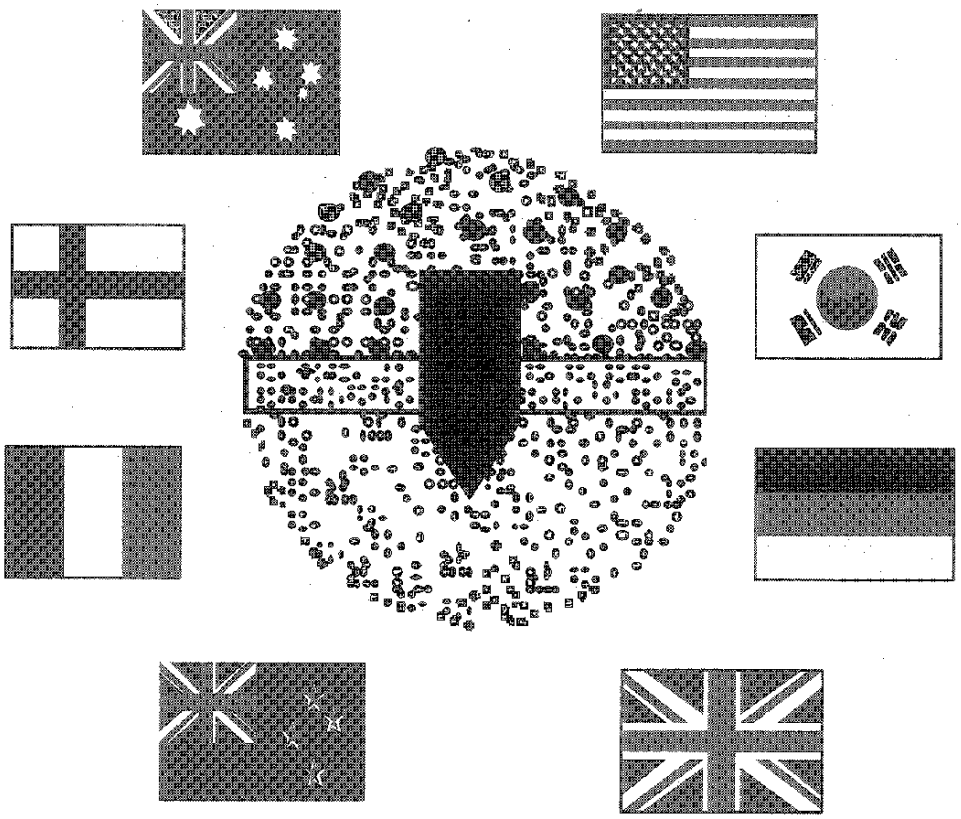
MEMBRANE BIOFOULING

An International Workshop



Sydney, Australia

November 16 -17, 1996

Sponsored by

National Water Research Institute

UNESCO CENTRE FOR MEMBRANE SCIENCE AND TECHNOLOGY

CRC FOR WASTE MANAGEMENT AND POLLUTION CONTROL LTD.

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Prof. Anthony Fane
Dr. René Schneider
Dr. Peter Beatson

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WORKSHOP ORGANIZATION

Background

The concept for this workshop had its origin at the 1995 International Desalination Association World Congress held in Abu Dhabi. While there, Professors Tony Fane (University of New South Wales [UNSW], Australia), Harvey Winters (Fairleigh Dickenson University, USA) and Mr. Ron Linsky (National Water Research Institute, USA) met on several occasions and discussed the International Membrane Science and Technology Conference (IMSTEC) to be held the following year in Australia. A proposal was put forth to hold a workshop following the conference that would focus on the identification of significant biofouling research issues that must be addressed if the technology was to move forward in a timely manner within the next five years. Inasmuch as IMSTEC attracts a significant number of membrane research leaders from throughout the world, the conference would present a significant opportunity for participants to also attend the workshop.

In support of the proposal, the National Water Research Institute (NWRI) provided a US$10,000 grant for the workshop.

Members of the Workshop Organizing Committee, appointed by Prof. Fane, were:

- Prof. Anthony G. Fane, Director, UNESCO Centre for Membrane Science & Technology (UNESCO CMS&T);

- Dr. René P. Schneider, Research Fellow, School of Microbiology and Immunology UNSW; and

- Dr. Peter J. Beatson, Research Associate, UNESCO CMS&T.

Additional financial support was provided by the Co-operative Research Center for Waste Management and Pollution Control (CRC WM&PC) - a Center established and supported under the Australian Federal Government's Co-operative Research Centres Programme.

The date of the workshop was set for November 15-17, 1996. The venue was the UNSW Institute of Administration located south of Sydney at Little Bay. This was an exceptional location which provided excellent facilities in a pleasant environment, all complemented with a fine menu.

Invited delegates were selected on the basis of their internationally-acknowledged expertise in microbiology, physical chemistry and engineering and their availability either from the local research community or attendance at the IMSTEC Conference.

Handout packets (Appendix C) distributed at the start of the workshop included:

- An Agenda.
- Information on the Workshop's organization.
- Significant Issues Worksheets.
- UNSW Institute of Administration Brochure.

At the opening of the workshop, the participants were challenged by the Workshop Facilitator, Ron Linsky, who posed the question, *"What are the most significant issues to be solved in membrane biofouling in the next five years in order to move membrane technology forward?"* The workshop then began with the presentation of five prepared papers (Appendix D) by the following invited speakers.

- *Mechanisms of Fouling Layer Formation*
  Prof. Harvey Winters
  Fairleigh Dickinson University, New Jersey, USA
- *Characterization and Monitoring of Biofilms*
  Prof. Hans-Curt Flemming
  University of Duisburg, Germany
- *Biofilm Prevention and Cleaning of Membrane Systems*
  Dr. Harry Ridgway
  Orange County Water District, USA
- *Cleaning and Disinfection of Biofilm Using Peroxygens*
  Dr. Michael Bird
  University of Bath, UK
- *Surface Interactions and Membrane Polymer Properties*
  Dr. Hans Griesser
  CSIRO Chemicals and Polymers, Australia

Participants were requested to capture on their worksheets any ideas or important issues that emerged during the presentations. In as much detail as

possible, participants were asked to describe the issue, its scientific/technical significance, and to suggest a research approach to solve the issue.

Identification of Concepts - Breakout Groups

The delegates were organized into four groups (see following table) based on similarity of expertise. Each group discussion was facilitated by one of the presenters. Each breakout group met for approximately three hours. Scribes were assigned to each group, and facilities for audio recording were provided.

BREAKOUT GROUPS (f) = Facilitator (s) = Scribe	
GROUP 1 - White ('Microbes')	**GROUP 2 - Orange ('Monitors')**
Mr. Kamran **CHIDA** Ms. Janine **FLOOD** (s) Ms. Lisa **HENTHORNE** Prof. Staffan **KJELLEBERG** Dr. Paul **MARCH** Ms. Andrea **SCHÄFER** Dr. Gabriella **SCHAULE** Ms. Gretel **SILYN ROBERTS** Prof. Harvey **WINTERS** (f)	Dr. Pierre **AIMAR** A/Prof. Nicholas **ASHBOLT** Dr. Peter **BEATSON** (s) Ms. Linda **DUDLEY** Prof. Hans-Curt **FLEMMING** (f) A/Prof. Chung-Hak **LEE** Dr. Greg **LESLIE** A/Prof Zbigniew **LEWANDOWSKI** Dr. Roya **SHEIKHOLESLAMI**
GROUP 3 - Green ('Engineers")	**GROUP 4 - Red ('Chemists')**
Prof. Roger **BEN AÏM** Dr. Michael **BIRD** Mr. Leonard **COSTER** (s) Prof. Anthony **FANE** Mr. Ian **FERGUS** A/Prof. Young Moo **LEE** A/Prof. Marianne **NYSTROM** Dr. Harry **RIDGWAY** (f) Mr. Michael **STEFANIC** Mr. Ye-Kang **YOO**	Mr. Tim **CHARLTON** (s) Dr. Hans **GRIESSER** (f) Mr. Peter **KINGSHOTT** Dr. Jaleh **MANSOURI** Dr. Keith **McCLEAN** Dr. Seyed **SADR GHAYENI** Dr. Rene **SCHNEIDER** Dr. Heather **ST. JOHN** Prof. David **WHITE**

Each group was asked to identify and prioritize key concepts in terms of importance and develop research approaches and solutions. The approach taken by each group varied. The breakout groups began their efforts by filling out the significant issues worksheets. This was followed by each group member presenting one issue to the group. Feedback from the group was added to the

form, where appropriate. The discussion moved around the table until all ideas had been exhausted. All issues were tabulated for presentation to the workshop participants.

