion behaviour of the metallic structures exposed to sea water will be governed by dynamic and complex interactions between the inorganic passive layers and the biological deposits.

Important improvements in analytical, microbiological, electrochemical and microscopical instrumentation allowed us to reach a better understanding of biofilm structure and dynamics. New insights into biofilm systems have changed classical concepts of diffusion control for interpreting mass transport within the biofilm, or the mechanisms explaining the action of some oxidizing biocides.

Taking into account the new perspectives in the study of biofilms, a brief overview on biocorrosion and biofouling interactions upon different metallic substrata (copper alloys, titanium, carbon steel , stainless steel) immersed in marine environments is presented. New strategies for monitoring biocorrosion and biofouling in offshore structures and coastal power plants are also discussed.

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Correlation of properties with performance of silicone foul release coatings

In an effort to better understand the effects of variables such as filler level, filler type and crosslink density on abrasion, tear strength, pseudobarnacle adhesion and release properties of silicone foul release coatings, formulations were prepared in which the crosslink density was varied at constant filler loading or the filler level was varied at constant crosslink density. The physical and foul release performance of these coatings was evaluated using a variety of test methods. Several trends were evident from these studies. For example, pseudobarnacle adhesion was found to increase with increasing filler levels but was relatively unaffected by polymer crosslink density. A detailed account of these studies will be presented.

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The relationship between SRB outer membrane protein profiles and their corrosion causing abilities: a comparative study

Sulphate Reducing Bacteria (SRB), present in biofilms formed on metal surfaces, are often involved in the anaerobic corrosion of iron and ferrous alloys. The understanding of the process of biofilm formation involving bacterial cell attachment to metal surfaces, along with Fe (II) adsorption to the bacterial cell, is necessary to elucidate mechanisms of SRB influenced deterioration of steel. Previous studies showed the importance of OM macromolecules such as lipopolysaccharides in the initial adhesion of SRB cells to mild steel (Beech and Gaylard, 1989). Recent investigation demonstrated the induction of proteins in exopolymers secreted outside the SRB cell wall in the presence of carbon steel surfaces (Zinkevich *et al.*, 1995). In this work both laboratory and wild SRB strains isolated from corrosion failures were examined to compare the bacterial outer membrane (OM) protein profiles between SRB strains varying in their aggressiveness towards mild steel. Corrosion rates of steel coupons were determined using weight loss measurements. Techniques of light and scanning electron microscopy were employed to study biofilm formation on steel surfaces. Bacterial counts were carried out in order to determine the density of sessile SRB population. OM were extracted from bacterial cells grown in the presence and in the absence of steel surfaces as well as in the presence of different concentrations of Fe (II). Protein profiles of isolated OM were obtained using SDS-PAGE electrophoresis. Each of SRB strains had a distinct protein pattern confirming the result of earlier work reporting that the OM protein profile could be used as a marker for each sulphate reducing bacterial strain (Feio *et al.*, 1995). In view of the results obtained the involvement of OM proteins in the process of SRB attachment to steel, subsequent surface colonisation and corrosion of steel in the presence of biofilms, are discussed.

SESSION XIII

PARALLEL B

Biological offered papers

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Settlement on concrete blocks immersed in the Ligurian Sea (N-W Mediterranean)

In the framework of a study on the behaviour in the sea of recycled materials, some cubic concrete blocks were immersed in two different places in the Ligurian Sea. These man-made objects, used as control material, had a side length of 15 cm in Lavagna (East Ligurian Sea) and 20 cm in Loano (West Ligurian Sea).

In both places the study started between November and December 1993 and ended in December 1994.

In order to gain a view of the different settlement periods and of the evolution of the population present, the substrata were taken away and replaced every three months. Each object was submitted in the laboratory to a careful analysis of its benthonic community, both in terms of quality (species present) and quantity (covering indices, wet weight, dry weight and ash weight).

In Lavagna, the area under study is located at a depth of 17 m on a sand - muddy bottom colonized by a spare *Cymodocea nodosa* and strongly influenced by the mouth of the river Entella. The structure from which the blocks hang is surrounded by 8 pyramids, each made up of 5 concrete cubes of 2 m side, which form a small artificial reef. After a year's immersion it was possible to highlight the different stages in the evolution of the association which had formed on the small blocks after 3, 6, 9 and 12 months. A total of 42 species, belonging to 17 taxa, were classified. The highest value of biomass settled on the objects was of 12.8 g/dm^2.

In Loano, the study site is located at a depth of 10 m on a sand - muddy bottom partially covered by *Posidonia oceanica* and *Cymodocea nodosa* meadows. The small blocks were positioned close to an artificial reef. A total of 45 species, belonging to 15 taxa, have been found; after one year's immersion the highest value of biomass settled on the substrata was of 15.6 g/dm^2.

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Inhibition of invertebrate larvae, algae and diatoms by a surface colonising bacteria

The isolation of natural antifoulants against marine invertebrate larvae has, so far, generally been obtained from marine eukaryotes. A recently isolated marine bacterium, *Alteromonas* sp. D2 was found to inhibit the settlement of invertebrate larvae. This organism has been found to produce at least three extracellular components which have properties that make them very promising antifouling agents. A high molecular weight component, a protein, inhibits the settlement of larvae of a tunicate and a barnacle, as well as inhibiting a range of marine bacteria. A low molecular weight component, less than 500 Da in size, is toxic to tunicate, barnacle and bryozoan larvae. Additional compounds other than the high and the low molecular weight components of *Alteromonas* sp. D2 have also been demonstrated to have inhibitory activity against the alga *Ulva lactuca,* the diatom *Amphora* sp. and a fungus species.

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Release of biocides from antifouling coatings

Increasingly, producers of antifouling coatings are requested by national authorities to generate and submit data on leaching rates of biocides from antifouling coatings. Such data are necessary for authorities to carry out realistic environmental risk assessments in relevant marine (and freshwater) regions. It is not the purpose of the present paper to question the request for leaching rate data. The basis on which data are generated for calculation of the environmental loading may, however, be discussed.

The methods for determination of leaching rates of biocides from antifouling coatings are many. Until now, no uniform method has been established, although an ASTM Standard: "Standard Test Method for Organotin Leaching Rates of Anti-fouling Coating Systems in Seawater" (D 5108-90) does exist. Recently, ISO has agreed that an ISO Standard on leaching rate of biocides from antifouling coatings should be developed. This initiative is indeed welcomed and will be supported by antifouling paint manufacturers world wide.

On the basis of results obtained from practice over an extended period of years, Hempel has established a calculation method by which leaching rates of biocides from antifouling coatings can be generated. The present paper describes the principles of the method and highlights the advantages compared to laboratory methods.

In summary, the information required by authorities in order to establish an average leaching rate of a given biocide into a given marine (or freshwater) region is the paint type, the concentration of biocide in the paint and the non-volatile concentration of the paint. This data can be easily derived from the paint declaration.

It is suggested to use calculation for determination of leaching rates whenever such data are requested since:

- The figures provided will be the most relevant for the calculation of environmental loading as they are based on data obtained from practice and following a simple principle: All the biocide, but not more than what has been applied on a ship for a given period, will leach out
- Products will be treated on an equal and comparable basis
- The equations used are valid for all biocides used in antifouling coatings both actual and future, without the need for development of complicated analytical methods
- The problems of measuring leaching rates of fast degradable organic biocides are avoided
- The calculation method is cheap and easily affordable also for smaller companies

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On-line fluorescence measurements as a bacterial biomass measure of prospective antifouling paints

Biofilm formation and activity were monitored non-destructively in a laminar-flow cell system using tryptophan fluorescence (TF) as a measure of biomass and *Vibrio harveyi* bioluminescence. By normalizing light measurements from candidate antifouling (AF) coatings to an upstream stainless steel surface, a quantitative ranking of AF efficacy against bacterial attachment and biofilm development was generated. Coatings which demonstrated a TF ratio of 0.65 or less were considered to be effective compared to stainless steel controls. It was hypothesized that coatings which exhibited low biomass ratios coupled with significantly greater bioluminescence ratios induced a sublethal effect in which biofilm cells were metabolically active but not actively reproducing. Similar results have been obtained with *V. harveyi* cells exposed to carbon monoxide and sodium azide. Fouling-release coatings were ranked according to the percent of biomass stripped from the coating surface after a 15 min exposure to a shear stress of 330 dynes cm^{-2}. Fluorescence and bioluminescence measurements were obtained to determine the percent of biofilm biomass stripped away as well as the final biomass and activity ratios. The antifoulant zosteric acid (ZA) inhibited *V. harveyi* biofilm development at a minimum bulk phase concentration of 0.05 mg mL^{-1}. Interestingly, ZA demonstrated little effect upon the growth rate of *V. harveyi* in batch cultures, even in the presence of up to 1 mg mL^{-1}.

CAMBON, C.

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New generation of antifouling paints for French Navy: high performance tin and copper free paints, anti-adhesive coatings, and a new accelerated test for comparative evaluation of erosion rates of self-polishing coatings

The DCN specifies the protective systems in service on French Navy warships (surface ships and submarines) and has been involved for a long time in different programmes for reducing the pollution of the environment, especially of harbours, by TBT and copper compounds.

The ambitious target is the complete replacement of TBT self-polishing antifouling by new generations of environmentally more friendly coatings which maintain the self-polishing behaviour, long-term biocide performances (3 years minimum between dry docking), and reasonable economic cost (product price, maintenance costs) of TBT coatings.

Two different and complementary methods are proposed:

1. Anti-adhesive coatings (silicones, fluorinated polymers);
2. An innovative 'tin and copper free' new class of antifouling coating developed for two years by DCN in Toulon's CTSN paint Laboratory.

These coatings are based on a chemically modified acrylic copolymer (chlorine free), containing quaternary ammonium groups and associated with an organizing biocide. This kind of formulation allows 'tailor-made' erosion rates according to the specific needs of the user. Both biostatic and biocide effects are combined to provide an excellent antifouling performance: 2 warm seasons in static immersion in the Mediterranean without any fouling but with slime; the third warm season of exposure is now in progress.

These coatings are experimental on 3 Naval surface ships based in Brest (French Navy experimental formulas EM212/EM 215).

Civilian applications on aluminium hulls and yachts are now possible (patent licences).

We also describe a new accelerated testing method for comparative evaluation of erosion rates of self-polishing coatings developed by the DCN laboratory. This test allows important time saving in new antifouling formulation and optimization programmes.