Some groups did not use worksheets and chose instead to present their ideas on flip charts or directly onto summary sheets.

Breakout Group Reports

When the workshop reconvened on Saturday, facilitators for Groups 1 and 2 (H. Winters and H.-C. Flemming) presented their lists of issues, significant issues, and approaches. The facilitators for Group 3 (H. Ridgway) and Group 4 (H. St. John and D.C. White) presented their results on Sunday morning.

Groups 2 and 4 provided additional material (see Appendix E) that they felt were relevant to the broader focus of the workshop.

Consolidated Issues

Following the last group presentation on Sunday, the issue title sheets were posted on the walls of the meeting room and numbered in sequence. The Chair then drew the delegates' attention to each issue sheet in turn, asking them to identify other sheets with similar or subordinate concepts. Each issue originator was assured that they would retain the right to either merge their issue into a group of similar issues, or to insist that their issue stand alone. Sheets that had already been subsumed were skipped when they were encountered later in the sequence.

Issues subsumed under a major issue group were included in their entirety in the final report. The consolidated issues can be found in Appendix F.

Ranking

The workshop participants identified 69 issues. However, due to a constraint of time, the priority ranking phase of the workshop was conducted via fax or e-mail in an eight-week following the workshop. The delegates were asked to prioritize the top ten issues from their own perspective. Results were received from 26 of the 37 participants. An analysis of the results is presented in this report in the section entitled "Priority Ranking of Issues and Data Analysis."

Twenty-two additional worksheets were received after the close of the workshop. These issues were not included in the priority ranking or consolidation process; however, they were of merit and therefore appear in this report in Appendix G.

Informal Presentations

Delegates were invited to bring brief reports on their recent and/or unpublished reports on membrane biofouling problems. Copies of the presentations by David C. White, Linda Dudley, and I.S. Chang/J.S. Kim/C.H. Lee appear in Appendix H. Informal presentations by Staffan Kjelleberg and Zbigniew Lewandowski were not made available for inclusion in this report.

PRIORITY ISSUES AND DATA ANALYSES

The rankings of issues were received from 26 of the 37 participants who attended the workshop and were analyzed to determine what differences in individual participants' perceptions of priorities existed among the three sub-groups: (1) chemical engineers, (2) microbiologists, and (3) engineers. A category could contain not less than two individuals to maintain the confidentiality of the ranking process. The smallest group in this workshop contained three individuals and the largest group contained twelve.

The 20 priority issues are presented herein. Also included are tables presenting the analyses of priority ranking and strength of feeling data. These tables contain three types of information, in addition to the title of the issues:

- The numerator of the fraction in the first column (i.e., *Times Picked/Pts.*) is the number of times which that issue was selected by the participants from each group.

- The denominator of the fraction is the total number of points the issue received - ten points being the highest score; nine points being the second, and so on. All issues not selected received zero points.

- The third item of information is given in the column titled *Strength of Feeling*. This is simply the percentage obtained by dividing the total number of points received by the total number of points which the issue could have received if all participants had selected that issue as their first priority.

PRIORITY 1

Analysis of Foulant-Polymer Molecular Interactions

Originator: Ridgway

Significance:

Molecular interactions between foulants (e.g., organics, bacteria, etc.) and separation membranes are poorly understood. These molecular interactions form the basis of bacterial adhesion and initial biofouling. Knowledge of foulant-polymer molecular interactions is needed for the rational design of new cleaning agents or feedwater additives to retard bacterial attachment.

Suggested Approach:

- A multi-disciplinary approach is proposed involving various surface analytical techniques such as ATR-FTIR spectrometry, NMR, and molecular modeling.
- Initial studies should probably be limited to no more than a few foulants (e.g., several EPS types or proteins) and membrane types and could benefit from model studies on well-defined surface chemistries.
- Include effects of water on foulant-membrane molecular interactions.

PRIORITY 2

Molecular Characterization and Modeling of Polymer Surfaces in Hydrated Conditions and Under Biofilms

Originator: Ridgway

Significance:

There is inadequate information concerning the surface molecular conformation of polymer membranes. Knowledge of surface conformations is essential to understanding foulant-polymer interactions and would greatly assist in the design of new polymer membranes with reduced biofouling potentials.

Suggested Approach:

- Application of modern surface analytical techniques to characterize the molecular structure of hydrated polymer membranes.
- Correlation of polymer surface chemistry and molecular structure with organics adsorption and bacterial attachment.
- Molecular modeling of polymer conformations would represent an important part of this study.
- Development of techniques which could characterize surface heterogeneity on a molecular scale, e.g. AFM mapping techniques.

PRIORITY 3

What Are the Molecular Mechanisms that Keep the Biofilm Structure Together ?

Originator: Schneider

Significance:

Knowledge of these mechanisms will help us to:

- Develop antifouling compounds on a rational basis.
- Develop membrane surface chemistries which produce minimal biofilm stability.

Suggested Approach:

Characterize the EPS in biofilms.

PRIORITY 4

Understanding the Use of Signaling Molecules in Biofilm Development

Originator: Kjelleberg

Significance:

It is clear that signaling molecules in the biofilm mediate the expression of many adhesion colonization phenotypes. The interference with these systems can be done in a non-toxic fashion. It is also clear that such signaling systems are wide spread, e.g., the homoserine lactone system.

Suggested Approach:

- Identify environmental conditions that regulate signaling mediated responses.
- Use bio-mimics or antagonists of homoserine lactones or homoserine lactone-like signals.

PRIORITY 5

Understanding Biofilm Formation at the Fundamental Level in Terms of the Exact Chemistry Involved in Cellular Responses

Originator: Kingshott

Significance:

Understanding what triggers a bacterium to change its phenotype as it adheres to the surface, i.e., what surface triggers the production of particular extracellular proteins or polysaccharides.

Suggested Approach:

Use surfaces with well-defined chemistry and structure. In particular, self-assembled monolayers (SAMs) on gold to particularly control the exact chemistry of the surface. Then study the effect of changing the chemistry on cellular adhesion. For example, mixed monolayers of carboxy-, methyl- and amine-terminated SAMs.

PRIORITY 6

Understanding Gene Expression/Biology of Biofilm, Particularly How Different Conditions (e.g., Flow Rate, Temperature, etc.) Regulate Specific Gene Expression via a Signal Transduction Pathway

Originator: Kjelleberg

Significance:

Not provided by originator.

Suggested Approach:

- Invest in molecular microbiology tools generally available.
- Set up a model system and understand genes expressed in a pure culture of an appropriate model organism. When specific expression has been identified, develop probes to see whether the same genes are expressed in more complex systems. The next step would be to determine the function of gene products with a long-term goal of blocking function.

PRIORITY 7

Development of New Membrane Configurations with Low Fouling Propensity and Enhanced Cleanability

Originator: Henthorne

Significance:

If module design could be improved to provide better hydrodynamics, significant reduction in biofouling could be realized. In addition, present module hydrodynamics makes cleaning ineffective. Existing infrastructure further restricts making drastic changes in module design >> must develop a module that can use existing infrastructure.

Suggested Approach:

Transverse flow hollow fiber configuration may provide a low fouling environment. Terrific hydrodynamics can be achieved but high surface area to

volume ratios must be optimized. Fiber potting techniques must be developed for high RO pressures (MF, UF, and NF presently achievable). Importantly - the configuration can be fitted into existing vessels.

PRIORITY 8

Worldwide Database of the Biology of Membrane Biofouling Organisms Should be Constructed

Originator: March

Significance:

It is important to know what is present in systems with problems and what is present in systems that work; to determine a baseline and characterization of tolerable biofilm fouling.

Suggested Approach:

- A committee should select reference sites around the world which routinely determine what organisms and other biofilm parameters are present in the membrane systems.
- The committee should also select standard methods to be employed at the sites. The methods should be modern, rapid, cost-effective, molecular probing, and should be correlated with membrane performance.

PRIORITY 9

Biofouling Control Database and Expert System

Originator: Fergus

Significance:

Knowledge gained worldwide by system users/designers/researchers on system design/membrane selection, operating procedures and experiences (including short-term and long-term shutdown, disinfection techniques) should be accessed to optimize system effectiveness.

Suggested Approach:

Establish database (and expert center/network) of operating/maintenance strategies for control of biofouling for reclamation and desalination applications.

PRIORITY 10

Polymer Properties vs Antifouling and Fouling Release Properties of Microbial Biofouling Biofilms

Originator: White

Significance:

What polymer properties, including heterogeneity, affect antifouling and fouling-release effectiveness? Systematically generate polymers with defined physical and chemical properties and test antifouling and fouling release.

Suggested Approach:

Make polymers with a gradient of properties including mobility effects over time and in the water. Measure antifouling and fouling-release rates in a quantitative way using a bioluminescent bacteria in a laminar flow apparatus as a quantitative initial screening system.

PRIORITY 11

Define the Receptors, Signals, and Blocking Agents Useful in Confusing the Sequential Reactions Necessary for Biofilm Formation and Maturation

Originator: White

Significance:

To stop biofouling before onset, with biodegradable molecule(s) that target receptors using coating polymer reservoirs with slow release properties.

Suggested Approach:

Use bioluminescence in a biofilm organism to identify the response on-line and non-destructively and follow molecular responses in a biofilm system that controls the inoculum, the substratum, and the bulk fluid.

PRIORITY 12

Enhanced Control of Concentration Polarization by Critical Flux and Module Design

Originator: Fane

Significance:

- Bacteria can move up salt concentration gradient by diffusiophoresis. This mechanism would be mitigated by enhanced control of concentration polarization.
- Different scenarios for seawater desalination and low TDS water reclamation.
- The observation of critical flux for biofouling may relate to this. Could be controlled by module (spacer) design.

Suggested Approach:

- Evaluate effect of flux on biofouling, in terms of salt, organics and biosolids polarization.
- Evaluate effect of module (spacer) design on biofilms in terms of salt polarization.

PRIORITY 13

Tailored Modification of Membrane Functional Sites to Prevent Attachment of Specific Foulants

Originator: Nyström

Significance:

Characterization of bonds between foulants and membrane to tell what part of membrane chemical groups should be modified.

Suggested Approach:

- Characterize the fouled condition.
- Modify in order to prevent that.
- Examples: Chelating agents like humic acid need a charged metal ion to chelate with, if that is covered - no fouling. (Inorganic membranes sputtered with neutral gold). Water should be retained at the membrane surface in order to prevent proteins from fouling.

PRIORITY 14

Environment Creation to Minimize/Avoid Fouling; Modification of Feedwater and Membrane Environment to Minimize Biofouling

Originator: Fergus

Significance:

Hypothesis: Biofouling mechanisms/morphology is known (!) so that an environment can be created to avoid or control unacceptable performance caused by biofouling.

Suggested Approach:

Create an environment (e.g., preconditioning feed stream temperature control, pH and maintain optimum conditions within membrane) which avoids biofouling.

PRIORITY 15

Effect of Abiotic Particles (in Particular Humic and Iron Colloids) on the Stability of Biofilms in Membrane Systems

Originator: Dudley

Significance:

Improvements in surfactants and dispersants are contingent upon an understanding of the materials that stabilize the biofilm. Knowledge in this area may influence the most suitable chemical cleaning practices and pre-treatment selection.

Suggested Approach:

- Characterize the effect/role of abiotics on biofilm stability.
- Assess the effects of cleaning solutions on the removal and dispersal of biofilms.
- Correlate cleaning efficiency with abiotic material in the presence of biofilms.
- Determine the mechanisms of abiotic fouling with biological fouling also present.

PRIORITY 16

Promoting the Aggregation of Bacteria for Preventing the Biofouling or Modifying the Nature of the Biofilm

Originator: Ben Aïm

Significance:

Probably not possible with spiral modules but with tubular or HF. Would concern mainly UF/MF more than RO. Could be focused on combined processes like flocculation/MF or PAC/UF used for water treatment.

Suggested Approach:

Looking to the best conditions for the use of flocculants and PAC addition when used in combination with membrane process for enhancing bacterial adhesion/aggregation: consequences on biofilm formation and characteristics.

PRIORITY 17

Need for More Research into the Biological Responses Invoked by Bacteria During Membrane Colonization

Originator: March

Significance:

During RO, bacteria move from the planktonic environment into a non-planktonic environment . It is known that there is surface-specific expression of physiological traits (exopolysaccharide for example), but the mechanisms regulating such expression are not known. The scientific significance is that it is necessary to understand biological responses that are essential for successful surface colonization.

Suggested Approach:

Set up a model system and understand genes expressed in a pure culture of an appropriate model organism. When specific expression has been identified, develop probes to see whether the same genes are expressed in more complex systems. The next step would be to determine the function of gene products with a long-term goal of blocking function.

PRIORITY 18

Create a Database of Membrane Fouling and Membrane Fouling Monitoring

Originator: Lewandowski

Significance:

Organizing information relevant to membrane fouling.

Suggested Approach:

Use existing published papers to organize a system where membrane operators could refer the problem they experience to other known situations.

PRIORITY 19

Adhesion of Bacteria to Membrane by Regulation of Flow Rates. Critical Flux Rates and Velocity of Feed / Brine

Originator: Winters

Significance:

If we knew what the critical flux rates are for RO membranes in relationship to the bacteria and organic concentration, then we could design membrane systems that would have low fouling potential and high repulsion barriers.

Suggested Approach:

Use RO membranes and calculate their critical flux rates and energy repulsion barriers to organisms and organics that are known to cause biofouling. These critical operating rates must be determined at different flux rates, different concentrations of bacteria and concentrations of organics. The effect of temperature and salinity of feed water must be studied.

PRIORITY 20

Controls of Membrane Properties by Tailoring pH and Ionic Environment

Originator: Fane

Significance:

Hans Griesser's observations that lower pH lowers contact angle implies the possibility of less fouling at lower pH. Surface charge will vary with pH.

Suggested Approach:

Evaluate lower pH operating on biofouling. (Possible economic penalty here - need optimization.)

Table 1
Top 20 Issues Ranked by All Participants (26)

RANK	ISSUE	TIMES PICKED/PTS	STRENGTH OF FEELING
1.	Analysis of Foulant-Polymer Molecular Interactions	10/77	29.6%
2.	Molecular Characterization and Modeling of Polymer Surfaces in Hydrated Conditions and Under Biofilms	9/68	26.2%
3.	What Are the Molecular Mechanisms that Keep the Biofilm Structure Together?	12/66	25.4%
4.	Understanding the Use of Signaling Molecules in Biofilm Development	10/58	22.3%
5.	Understanding Biofilm Formation at the Fundamental Level in Terms of the Exact Chemistry Involved in Cellular Responses	8/53	20.4%
6.	Understanding Gene Expression/Biology of Biofilm, Particularly How Different Conditions (e.g., Flow Rate, Temperature, etc.) Regulate Specific Gene Expression via a Signal Transduction Pathway	7/52	20.0%
7.	Development of New Membrane Configurations with Low Fouling Propensity and Enhanced Cleanability	9/51	19.6%
8.	Worldwide Database of the Biology of Membrane Biofouling Organisms Should be Constructed	10/50	19.2%
9.	Biofouling Control Database and Expert System	8/48	18.5%
10.	Polymer Properties vs Antifouling and Fouling Release Properties of Microbial Biofouling Biofilms	7/48	18.5%
11.	Define the Receptors, Signals, and Blocking Agents Useful in Confusing the Sequential Reactions Necessary for Biofilm Formation and Maturation	6/41	15.8%
12.	Enhanced Control of Concentration Polarization by Critical Flux and Module Design	6/37	14.2%
13.	Tailored Modification of Membrane Functional Sites to Prevent Attachment of Specific Foulants	6/37	14.2%
14.	Environment Creation to Minimize/Avoid fouling; Modification of Feedwater and Membrane Environment to Minimize Biofouling	6/35	13.5%
15.	Effect of Abiotic Particles (in Particular Humic and Iron Colloids) on the Stability of Biofilms in Membrane Systems	6/30	11.5%
16.	Promoting the Aggregation of Bacteria for Preventing the Biofouling or Modifying the Nature of the Biofilm	4/29	11.2%

Table 1 (continued)

RANK	ISSUE	TIMES PICKED/PTS	STRENGTH OF FEELING
17.	Need for More Research into the Biological Responses Invoked by Bacteria During Membrane Colonization	4/29	11.2%
18.	Create a Database of Membrane Fouling and Membrane Fouling Monitoring	4/24	9.2%
19.	Adhesion of Bacteria to Membrane by Regulation of flow Rates. Critical Flux Rates and Velocity of Feed/Brine	3/24	9.2%
20.	Controls of Membrane Properties by Tailoring pH and Ionic Environment	3/22	8.5%