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Method for preventing aquatic organisms in active dissolving anodic electrolysis of metal

Marine aquatic organisms (bacteria, seaweed, shellfish) attach and breed on various sections of equipment that have contact with water in steam or atomic power stations. Therefore, the maintenance and management of such intake equipment is of great importance. The most essential points which need to be considered are the control of corrosion of the facilities and attachment of aquatic organisms.

Excellent corrosion prevention engineering methods have been developed, such as corrosion resistant materials and progress in coating and cathodic protection. These methods are effective in preventing attachment of marine organisms, but anti-fouling methods produce toxic ions such as chlorine, hypochlorite, copper, mercury and tin, which cause secondary environmental pollution.

We have paid special attention to the fact that attachment of marine organisms cannot be observed on the surface of an electrode functioning as an anode in conventional cathodic protection. This is based on the concept that attachment of aquatic organisms is unobservable on the active dissolving portions and is controlled by anodic electrolysis of metal generating non-poisonous ions. This did not result in the generation of chlorine or toxic ions. Our report is the result of an experiment we conducted using steel as an anode in sea water over the period of a year.

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The use of pyrolysis-mass spectrometry for the analysis of sulphate-reducing bacteria

This study was undertaken to determine whether the presence of carbon steel surfaces in microbial growth media had an influence on the chemical composition of planktonic cells of marine sulphate-reducing bacteria (SRB). The investigation was carried out in order to gain a better understanding of the interactions between SRB and steel structures in marine environments.

Three SRB isolates recovered from corrosion failures in Alaska, England and Indonesia were grown as batch cultures in 2 different nutrient media in the presence and in the absence of mild steel coupons. It was previously shown that these isolates produced extracellular polymeric substances (EPS) [1]. The EPS composition varied with the type of SRB and growth conditions (i.e. coupon free and coupon containing media).

Bacterial cells harvested from 5 day old cultures were analysed using pyrolysis mass spectrometry (Horizon RAPyD-400). The results demonstrated differences between SRB isolates grown under identical conditions. Furthermore, the presence of mild steel surfaces induced changes in the cellular composition of all three strains. An increase in the ion at m/z=60, corresponding to acetic acid, was found to be statistically significant. This ion, shown to be derived from acetyl groups, has been implicated in binding of bacterial cells to steel surfaces (Ford et al, 1990) [2].

The implications of our findings for the phenomenon of steel deterioration in the presence of SRB are discussed.

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Fouling communities on seagrasses in the Gulf of Aqaba (Red Sea)

During the period May 1992 to April 1993, a study was undertaken of the marine fouling communities of seagrasses in the Gulf of Aqaba, Red Sea. Monthly samples of shoots of five species of seagrass (*Halophila stipulacea, Halophila ovalis, Halodule uninervis, Thalassia hemprichii* and *Thalassodendron ciliatum*) were collected and brought back to the laboratory for detailed examination. Light microscopy was used to identify the macroscopic plant and animal epiphytes whilst scanning electron microscopy was used to study the microfouling communities. Biomass data were also obtained for the epiphyte communities.

Thalassodendron ciliatum was the most heavily fouled seagrass, supporting a wide range of plant and animal communities. In general, algal epiphytes were more abundant than zoo-epiphytes. For example, 33 species of fouling macroalgae were recorded on the stem and leaves of *Thalassodendron ciliatum*, comprising 3 Cyanophyta, 3 species of Chlorophyta, 5 species of Phaeophyta amd 18 species of Rhodophyta, whilst zoo-epiphytes were restricted to rare occurrences of a small number of foraminiferans, spirorbid tube worms, hydrozoans, bryozoans and molluscan egg masses. Whilst some algal epiphytes (mainly Rhodophyta) occurred all year round, others (mainly Phaeophyceae and Chlorophyceae) were seasonal in occurrence. Differences were also observed in fouling communities on individual species occurring in different localities. In general, epiphyte communities showed low abundance, variety and biomass in autumn and winter, which increased to a maximum through spring to summer. Observations on the colonisation process revealed a basipetal mode of attack by the epiphytes with the oldest, more distal regions of the host plant supporting the greatest diversity and abundance of epiphytes. Leaf edges also appeared to be more epiphytised than leaf faces whilst broad leaves were shown to harbour relatively more epiphytes than narrow leaves.

SESSION XIV

Corrosion

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New anticorrosive paints; use of n-semiconductor pigments

Among the several methods of protection of metals against corrosion there are some, such as cathodic protection by sacrificial anodes (in indirect or direct contact with metal surface), by external imposed current, by sacrificial metals powders in anticorrosive paints and by using atmospheric electricity [needle-diodes methods (1-3)], that they act by decreasing the corrosion potential and current. For the same purpose we introduced a new system using n-semiconductor pigments in polymeric vehicles. The n-semiconductors, having the predisposition to offer electrons (they promote reduction reactions), could impose a type of cathodic protection, they do not get exhausted and, depending on their structure, they can protect their polymeric supports from UV light degradation acting as sensitisers.