Table 2
Top 20 Issues Ranked by Chemical Engineer Participants (12)

RANK	ISSUE	TIMES PICKED/PTS	STRENGTH OF FEELING
1.	Analysis of Foulant-Polymer Molecular Interactions	6/43	35.8%
2.	Molecular Characterization and Modeling of Polymer Surfaces in Hydrated Conditions and Under Biofilms	5/38	31.7%
3.	Promoting the Aggregation of Bacteria for Preventing the Biofouling or Modifying the Nature of the Biofilm	4/29	24.2%
4.	Worldwide Database of the Biology of Membrane Biofouling Organisms Should be Constructed	5/28	23.3%
5.	Development of New Membrane Configurations with Low Fouling Propensity and Enhanced Cleanability	4/27	22.5%
6.	Enhanced Control of Concentration Polarization by Critical Flux and Module Design	4/24	20.0%
7.	Understanding Biofilm Formation at the Fundamental Level in Terms of the Exact Chemistry Involved in Cellular Responses	4/23	19.2%
8.	What Are the Molecular Mechanisms that Keep the Biofilm Structure Together?	5/23	19.2%
9.	Environment Creation to Minimize/Avoid Fouling; Modification of Feedwater and Membrane Environment to Minimize Biofouling	3/22	18.3%
10.	Effect of Abiotic Particles (in Particular Humic and Iron Colloids) on the Stability of Biofilms in Membrane Systems	4/21	17.5%
11.	Understanding the Use of Signaling Molecules in Biofilm Development	3/20	16.7%
12.	Biofouling Control Database and Expert System	4/18	15.0%
13.	Identify Primary Trigger Foulant(s) in Each Particular System and Understand How it Interacts with Membrane Surfaces in Order to Design Strategies to Prevent Fouling	3/18	15.0%
14.	Tailored Modification of Membrane Functional Sites to Prevent Attachment to Specific Foulants	2/17	14.2%
15.	Define the Receptors, Signals, and Blocking Agents Useful in Confusing the Sequential Reactions Necessary for Biofilm Formation and Maturation	2/17	14.2%
16.	Controls of Membrane Properties by Tailoring pH and Ionic Environment	2/14	11/7%

Table 2 (continued)

RANK	ISSUE	TIMES PICKED/PTS	STRENGTH OF FEELING
17.	*In Situ* Measurement of Changes in Optical Density at the Biofilm/Bulk Water Phase Interface	2/14	11.7%
18.	Study of the Relationship Between Membrane Autopsy Results and Plant Fouling/Performance Characteristics	2/12	10.0%
19.	Life-Cycle Characterization of Biofilm Development vs. Performance Under Industrial Conditions	2/11	9.2%
20.	Need for More Research into the Biological Responses Invoked by Bacteria During Membrane Colonization	2/11	9.2%

Table 3
Top 20 Issues Ranked by Microbiologist Participants (11)

RANK	ISSUE	TIMES PICKED/PTS	STRENGTH OF FEELING
1.	What Are the Molecular Mechanisms that Keep the Biofilm Structure Together?	7/43	39.1%
2.	Understanding Gene Expression/Biology of Biofilm, Particularly How Different Conditions (e.g., Flow Rate, Temperature, etc.) Regulate Specific Gene Expression via a Signal Transduction Pathway	5/39	35.5%
3.	Understanding the Use of Signaling Molecules in Biofilm Development	7/38	34.5%
4.	Polymer Properties vs Antifouling and Fouling Release Properties of Microbial Biofouling Biofilms	3/27	24.5%
5.	Define the Receptors, Signals, and Blocking Agents Useful in Confusing the Sequential Reactions Necessary for Biofilm Formation and Maturation	4/24	21.8%
6.	Molecular Characterization and Modeling of Polymer Surfaces in Hydrated Conditions and Under Biofilms	3/21	19.1%
7.	Understanding Biofilm Formation at the Fundamental Level in Terms of the Exact Chemistry Involved in Cellular Responses	3/20	18.2%
8.	Analysis of Foulant-Polymer Molecular Interactions	2/18	16.4%
9.	Need for More Research into the Biological Responses Invoked by Bacteria During Membrane Colonization	2/18	16.4%
10.	Worldwide Database of the Biology of Membrane Biofouling Organisms Should be Constructed	4/17	15.5%
11.	Tailored Modification of Membrane Functional Sites to Prevent Attachment to Specific Foulants	3/16	14.5%
12.	Adhesion of Bacteria to Membrane by Regulation of flow Rates. Critical Flux Rates and Velocity of Feed/Brine	2/16	14.5%
13.	Identification of the Mechanisms of Action of Natural Antifoulants	3/15	13.6%
14.	Development of Useful *In Situ* Detection Technique to Study the Mechanism of biofouling and Interaction Between Membrane Surface and Solution	2/15	13.6%
15.	Obtaining Best Information from Membrane Autopsy	2/14	12.7%
16.	Development of New Membrane Configurations with Low Fouling Propensity and Enhanced Cleanability	3/13	11.8%

Table 3 (continued)

RANK	ISSUE	TIMES PICKED/PTS	STRENGTH OF FEELING
17.	Biological Control of Biofouling Using Grazing Organisms to Increase Biofilm Permeability	2/13	11.8%
18.	Enhance the Possibility of Communication Between Microbiologists and Engineers	2/11	10%
19.	Biofouling Control Database and Expert System	2/11	10%
20.	Add "Probiotic" Bacteria to Establish a Biofilm that Remains Thin and Deters Other Bacteria from Colonizing	2/11	10%

Table 4
Top 20 Issues Ranked by Engineer Participants (3)

RANK	ISSUE	TIMES PICKED/PTS	STRENGTH OF FEELING
1.	Biofouling Control Database and Expert System	2/19	63.3%
2.	Analysis of Foulant-Polymer Molecular Interactions	2/16	53.3%
3.	Polymer Properties vs Antifouling and Fouling Release Properties of Microbial Biofouling Biofilms	2/15	50.0%
4.	Understanding Gene Expression/Biology of Biofilm, Particularly How Different Conditions (e.g., Flow Rate, Temperature, etc.) Regulate Specific Gene Expression via a Signal Transduction Pathway	2/13	43.3%
5.	Development of New Membrane Configurations with Low Fouling Propensity and Enhanced Cleanability.	2/11	36.7%
6.	Understanding Biofilm Formation at the Fundamental Level in Terms of the Exact Chemistry Involved in Cellular Responses	1/10	33.3%
7.	Design a Simpler Cleaner - Low Ionic Strength and Acid/Base Cycling by *in situ* Electrolysis	2/9	30.0%
8.	Molecular Characterization and Modeling of Polymer Surfaces in Hydrated Conditions and Under Biofilms.	1/9	30.0%
9.	Create a Database of Membrane Fouling and Membrane Fouling Monitoring	1/8	26.7%
10.	Environment Creation to Minimize/Avoid fouling; Modification of Feedwater and Membrane Environment to Minimize Biofouling	1/7	23.3%
11.	Effect of Abiotic Particles (in Particular Humic and Iron Colloids) on the Stability of Biofilms in Membrane Systems	1/7	23.3%
12.	Identification and Evaluation of Chemical Dispersants to Maintain Bacteria in Suspension and Prevent their Attachment to Membrane Surfaces	2/6	20.0%
13.	Study the Relationship of Shear Effects to the Velocity and Laminar/Turbulent Flow of the Feedwater in Spiral Wound Element Brine Channels to Adhesion of Biofilms	1/6	20.0%
14.	Expert System to Evaluate Optimal Parameter Selection in Module Autopsy	1/5	16.7%
15.	Worldwide Database of the Biology of Membrane Biofouling Organisms Should be Constructed	1/5	16.7%

Table 4 (continued)

RANK	ISSUE	TIMES PICKED/PTS	STRENGTH OF FEELING
16.	More Intensive Studies on the Relationship of Bactericides and Pretreatment Chemicals with Microbiological Matter which Create Biofilms and Biofouling	2/5	16.7%
17.	Enhanced Control of Concentration Polarization by Critical Flux and Module Design	1/4	13.3%
18.	Tailored Modification of Membrane Functional Sites to Prevent Attachment of Specific Foulants	1/4	13.3%
19.	Which Biofilm Parameters are Strongly Correlated to Which Aspects of Membrane Performance (Transverse Pressure Drop, Flux Decline, Permeate Quality)	1/2	6.7%
20.	Alternate Non-Chemical Biocides	1/2	6.7%

APPENDIX A

LIST OF ACRONYMS

Å	Angstrom Unit (10^{-10} m)
AM	Atomic Force Microscopy
ATR-FTIR	Attenuated Total Reflection Fourier Transform Infrared (Spectroscopy)
AOC	Assimilable Organic Carbon
BAC	Biological Activated Carbon
BDOC	Biodegradable Dissolved Organic Carbon (= dissolved AOC)
cfu	Colony forming unit (in bacterial plate count)
CRC	Co-operative Research Centre (Australian Govt. research foundation)
CAIRO	Commonwealth Science and Industry Research Organization (Australia)
CSLM	Confocal Scanning Laser Microscopy
CTC	A fluorescent redox dye used to detect electron transport activity
DAPI	A DNA-specific fluorescent stain used for microscopic cell counts
ECPO	Extra Cellular Polymer and other Organics
EPS	Extracellular Polymeric Substances
GFP	Green Fluorescent Protein
HPC	Heterotrophic Plate Counts
HPLC	High Performance Liquid Chromatography
IDA	International Desalination Association
IMSTEC	International Membrane Science and Technology Conference
MF	Microfiltration
mRNA	messenger RNA (ribonucleic acid)
MRD	Modified Robbins Device
NF	Nanofiltration
NGT	Nominal Group Technique
NMR	Nuclear Magnetic Resonance
NWRI	National Water Research Institute
PAC	Polyvalent Aluminum Chloride
PEO-PPO	Poly Ethylene Oxide - Poly Propylene Oxide copolymer
RO	Reverse Osmosis
SAM	Self-Assembling Monolayer

List of Acronyms (continued)

SDI	Silt Density Index
SEM	Scanning Electron Microscopy
SLB	Signature Lipid Biomarker
SSIMS	Static Secondary Ion Spectroscopy
TDS	Total Dissolved Solids
TMP	Transmembrane Pressure
UF	Ultrafiltration
UNESCO-CMS&T	United Nations Education, Science and Culture Organization-Centre for Membrane Science & Technology
UNSW	University of New South Wales
UV	Ultra-Violet
XPS	X-ray Photoelectron Spectrometry

APPENDIX B

LIST OF WORKSHOP PARTICIPANTS

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Dr. Pierre Aimar's (Universite Paul Sabatier in Toulouse, France) expertise with MF, UF and NF membranes includes their fouling and cleaning; their applications in the dairy and water industries as well as in biotechnology; the spinning of novel hollow fiber membranes; and the theory of mass transfer in membrane equations, in particular the concept of critical flux.

A/Prof. Nicholas Ashbolt (School of Civil Engineering, University of New South Wales) is a microbial ecologist with interests in the application of rRNA probes to identify biofilm community structure, using cryosectioning, in drinking and wastewater systems, health-related water microbiology (viruses, bacteria, parasitic protozoa), compost pathogens, and the application of flow cytometry to microbiology.

Dr. Peter Beatson *(Workshop Organizer* - UNESCO Centre for Membrane Science and Technology, UNSW and CRC for Waste Management and Pollution Control) is interested in behavior of bacteria at surfaces, currently researching biofouling in a dual (MF-RO) process for extracting high purity process water from untreated domestic effluent. He is collaborating with A. Fane, H.-C. Flemming, R. Schneider, and N. Ashbolt in future laboratory investigations of the development of sewage biofilms on operating RO membranes.

Prof. Roger Ben Aïm (Lab. of Engineering of Environmental Processes, INSA, Toulouse, France) has the following fields of interest related to biofilms: attached biomass biofilters for water and wastewater treatment, membrane bioreactors, and the influence of hydrodynamics on fouling, biofouling, and deposit formation (mainly for microfilters and ultrafilters).

Dr. Michael R. Bird (School of Chemical Engineering, University of Bath) has investigated cleaning of surfaces in many situations, including the effect of peracetic acid and hydrogen peroxide upon the deactivation kinetics of *Pseudomonas aeruginosa* and *Staphylococcus aureus* biofilms established and cleaned in modified Robbins' Device-based flow systems.

Tim Charlton (Centre for Marine Biofouling and Bio-Innovation, UNSW) has expertise in quantitative gas chromatography-mass spectrometry and associated sample preparation methods. He assisted with the recording and collating of information during the workshop.

Mr. Leonard Coster (School of Biophysics, UNSW) is expert in micromanipulation of cells using RF electric fields. He is employed by FuCell Pty. Ltd. to create new types of immune cell hybridomas that produce monoclonal antibodies vital for medical research. He assisted with the recording and collating of information during the workshop.

Linda Dudley (Houseman Ltd. UK) is a Chemical Engineer with Houseman Ltd., a supplier of antiscalants, membrane cleaners, biocides, and technical support services to the Reverse Osmosis industry. She uses her expertise to provide advise and assistance in the control/minimization of biofouling problems in RO plants.

Prof. Tony Fane *(Workshop Chairman* - UNESCO Centre for Membrane Science and Technology, UNSW) is the director of the chemical engineering activities of the UNESCO Membrane Centre. In this group, there are over 20 researchers who work on about 15 projects. His interest in biofouling includes projects funded by the CRC for Waste Management and Pollution Control ('Membrane Systems for Waste Water Reuse') and the Australian Research Council ('Integrated Biofilm Management for Membranes used in Water Reclamation').

Ian Fergus (CRC for Waste Management and Pollution Control) is Program Manager for Water Reuse/Waste Water at CRC WM&PC. He has over 20 years experience with the implementation of membrane technology (RO/CMF/UF/EP) in large systems in Australia for a wide range of applications and is currently accountable for two membrane research projects, 'Membrane Systems for Waste Water Reuse' and 'Conducting Membranes', lead by Memtec Ltd. and the UNESCO Membrane Centre. His interests include control or biofouling in a wide range of applications and networking with 'biofouling' technologists.

Prof. Hans-Curt Flemming (Institute of Water Chemistry and Water Technology, Mülheim a.d. Ruhr Germany) has studied biofouling on reverse osmosis membranes, heat exchangers, and drinking water systems; microbially influenced corrosion of mineral and metallic materials; biological wastewater and solid waste treatment; biological air purification; and microbial ecology of biofilms. He advocates practical anti-fouling strategies: some common mistakes- monitoring of biofilms is not performed on surfaces, countermeasures only rely on biocides, and nutrients are not considered as potential biomass.

Janine Flood (School of Civil Engineering/Centre for Wastewater Treatment, UNSW) is completing a Ph.D. investigation upon biofilms of macrophytes, as used in artificial wetlands for wastewater treatment, incorporating cryosectioning and specific phylogenetic and activity staining techniques. She assisted with the recording and collating of information during the workshop.

Dr. Seyed Bagher Sadr Ghayeni (UNESCO Centre for Membrane Science and Technology, UNSW) has interests including the chemistry of biofilms and membranes; biofilms on membranes in water and wastewater applications; development of low fouling membrane polymers; and operation of membranes under conditions conducive to fouling prevention (e.g., below 'critical flux'). He provided general assistance during the workshop.

Dr. Hans J. Griesser (Division of Chemicals and Polymers, CSIRO Australia) has expertise in polymer surface science; surface modification; thin polymeric coatings, surface analysis; interfacial interactions between polymers and contacting media, especially proteins; biomaterials science; adsorption phenomena; and, surface fouling.

Lisa Henthorne (US Bureau of Reclamation) has expertise in the evaluation of biocides for use with cellulose acetate and thin film composite membranes.

Peter Kingshott (CRC for Eye Research and Technology and CSIRO Division of Chemicals and Polymers) is a Ph.D. student with expertise in surface characterization of polymers and is investigating the interfacial interactions between modified polymer surfaces and the proteins and lipids present in biological fluids. He has a B.Sc.(Hons) in Chemistry from Murdoch University, Perth, Western Australia.

Prof. Staffan Kjelleberg (School of Microbiology and Immunology, UNSW) is conducting research in bacterial adaptation to starvation, and signaling molecules they produce during stresses such as starvation; and bacterial interference in the settlement of marine eukaryotes.

A/Prof. Chung-Hak Lee (Department of Chemical Technology, Seoul National University South Korea) is Research Manager at the Institute of Environmental Science and Engineering, Seoul National University.