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A study of the corrosion behaviour of laser treated cast iron alloys

In this study the corrosion behaviour, in a laboratory scale, of chromium cast iron alloys modified by laser treatment is presented. Five alloys of chromium cast iron have been studied with the following alloying elements Fe+(Cr), Fe+(Cr, Mn), Fe+(Cr, Mn, Nb), Fe+(Cr, Mn, W), Fe(Cr, Mn, Ti). The laser surface treatment was provided by a continuous-wave CO2 laser and two energy inputs were tested: 202 W.s/mm and 302 W.s/mm on the surface of the specimen. The corrosion testing environment was a 3.5% NaCl solution. Electrochemical tests were also performed on the specimens and Tafel and polarization curves were obtained. Also the results of the electrochemical tests are compared to iron dissolution rates, measured by atomic absorption of the dissolved iron ions in the solution. The surfaces of all exposed and unexposed alloys were checked by SEM and EDAX analysis, to observe the superficial changes on the laser modified surface, caused by the corrosion process. The results show that the addition of Ti and W did improve the corrosion resistance of the alloys. Also the modified surface by the laser energy input of 302 W.s/mm is more susceptible to corrosion than the one modified by the laser input of 202 W.s/mm. Some of the results of the electrochemical measurements are questionable as far as extraction of quantitative results.

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Electrochemical evaluation of coatings for marine corrosion control

The long term protection of metal structures exposed to marine environment is a problem to be approached by many different points of view and involving many disciplines. A reliable set up of good metal substrate, suitable surface treatment, high performance coating, carefully designed cathodic protection, detailed maintenance plan and, last but not least, a complete testing and survey schedule can provide a safe and long service life. The use of Electrochemical Impedance Spectroscopy (E.I.S.) for the study of marine corrosion systems (made up of metal, possible pretreatments, organic coating cycle, mechanical impacts, aggressive environment) allowed to find out the relationship of some physico-chemical parameters with the values of corresponding components of the equivalent electrical circuit and with the trend towards degrade. In particular, water absorption, porosity, delamination, amount of undermining corrosion were evaluated as a function of either natural or artificially induced degradation phenomena, including mechanical deformation. Also the performances of sacrifice anodes are studied by means of E.I.S.

Another important field of application of E.I.S. techniques is the study of the features and performances of the calcareous coatings obtained on an immersed structure under the effects of cathodic protection.

A survey of the testing procedures and of the results obtained in the study of high built epoxy, fluoropolymers and other organic coatings for marine corrosion control will be presented, together with some results related to cathodic protection.

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Influence of inhibitors on the performance of reinforced light mortar in the marine environment

A series of mortars and grouts are commonly used for rendering of horizontal or filling of vertical elements of reinforced masonry. They are generally composed of cement, sand, lime and water or cement, sand, hyperplasticiser and water.They exhibit porosity values in the range of 13-20% and possess high thermal conductivity creating thermal bridges. The use of porous light weight aggregates, with a diameter up to 6mm, results in porosity increase (35-40%) and in a significant reduction in thermal conductivity. Durability of these mortars is questionable as far as the corrosion of the reinforcements is concerned and taking into consideration that the rest of the construction (bricks) has also high porosity. Attempt is made light weight concrete to combine the advantages of low density with the constructability, mechanical and durability properties of these mortars. Addition of inhibitors to mixing water provides a rather convenient method for deterioration prevention of concrete structures. The aim of the present work is the examination of the performance, in marine environment, of mortars specimens comprising fine-pumice aggregates (max. diameter 8mm) together with corrosion inhibitors, in an effort to lower the corrosion rate of steel reinforcements. More specifically the following corrosion inhibitors were used: sodium nitrite (2 or 5%wt), sodium benzoic (1 or 2%wt) and ferric oxide (5 or 10%wt). The ratio cement / water / porous light weight aggregate was kept stable and equal to 1/0,9/3, varying only inhibitors type and concentration. Specimens with quartz sand and others with fine pummice aggregates without inhibitors were cast for reference. The specimens durability was evaluated by measuring the corrosion potential and the steel rebars mass loss time dependence, the carbonation depth, the porosity and the mechanical strength of the specimens. Correlation of the above results revealed that the corrosion inhibitors examined exert protective action to the steel rebars. The best of them being sodium nitrite, while changes of mechanical strength are unsignificant compared to the practical needs.

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Metal-filled composites as protective coatings against marine corrosion

Epoxies represent perhaps the best combination of corrosion resistance and mechanical properties providing thus outstanding service under severe conditions. Recent increasing technical demands justify recognition of composite coatings, such as coatings reinforced with glass, polyaramide or graphites fibres or flakes, as well as particulate polymeric composites with conductive fillers which exhibit increased thermal and mechanical properties. Additives incorporated within the coating offer protection either by exerting a barrier effect or acting as a sacrificial anode or an inhibition agent. The dispersion of conductive particles in an epoxy matrix changes the transport properties of the coating because it leads to the decrease of the coating resistance and to the easy creation of conductive paths, which in the case of powder metal less electroactive than the metal substrate, accelerates the corrosion rate of the substrate due to galvanic corrosion. This behaviour is counterbalanced by a barrier effect created in the coating because the microprores of the polymer are filled either by the metal particles or by their bulky corrosion products. The transport pattern which determines the conductivity in such conductive polymeric composites undergoes an insulator-conductor transition which depends on the particles concentration. The effect of the presence of metal powders in the coating, as well as, the influence of their concentration on the assessment of the coatings behaviour in marine environment has been studied. Pretreated steel samples have been coated with a layer of metal-filled epoxy resin and the electrochemical and dielectric behaviour of these specimens in corrosive environment (3.5% wt NaCl) has been investigated. Corrosion Potential, Corrosion Current density (Tafel method), Electrochemical Impedance Spectroscopy and Dielectric Measurements were performed. In an effort to examine the influence of the presence of more and less electroactive metal powders than the steel substrate, Zn, Al, Fe, Cu, Ni in powder form were used as fillers in the polymeric coatings. Metal powder addition does not generally seem to worsen the protective performance of the coatings, compared to the coatings with pure epoxy resin, except for the composite with copper particles which shows a reduced protection performance, but it still continues to be protective.

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Corrosion of high-grade alloys in marine conditions containing the sulphate reducing bacteria

Results from a series of electrochemical tests in seawater containing the sulphate reducing bacteria (SRB) will be reported. Laboratory conditions were set up to initiate and sustain growth of a culture of SRB on a batch culture basis with the aim of assessing the effect of SRB presence and metabolic products on the corrosion behaviour of high grade alloys.

This work represents part of a wider study into the effects of marine organisms (aerobic and anaerobic) on a range of alloys including stainless steels, cobalt based and nickel based alloys. All materials are currently widely used in marine environments and in particular oilfield applications where incidences of reservoir souring increase the need for materials to operate in aggressive conditions.

Electrochemical potentiodynamic and potentiostatic tests were conducted in SRB-containing seawater. Levels of H_2S ranged from a few ppm to 300ppm. Anodic and cathodic electrochemical features due to the presence of SRB have been identified and characterised using microscopical and microanalytical techniques.

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Corrosion risks in the IMO Pathogen Guidelines

The last few years have seen an increasing incidence of serious wastage of ships ballast tanks which has been attributed to the action of sulphate-reducing bacteria, SRB. Since SRB have been with us for many years, it is far from clear just why they should have become such a problem. Nevertheless, the cause has been recognised and preventative measures may be taken. Almost concurrently, the threat to aquatic environments due to the importation of non-native unwanted pathogens has been recognised. Concerted international action has prompted the publication of guidelines aimed at countering this threat. However, the context in which these guidelines were written was very specific and they have potentially dangerous implications for the ballast water-SRB-ship interface. The object of this paper is to focus attention on the implications of the guidelines on unwanted pathogens on this interface.

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Corrosion and hydrogen absorption inhibitors with biocyde action against sulphate reducing bacteria

In order to develop our idea about compatibility in one and the same compounds the functions of corrosion inhibitor (CI), hydrogen absorption inhibitor (HAI) and biocyde against sulphate reducing bacteria (SRB), new organic compounds were synthesized and investigated. In the ideal case the organic substance used in corrosion medium as CI should be useful in suppression the SRB reproduction in this medium and hydrogen absorption by corroding steel.

For corrosion experiments the polycristalline steel plates 50*20*1.5 mm were used. These samples were cut from sheet mild steel ($C< 0.2\%$, $S <0.02\%$, $P<0.02$), mechanically polished and cleaned with slurry of $CaO+CaCO_3$, followed by UV sterilisation. Weighed specimens were placed in the test tubes containing 100 ml of corrosive medium. Several experiments were carried out using a solution of inorganic salts in water (PostgateB medium). Another series of experiments were performed with artificial sea water (3% NaCl solution). Corrosive medium was sterilised by boiling during 2 h before the experiments; 2 ml of 2-days-old accumulation of bacterial culture were introduced under sterile condition. The test tubes were hermetically sealed and thermostatically controlled at 310 K. Every 24 h the cell number was counted using a microscope equipped with a Goryayev camera and phase contrast addition FK-4; pH and redox potential E_h values of media were measured using RADELSKIS OP 210/3 microanalyser.

The organic substances were therefore added after 48 h to test their biocyde efficiency and inhibiting properties against corrosion and steel hydrogenation. After 200 h the specimens were removed from the corrosive medium, the black sediment of FeS was washed out and the specimens were dried and weighed. The anodic dissolution of steel specimens was used to estimate the quantity V_h of hydrogen absorbed by steel specimens during corrosion.. In the reference probes where no inhibitor was added, the redox potential E_h changed slightly to more negative values (up to tens of millivolts) during the first few days, then shifted to the positive direction, usually reaching values of -230...-250 mV; pH value increased to 0.2...0.8. The number of SRB cells increases up to 8*10^7 l^-1 during the first 48 hours and then steadily decreases. The addition of alkyl-aromatic compounds with diethylamine group to the corrosion medium results in a rapid shift of E_h to more positive values followed by a rather slow changing of Eh in the same direction. The number of moving bacterial cells n was already significantly decreased on the 3rd day of exposure and at the end of the 8th day of exposure. The comparison of the time dependences E_h (t) and n(t) for the different inhibitors suggest the sinbat behaviour of $E_h(t)$ and n(t). The corrosion rate of mild steel was less in the corrosion medium with addition of inhibitor. There is sinbat behavior of K(c) and n(c). Electrode potential of steel after addition to corrosion media of alkylaromatic compounds shifts to more positive values. The quantity of hydrogen absorbed by the subsurface layer of steel was less in the specimens corroded in media with added inhibitors than in the reference media. The best HAI in the presence of SRB proved to be that of CI with $R= p\text{-}BrC_6H_4$. This compound was also the best CI and the best biocyde against SRB. The greater inhibiting efficiency of the compounds with $R=p\text{-}ClC_6H_4$ in comparison with that of $R=p\text{-} CH_3C_6H_4$ is in agreement with previous results.