A/Prof. Young Moo Lee (Department of Industrial Chemistry, Hanyang University South Korea) is mostly interested in modification of polymer membranes to prevent bacterial fouling. Modification of polymer membranes includes an environmentally responsive grafting technique that will allow bacterial cleaning in response to environmental signals such as a change in temperature or pH, or electricity.

Dr. Greg Leslie (Orange County Water District, California) is a Senior Engineer with responsibility for coordinating the demonstration and pilot testing of UF, MF, and RO membranes for water reclamation. He has published work on biofouling and biocolloids in the Journal of Membrane Science and the Journal of Colloids and Surfaces.

A/Prof. Zbigniew Lewandowski (Center for Biofilm Engineering, Montana State University) has expertise in biofilm structure and function, water chemistry, electrochemistry, chemical sensors, and microbially influenced corrosion.

Ronald Linsky (*Workshop Facilitator* - National Water Research Institute) has been associated with water science technology in public and private sector programs and projects for nearly 25 years. During that period he has served as Director of Research, Chief Technical Advisor, and/or Executive Director at the national and international levels. For the last five years has been the creative force behind the development of the National Water Research Institute in California, USA, serving as its Executive Director.

Dr. Jaleh Mansouri (UNESCO Centre for Membrane Science and Technology, UNSW) has interests in the surface characterization of membranes, and the prevention of biofouling by membrane modification through new polymer design or treatment of available commercial membranes. She provided general assistance during the workshop.

Dr. Paul March (School of Microbiology and Immunology, UNSW) has expertise in microbial genetics and physiology. His broad area of interest is the molecular mechanisms employed by marine bacteria to colonize surfaces. He is leading projects on differential gene expression by bacteria observed upon initial colonization of a surface and the development of genetic screens and selection procedures to obtain mutated bacterial strains defective in the ability to colonize surfaces.

Dr. Keith McClean (Division of Chemicals and Polymers, CSIRO, Australia) is a microbiologist with expertise in the biofouling of industrial systems, especially in the petroleum industry and anaerobic digestion. He is currently working as a surface scientist on polymer surface modification and analysis.

A/Prof. Marianne Nyström (Lappeenranta University of Technology, Finland) has interests in the interaction of particles with membranes; fouling of membranes, and the characterization of the membrane and fouling layer in terms of charge, structure, and hydrophilicity; the modification of membranes; applications of membranes to protein fractionation and pulp and paper effluents; and the MF, UF, and NF processes.

Dr. Harry F. Ridgway (Orange County Water District, California) has interest and expertise in membrane biofouling, biodegradation, and molecular modeling.

Gretyl Silyn Roberts (School of Biological Sciences, University of Auckland) is a Ph.D. student. Her thesis topic is "Development of biofilms on rock substratums in wetlands used in wastewater treatment," an ecological study of the bacterial populations which establish in the first two months of wetland operation. It includes surface characterization of the rock substratum and bacterial cells. Biofilm population dynamics are followed by *in-situ* hybridization with confocal scanning laser microscopy and cryosectioning.

Dr. Heather A.W. St. John (Division of Chemicals and Polymers, CSIRO, Australia) has expertise in surface characterization and modification of polymers by techniques such as XPS, SSIMS and SEM, the development of non-fouling coatings, and development of methods to characterize the initial stages of biofouling.

Andrea Schäfer (UNESCO Centre for Membrane Science and Technology, UNSW) is undertaking a Ph.D. on the organic fouling of membranes. She provided general assistance during the workshop.

Dr. René Schneider *(Workshop Organizer* - School of Microbiology and Immunology, UNSW) has expertise in microbial physiology; microbial adhesion; the role of conditioning films in adhesion, and the effects of their composition on microbes; the biofouling of MF and RO membranes in wastewater treatment; and the biodegradation of pollutants.

Dr. Gabrielle Schaule (Institute of Water Chemistry and Water Technology, Mülheim a.d. Ruhr Germany) is a chemist and microbiologist investigating biofilms (biofouling) in technical water systems, in particular on membranes; the characterization and analysis of biofilms; and health-related water microbiology (waterborne pathogenic microorganisms).

Dr. Roya Sheikholeslami (School of Chemical Engineering and Industrial Chemistry) is interested in the interactive effects of inorganic fouling and biological fouling.

Michael I. Stefanic (Fluid Systems Corp., USA) is International Sales Manager for FSC, a manufacturer of UF, NF, and RO membrane elements. They assist RO equipment manufacturers with the application and design of RO units and systems.

Prof. David C. White (Oak Ridge National Laboratory, USA) is a Distinguished Scientist with the University of Tennessee. His expertise includes the study of the microbial ecology of biofilms in biofouling, bioremediation (groundwater), rhizospheres, and indoor air using on-line, non-destructive and high resolution signature biomarker analysis.

Dr. Harvey Winters (Fairleigh Dickinson University, USA) has been actively engaged in the study of biological and organic fouling of reverse osmosis (RO) membrane surfaces. Dr. Winters has extensively published research papers in the Journal of Desalination. At the 1995 Desalination Conference in Abu Dhabi, Dr. Winters' paper was recognized as the outstanding research paper in the conference. Currently a Professor in the Department of Biological Sciences, Dr. Winters has been at Fairleigh Dickinson University since 1972 where he has been both Director of the Desalination Technology Transfer Center and Director of the Biology Program.

Je-Kang Yoo (Sunkyung Engineering & Construction Ltd.) is Manager at the Research and Development Technical Centre of one of South Korea's largest engineering firms.

APPENDIX C

CONTENTS OF HANDOUT PACKETS

- **Agenda**
- **Information on Workshop's Organization**
- **Significant Issues Worksheet**
- **UNSW Institute of Administration Brochure**

NWRI-UNESCO CMS&T BIOFOULING WORKSHOP PROGRAM

FRIDAY 15TH NOVEMBER

17.00 on	Arrival, assignment of rooms (please go to Reception)
17.30	Pre dinner drinks, followed by a word from the Venue Manager
18.30	Dinner, organizing the meeting

SATURDAY 16TH NOVEMBER

07.30	Breakfast
08.00	Registration
08.20	**Opening** (T. Fane), and **'The Challenge'** (R. Linsky)
08.30	**Invited Presentations** ('Thought Provokers')
08.30	1) Mechanisms of Fouling Layer Formation. H. Winters
08.55	2) Characterization & Monitoring of Biofilms. H. Flemming
09.20	3) Prevention and Cleaning - I: H. Ridgway,
09.45	II: M. Bird
10.00	4) Surface Interactions & Polymer Properties. H. Griesser
10.25	Tea/Coffee
11.00	**Breakout Groups**
13.00	Lunch and Relaxation
14.30	**Open Discussion** (presentation from Group 1)
16.00	Tea/Coffee
16.30	**Open Discussion** (presentation from Group 2)
18.00	Pre-dinner drinks
18.30	Formal Dinner

SUNDAY 17TH NOVEMBER

08.00	Breakfast
08.30	**Open Discussion** (presentation from Group 3)
10.00	Tea/Coffee
10.30	**Open Discussion** (presentation from Group 4)
12.00	Lunch
14.00	**Open discussion** - prioritize/consolidate issues, identify future work (leaders - R. Linsky, T. Fane, +?)
16.00	Tea/Coffee and Close of Workshop: R. Linsky

Breakout groups (in no particular order):

'Cleaners'	Scribe - Leonard Coster
'Monitors'	Scribe - Peter Beatson
'Chemists'	Scribe - Tim Charlton
'Microbes'	Scribe - Janine Flood

General Assistants- Jaleh Mansouri, Seyed Ghayeni, Andrea Schäfer

MEMBRANE BIOFOULING

How Can We Beat It?

International Workshop November 16-17th 1996

INTRODUCTION

Managing biofouling of membranes is vitally important for the successful use of membranes, particularly in water production and reuse. Although the topic has received considerable attention it is generally agreed that the problem is far from being solved. This meeting brings together a group of people with a range of expertise that could be applied to overcome the membrane biofouling problem.

Our **aim** is to answer a **single question** from the following perspectives:

- Membrane Structure and Dynamics
- Bacterial Adhesion
- Biofilm Analysis - in situ, continuous, non-destructive techniques
- Surface and Thin Film Chemistry
- Field experience of Membrane Biofouling situations

Our **approach** is to examine ways to better evaluate parameters that are not well understood in the development of biofouling (membrane properties and the microenvironment); new and emerging techniques for biofouling characterization; new techniques for biofouling monitoring; and the most effective techniques for controlling biofouling of membranes.