SESSION XV

Posters

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Efficacy of non-oxidizing antimicrobial agents to control hydrozoans

Hydrozoan growth can pose a serious fouling threat to industrial cooling systems that utilize brackish water and seawater. Continuous exposure treatment with oxidizing biocides such chlorine, bromine, and chlorine dioxide have proven to be most effective and poplar methods for controlling hydrozoan fouling. However, increased environmental constraints on the continuous use of oxidizing biocides have imposed stringent limits to this practice. Non-oxidizing antimicrobial agents which are commonly used to control undesirable microbiological growth of algae, bacteria and fungi in industrial cooling water systems may provide an alternative means for controlling hydrozoans. However, their effectiveness to eradicate hydrozoans is relatively unknown. Thus, the objective of this study was to the determine the efficacy of 24 to 48 hour exposures of several non-oxidizing antimicrobial agents to eradicate hydrozoans, *Garveia franciscana,* and prevent their regeneration and growth.

The efficacy of a broad range of non-oxidizing antimicrobial agents, which represent both phosphorous and nitrogen-containing compounds, was investigated and compared to chlorine. Established hydrozoan colonies were exposed to the antimicrobial agents under either static or static renewal conditions. At the end of the exposure, the hydrozoans were stained with trypan blue dye and then placed into continuously flowing seawater for up to seven days. The staining procedure provided a method to distinguish existing growth (stained) from new growth that was unstained. Periodically, hydrozoan colonies were examined to monitor for the presence of hydranths and new growth. Preliminary results have shown that chlorine fed at 2.0 mg/L free residual oxidant as well as the majority of the non-oxidizing antimicrobial compounds fed at up to 10 mg/L active ingredient were effective at destroying hydranths. However, these treatments were ineffective at controlling hydranth regeneration and new growth (elongation of colonies) which occurred within seven days. A 24 to 48-hour biocide exposure does not offer a viable treatment strategy for controlling *Garveia franciscana*. This presentation will discuss the results of these studies and plans to further investigate the efficacy of selected non-oxidizing antimicrobial agents to control hydrozoans.

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Evaluation of silicone technology for fouling release coating systems

The marine environment is extremely diverse and harsh, requiring a varied technology base for protection against biofouling of ships and other submerged structures. The next generation of materials in the battle against marine biofouling are fouling release coatings based on silicone elastomers. While silicones have been investigated for approximately 25 years, commercial application of fouling release coatings is recent. Although there is no substitute for actual field testing of potential coatings, it can be time-consuming, costly, and subject to uncontrolled parameters. Controlled procedures are required during development to generate candidate materials for field testing. The change from toxic based coatings to fouling release coatings has left a void in the test methods for the marine paint developer.

Outlined here are some laboratory evaluation methods for screening silicone based materials. The major emphasis is on measuring toughness, with some attention given to release measurement and adhesion. The standard hardness and other coating measurements are not applicable for defining toughness of silicone elastomeric coatings. A combination of traditional elastomeric techniques (Instron - tensile properties), coating methods (taber shear/scratch test), and new techniques (microhardness) can be used to elucidate the parameters responsible for operational toughness. Toxicant leaching rate is not suitable for predicting the fouling release performance of silicone coatings. Rather, release capabilities can be partly predicted by surface energy measurements in air or underwater. Finally, the methods for measuring adhesion of the multicoat systems must adapted when dealing with silicone elastomeric top coats. A series of silicone coating families will be used to illustrate the techniques necessary to fully evaluate candidate fouling release coatings.

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The use of viability and immunolabelling techniques to determine the presence of pathogenic bacteria, cyanobacteria and parasites in biofilms

Biofilms were developed on the surface of glass and plastic surfaces using three-stage chemostat modelling. *Pseudomonas aeruginosa, Legionella pneumophila, Escherichia coli, Microcystis aeruginosa* and *Cryptosporidium parvum* were present the consortium of biofilm microorganisms. The use of direct immunofluorescence provided a more rapid staining method than indirect immunofluorescence. In the case of samples containing parasites, the background fluorescence was sufficiently low to enable screening, even without washing. Viability of these organisms was determined using propidium iodide and DAPI, combined with examination of the parasite structure using Hoffman modulation microscopy. The biofilms could be screened rapidly using FITC labelling but details of the biofilm structure were not evident using this method. The combined use of immunogold labelling and examination by differential interference contrast microscopy enabled simultaneous visualisation of the total biofilm flora and immunolabelled organisms. Screening of samples using immunogold labelling techniques were however, more time consuming and in some samples there was interference by metal deposits.

MOATE, R.M. and M.B. JONES

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Mechanisms of substrate attachment and settlement in *Tubularia larynx* (Ellis and Solander)

Temporary attachment of the actinula larva of *Tubularia larynx* is brought about by the discharge of nematocysts from almost all the aboral tentacles. Granule containing columnar ectodermal cells line the aboral pole. These cells form the 'stolon bud' on permanent settlement and more than one stolon bud may be formed. Two types of ectodermal granules were identified and are suggested to be important in the 'settlement attachment' or permanent attachment to the substratum.

MOLITORIS, H. P., S. MOSS, S. NEUMEIER, K. RATHBERGER, E. STRAUBINGER and B. ZAUNSTÖCK

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Decomposition of thermoplasts in the sea

Thermoplasts, mostly derived from fossile resources, today are unrenouncable materials because of their recalcitrance, multiple applicability and relatively low price. Increasingly, however, they are causing problems due to exhaustion and price increases of the fossile resources, pollution due to their missing biological degradability and partially by their toxic endproducts. Biologically degradable, biosynthetic plastics with no toxic endproducts would be the solution. A number of such biosynthetic materials such as the bacterial Polyhydroxyalkanoates (PHA, e.g. poly-hydroxybutyrate = PHB, poly-hydroxyvalerate = PHV, poly-hydroxyoctanoate = PHO), fungal Poly-L-Malate (PMA) or even chemosynthetic plastics such as Poly--caprolactone (PCL) could fulfill these postulates. Some of these materials (PHB, "BIOPOL", PCL) are already commercially produced and used. Their biodegradability by bacteria has been shown in most cases, but biodegradation by fungi, the other large group of degrading microorganisms, is still an open question. Since our oceans are increasingly misused as wastedumps, in this study the degradation of plastics is studied especially for marine fungi and in seawater media. Since preferentially oceanic dump grounds belong to the deep sea area, it would hence be very important to know whether these materials could be degraded also under deep sea conditions. Therefore, for the first time, degradation studies of thermoplasts with marine fungi have been performed also under simulated deep sea conditions using recently developed apparatus and methods.

Under surface conditions (atmospheric pressure = 0.1 MPa) degradation studies were conducted on seawater and freshwater media using more than 360 marine and terrestrial yeasts and filamentous fungi of different ecological and systematic groups. Highest degradation rates were found for the chemosynthetic thermoplast PCL. It is used as carbon source and degraded by over 85% of the more than 230 fungi tested. No significant differences in PCL degradation appeared between seawater and freshwater medium and among the different groups of fungi tested. Second in biodegradability were the biosynthetic PHAs (PHB, BIOPOL, PHV, PHO) which are used as carbon source and degraded by terrestrial fungi on freshwater media up to over 50%, by marine fungi, however, by only over 5% of the strains tested. No significant differences in PHA degradation appeared between seawater and freshwater medium and among the different groups of fungi tested. Preliminary tests with the new fungal material PMA showed that only 6% of the strains degraded it on freshwater media, degradation on seawater media is expected not to exceed this figure.

Under simulated deep sea conditions (pressure of 10 MPa = 1000 m, 27°C) the degradation of PHB powder (enzymatic clearing test on agar plates) and PHB sheets (weight loss and enzymatic clearing test) in freshwater media by three highly active terrestrial strains was tested. With all fungi except one, PHB degradation occurred also under deep sea conditions, however, considerably slower and to a lesser extent. Experiments on seawater media, using marine strains, are under way.

From the experiments it is evident that thermoplasts are degraded by fungi also under marine conditions, however, the degree of degradation is strain-specific, is less pronounced among marine strains and decreases with increasing pressure.

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The effect of *Delisea pulchra* secondary metabolites on the attachment, survival and growth of marine bacteria

In the marine environment, bacterial fouling is rapid, ubiquitous and has important consequences for both natural communities and artificial surfaces. Bacteria cause corrosion and loss of efficiency for shipping and aquaculture, and as primary colonisers they affect subsequent macrofouling. Recently, the observed lack of fouling of some marine organisms has been investigated in relation to possible antifouling applications. We have studied a range of unique structurally similar secondary metabolites from the red alga *Delisea pulchra* which are highly effective in inhibiting fouling by a range of organisms including marine bacteria. The effects of *D. pulchra* secondary metabolites against bacteria isolated from a range of sources was investigated with respect to attachment, survival and growth in laboratory media as well as under field conditions. In addition, SEM was used to determine the distribution and abundance of bacteria on the surface of *D. pulchra* and four co-occurring algal species. Extracts and purified metabolites selectively inhibited the attachment of bacteria without broad biocidal effects. Bacterial strains displayed a range of growth responses, from strong inhibition of some strains, comparable with the antibiotic gentamicin, to minimal inhibition of other strains at 50 to 500μg/ml. The sensitivity of bacteria to *D. pulchra* secondary metabolites appears to be source dependent. Strains isolated from rock surfaces are, in general, resistant in terms of growth, while strains isolated from the surface of *D. pulchra* plants display sensitivity. The implications of these differential responses will be discussed.

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A computerised annotated bibliography of marine fouling algae

An interactive programme has been written to provide easy and rapid access to an annotated bibliography of marine fouling algae. The programme has been written in C language, using the software Borland C++, version 3.1 and employs a modern, menu driven interface which can be run with mouse support. Over 500 journal articles, books, chapters, conference and symposia proceedings and reports pertaining to marine algae as fouling organisms are stored in a bibliographic database. Pop-up windows also allow access to a complete checklist of marine fouling algae distributed in the main taxonomic groups Chlorophyta, Phaeophyceae, Rhodophyta, Cyanophyta, Bacillariophyceae Tribophyceae, Dinophyta and Chrysophyceae, as well as alphabetical listings of the full range of the Sea Areas, Countries, Structures and Materials involved. The main function of the programme is to access fouling literature relating to any one or combination of the listed taxonomic groups, species, sea areas, countries, structures and materials. As an example, it is possible to stepwise locate all the bibliographic references pertaining to the occurrence of the fouling species *Ectocarpus siliculosus*, in the taxonomic group Fucophyceae, in the North Atlantic, on the coasts of the British Isles, on panels which are made of tufnol. Using the instructions provided, delegates are invited to examine the demonstration version of the programme.