WORKSHOP STRUCTURE

PART ONE - Opening (Prof. Tony Fane)

The Challenge (Ron Linsky)

"What are the most significant issues to be solved in the next five years in order to move Membrane Technology forward?"

PART TWO - Thought Provokers (2 hours)

Designated speakers will deliver prepared papers, each approx. 15-30 minutes. They will get the workshop participants thinking on the following key areas:

Topic 1: Mechanisms of Fouling Layer Formation.
Prof. Harvey Winters *(Fairleigh Dickinson University, New Jersey USA)*

Topic 2: Characterization and Monitoring of Biofilms.
Prof. Hans-Curt Flemming *(University of Duisburg, Germany)*

Topic 3: Prevention and Cleaning.
Dr. Harry Ridgway *(Water Factory 21, Orange County Water District USA)*
Dr. Michael Bird (University of Bath UK)

Topic 4: Surface Interactions and Membrane Polymer Properties.
Dr. Hans Griesser *(CSIRO Chemicals and Polymers, Australia)*

TASK - delegates to contribute their ideas **on the sheets provided** during the talks, or as they think of them:

- Issue
- Scientific/Technical Significance of the issue
- Suggested Approaches (?solutions - may not come out at this stage)

PART THREE - Breakout Groups (2 hours)

The delegates break out into four groups of similar expertise. They pool their idea sheets and consider each issue, with the aim of...

TASK - Identifying a few **key issues**, PRIORITIZING them in terms of importance, coming up with some research approaches and solutions.

Tools - flip chart, someone to write, audio recording & assistant.

PART FOUR - Open Discussion (Four 1.5 hour sessions)

The groups come back together. **"Round Table" discussion:** In each session one of the four groups will present their lists of priority problems and suggested answers. These sessions will be moderated by the reporter(s) of each group. Other delegates add their comments, new answers, etc.

TASK - To attack problems identified as important to each discipline (microbiologists, membrane manufacturers, etc.) from numerous perspectives other than that of the proposers.

Tools - as above.

PART FIVE - Summary (2 hours)

General Discussion, chaired by Ron Linsky, Tony Fane, and... ?

TASK - "Answer" the Question! Consolidate issues, agree on a **prioritized list** of **key issues**, how they could be investigated, who has the expertise and resources, recommend approaches to combating membrane biofouling. The **result** should in essence be a point form outline of the planned **Proceedings**, the 'duties' for which will also be assigned at this time.

Tools - as above.

INFORMAL PRESENTATIONS

Delegates have been invited to bring **brief reports** on their recent or unpublished research, or on a membrane biofouling problem, to present to the meeting in the relevant session.

Please note:

- Presenters must notify the Discussion Leaders before the session starts.
- Permission to present is at the discretion of the Discussion Leaders.
- *This is not a Conference. Presentation must be limited to 3 or 4 minutes and a couple of slides or overheads.*
- A written summary (1-2 typed pages) for the Proceedings would be appreciated.

A white board, overhead projector, slide projector, VHS/NSTC video projector, and flip chart are available for presenters. An assistant can be made available as note-taker, as well as operate A/V aids.

From the Organizers: *The "germ" of this Workshop came from the meeting of Dr. Ronald Linsky, Prof. Harvey Winters, and Prof. Tony Fane at the 1995 IDA World Congress at Abu Dhabi. The Organizers would particularly like to thank Dr. Linsky for his enthusiastic support, and for the generous donation from the* ***National Water Research Institute*** *that has financed the bulk of this meeting. The financial support of the* ***CRC for Waste Management and Pollution Control Ltd.*** *is also warmly acknowledged (CRC WM&PC is a center established and supported under the Australian Federal Government's Co-operative Research Centers Program). To the delegates, thank you for your*

priceless contributions, we hope that you will have an enjoyable, challenging, and fruitful weekend.

With best regards -

Prof. Anthony G. Fane (Director, UNESCO Center for Membrane Science & Technology);

Dr. René P. Schneider (Research Fellow, School of Microbiology and Immunology UNSW);

Dr. Peter J. Beatson (Research Associate, UNESCO CMS&T).

NWRI-UNSW/UNESCO CENTRE FOR MEMBRANE SCIENCE AND TECHNOLOGY

INTERNATIONAL BIOFOULING WORKSHOP

Significant Issues

Originator Name: _____

Issue: _____

_____

_____

Scientific/Technical Significance:

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Suggested Approach:

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Signature: _____

Information and Facility Guide

INSTITUTE OF
ADMINISTRATION

The mission of the Institute of Administration is to improve the quality of management in Australia and Worldwide through excellent courses conducted within a creative and friendly learning environment.

THE UNIVERSITY OF NEW SOUTH WALES

Welcome to the unique environment of the Institute of Administration's Executive Retreat at Little Bay.

The institute of Administration, situated over the water's edge in the grounds of Prince Henry Hospital, is strategically located and custom-built as a fully residential training facility, the peaceful environment is conducive to stretching the individual's imagination and perspective in both a professional and personal sense.

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Memorabilia, fax, dry cleaning and bar accounts should be settled at the Administration Manager's Office. Payment may be made by cash, cheque, Mastercard, Bankcard or Visa Card. Please settle accounts on the final day of your stay.

Alarm

The security alarm for the lower level of the building is set at 11:00 pm and switched off at 6:00 am each day. The stairway doors to the accomodation level can be used at any time without activating the alarm system.

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A bar is located in each Common Room and is open from 9:00 pm to 11:00 pm.

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A common room/lounge area is allocated to each group in residence, where participants can relax with the daily newspaper and enjoy morning and afternoon tea along with supper. These rooms also have self service bar facilities.

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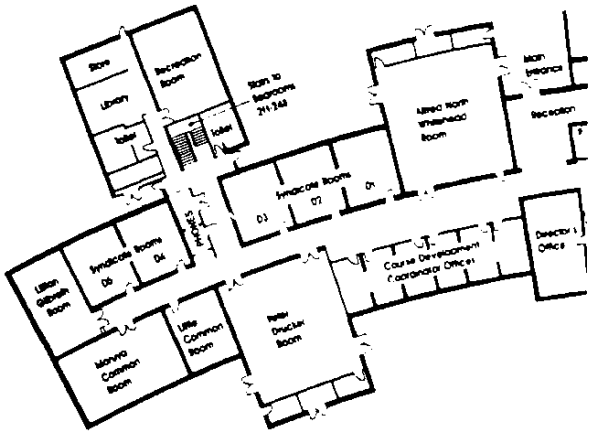
Breakfast:	7:30 am - 8:00 am
Lunch:	12:30 pm - 1:15 pm
Dinner:	6:00 pm - 6:45 pm

Meal times may vary according to program requirements.

Wines are served with evening meals and we request that they not be removed from the Dining Room. Iced water and juices are also available.



Special Dietary Arrangements

If you have requested a special diet, please advise the Dining Room staff as your table is being served.

If you have not advised us of your special diet requirements, please inform your Course Coordinator, prior to your first meal.

Dry Cleaning/Laundry

The accomodation level of the Institute has two laundries equipped with washing machines, dryers, irons and ironing boards.

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Fax facilities are available at Reception.

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Your key ring contains a key for your room and a key for the car park access doors on the accomodation level. Please return keys to reception on the final day of your program.

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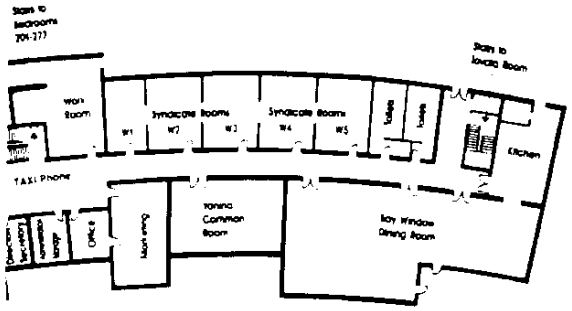
The institute maintains a small library of general reference books, management magazines and journals.

LOWER LEVEL



Mail

The mail courier collects from reception at approximately 9:30 pm and 1:30 pm on weekdays. Stamps are available at reception.

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Incoming messages, faxes and mail are left at the Message Board located on the wall opposite the Alfred North Whitehead Seminar Room. The Duty Manager is available to take urgent messages outside normal business hours.

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A range of memorabilia for sale is on display in the cabinet opposite the Administration Manager's Office.

Order forms are located in your room. Completed forms should be given to your Course Coordinator who will hand you the items requested.