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Enzymic characterization of sulfate reducing bacteria involved in biocorrosion

Sulfate Reducing Bacteria (SRB) are found in many different environments and have economic and ecological impact. They are involved in the microbial metal corrosion that occurs at neutral pH in anaerobic environments, with production of large amounts of sulfide ion. The consumption of hydrogen from either metal surface or from the ferrous sulfide film on the metal by the action of the hydrogenase enzyme has been described as the major reaction implicated in the corrosion process.

Desulfovibrio desulfuricans subspecies *desulfuricans* strain New Jersey (NCIMB 8313) (D*d*NJ) and *Desulfovibrio* ID-408/94, isolated from environments under active biocorrosion, were grown in large scale.

The present work describes the purification and the characterization of several proteins involved in the dissimilatory sulfate reduction (characteristic metalloproteins of the *Desulfovibrio* genus): **Hydrogenase** (hydrogen:ferricytochrome c_3 oxidoreductase);**Cytochrome c_3** (13 kDa, tetraheme His-heme-His); **Adenylyl sulfate reductase** a key enzyme in microbial dissimilatory sulfate reduction (activation of the sulfate molecule); **Sulfite reductase** that catalyses the six-electron reduction of sulfite to sulfide, another key reaction in sulfate respiration, following the activation of sulfate and its reduction to sulfite and **Ferredoxin**, a simple iron-sulfur protein.

All sulfate reducers are classified by phenotypic characteristics, namely nutrition and morphology criteria. The identification of sulfite reductase is also important to support this classification. We propose a reclassification of D*d*NJ bacterium based on the presence of desulforubidin (sulfite reductase) and spectroscopic and biochemical properties of the proteins isolated that reveal a high phylogenetic similarity with *Desulfomicrobium baculatus* Norway 4.

This work was supported by JNICT (STRIDE), GBF and EUREKA-CAMICO

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Influence of micronutrients concentration on growth and activity of sulfate reducing bacteria

Microorganisms can significantly influence a variety of environmental and economical processes. The role of anaerobic sulfate reducing bacteria (SRB) in corrosion of iron and ferrous alloys is well documented. Many industrial sectors suffer great economic losses and experience health and safety hazards as a result of SRB activities. SRB produce metabolic products such as volatile fatty acids and H_2S which enhance the corrosion process. In addition the involvement of the enzyme hydrogenase of SRB in the cathodic depolarization process is now accepted. The elemental material composition as well as the environmental conditions determine the extension of deterioration.

The aim of this study is to determine the influence of concentration of selenium, nickel and iron on the growth and activity of SRB. As a model microorganism, we have chosen *Desulfovibrio desulfuricans* strain New Jersey (D*dd* strain NJ), a member of the SRB group and hence an important corrosion-inducing species.

Experiments were carried out in batch and continuous reactors with pH and temperature control. Kinetics of growth, sulfate reduction and production of metabolic products for the different concentrations is evaluated. The influence of selenium concentration in the hydrogenase ([NiSe Fe] type) activity of the D*dd* strain NJ is also determined.

This work is supported by the EUREKA Programme (Camico) and Praxis XXI

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Biological aspects on marine microfouling treatments inside simulated industrial pipes

Many industrial plants use enormous quantities of running water in cooling pipes for production cycles. Thus, it is essential to prevent the settlement of fouling organisms which can penetrate pipes, adhere to pipe walls and form large colonies which may partially obstruct piping and slow down the flow rate. Consequently, antifouling treatment which is both technically feasible and economically viable is necessary in order to prevent (or at least slow down) the development of these organisms.

This work aims to identify both which microorganisms are able to penetrate and adhere to piping and assess their response to biocides (in particular chlorine dioxide - ClO_2). Working in collaboration with Caffaro S.p.A. (Gruppo SNIA B.P.D., Brescia, Italy), four experimental cycles were carried out at an experimental plant in the Gulf of Trieste (North Adriatic Sea). The cycles were repeated during all four seasons in order to take into account specific biotic and abiotic factors which may greatly influence colonisation. Glass slides were used as substrate since observation could be made directly and invasive treatment avoided. Species were identified via an optical microscope and, when possible, some species were observed via a scanning electron microscope.

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A biological study of the important marine fouling green alga *Ulothrix flacca* (Dillw.) Thur. in Le Jol

Field studies in the Solent region of the British Isles have revealed the green filamentous alga *Ulothrix flacca* to be an important contributor to a wide range of marine fouling communities. It particularly occurs as a pioneering organism on newly immersed structures and is usually one of the first macroalgal colonisers of toxic antifouling coatings. The present poster is concerned with a light and electron microscopical study of the attachment, germination and growth characteristics of *Ulothrix*. The rationale behind these studies is to contribute towards an understanding of the biology of this alga which might be useful in the future development of more selective control methods. Particular attention has been given to an examination of the zoospore attachment process, the early stages of germination and the growth of the erect filaments under different conditions of temperature, salinity and irradiance. Observations are also presented on the effect of copper on the germination and growth of *Ulothrix flacca*.