Reception

The Front Desk is open from 8:30 am to 5:30 pm Monday to Friday and as required for new arrivals.

Recreation

Golf & Tennis

The Institute is surrounded by the Coast Golf and Recreation Club, where through cooperation, participants are offered member concessions for use of the golf course, by prior booking with the Golf Club Professional. We can also, by prior arrangement, gain the use of tennis courts within the Prince Henry Hospital Grounds.

Little Bay Walks

We recommend that you use the guide "Cape Banks and Little Bay Walks" located in your room. It provides a brief history of the area and details of walking and jogging tracks.

Shops

At the Hospital entrance you will find a Post Office and General Store/Newsagent. The nearest chemist is at Malabar Junction, approximately two kilometres north along Anzac Parade.

Eastgardens, a Westfield Shoping Centre, is located on Bunnerong Road, Pagewood, a short drive from the institute.

Smoking Policy

The institute is a TOTAL smoke-free zone.

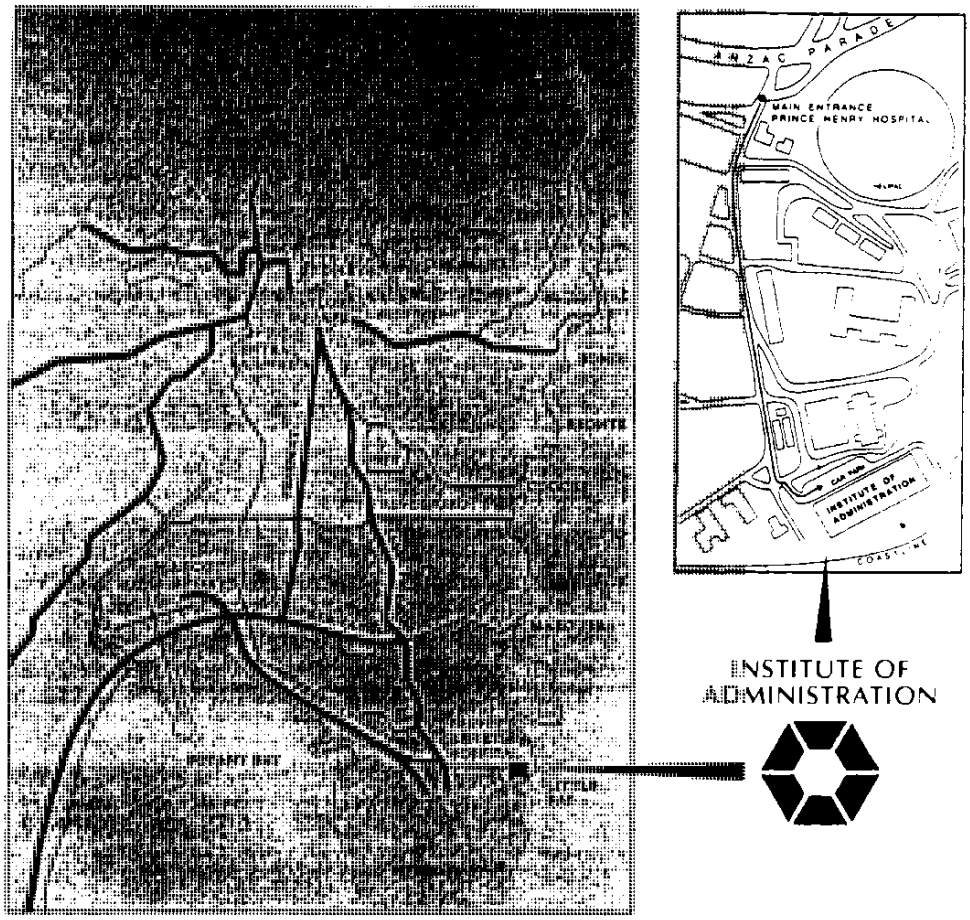
Storage Facilities

Storage facilities are located on the accomodation level.

Note: Valuables may be secured in the Institute's safe by arrangement with your Course Co-ordinator.

Telephones

Public Telephones, including a credit card access phone, are located on the lower level opposite the Peter Drucker Seminar Room. Telephone change and phone cards are available from reception.



The Institute of Administration is conveniently located for local, interstate and international participants.

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APPENDIX D

FORMAL PRESENTATIONS

Mechanisms of Fouling Layer Formation

by

Harvey Winters

School of Natural Sciences, Fairleigh Dickenson University

Teaneck, New Jersey USA

Characterization and Monitoring of Biofilms

by

Hans-Curt Flemming

Institute for Water Chemistry and Water Microbiology

University of Duisburg, Germany

Biofilm Prevention and Cleaning of Membrane Systems

by

Harry F. Ridgway

Orange County Water District

Fountain Valley, California USA

Cleaning and Disinfection of Biofilms Using Peroxygens

by

Alex Blanchard*, Michael Bird**, John Wright*

*School of Biological Sciences, University of Bath, UK

**School of Chemical Engineering, University of Bath, UK

Surface Interactions and Polymer Properties

by

Hans J. Griesser

CSIRO Chemicals and Polymers

Clayton, VIC, Australia

Mechanisms of Fouling Layer Formation

Harvey Winters
School of Natural Sciences, Fairleigh Dickinson University

If a solid surface is immersed into a body of natural water, a series of events occur, which leads to the formation of a microbial film. The film, at first, is simple consisting of a few species of primary periphytic bacteria, but later it becomes more complex, containing an assortment of microorganisms and complex organic macromolecules. Such a film can now be detected with the unaided eye.

A microbial film will always appear on a solid surface immersed in natural water. However, it is the "rate" of formation which will vary depending on the nature of the solid surface and the natural water environment.

ZoBell and Allen were the first to observe the consistent and rapid formation of the microbial film on solid surfaces immersed in seawater. ZoBell noticed the firm irreversible nature of the bacterial attachment, which has been confirmed by other researchers, such as Marshall, Stout, and Mitchell.

Stark and Harvey in the early 1940's suggested that solid surfaces served to concentrate nutrients from natural waters, which then promoted microbial activity. ZoBell experimentally supported these early studies by showing that inert surfaces adsorbed and concentrated nutrients in seawater, thereby promoting attachment and growth of primary periphytic bacteria. Do primary periphytic bacteria attach to surfaces because that is the site of the greatest nutrient concentration?

Chemical Conditioning of Surface

The earliest event in the mechanism of bacterial attachment is the so-called "chemical conditioning" or "molecular fouling." Baier and Loeb & Neihoff have shown that immediately after a clean solid surface is exposed to natural water

and seawater polymeric organic compounds are adsorbed. This "chemical conditioning" or "molecular fouling" imparts a negative surface charge to the "fouled" substrate which alters the physical and chemical characteristics of the surface. A "conditioned" surface can now concentrate low molecular weight substances used as a food supply for periphytic bacteria. A "conditioned" surface is presumed to also facilitate a strong adhesion between the periphytic bacteria to the surface. Does a "non-conditioned" surface form a biofilm?

The polymeric organic compounds that are adsorbed to the surface are believed to be humic-like material or some sort of glycoproteinaceous material. Membranes exposed to seawater immediately develop a humic acids fouling layer. This suggests that humic acids or humic acids-like material may be the important "conditioning" agent present in seawater. Because of the ubiquitous nature of humic acids in natural waters, can these compounds be the universal "conditioning" agent?

The rate of microfouling may well be related to the concentration of "conditioning" or humic-like material in the water and its adsorption rate onto the surface. Is it possible to measure the concentration of "conditioning" or humic material?

Nutrient Adsorption to the Surface

Does the adsorption of "conditioning" agent or humic material have a positive effect upon adsorbing nutrients (sugars, amino acids, etc.)? Mitchell and his coworkers have shown that chemical substances in natural waters can have either positive or negative chemotactic effect upon primary periphytic bacteria. Do humic acids have a positive chemotactic effect upon the attachment of primary periphytic bacteria?

What appears certain is that microbial films are formed as a matter of bacterial survival. The bacteria will migrate to the site where the nutrient concentration is the greatest. This is the important characteristic of primary periphytic bacteria; they will adsorb to a surface, if the nutrient concentration there is greater than in the bulk liquid. ZoBell showed that the ratio of the numbers of bacteria in water and on the solid surface was influenced by the concentration

and kind of organic matter present. All of this confirms the early observations of ZoBell which described the importance of surfaces for growth and survival of aquatic bacteria.

Corpe proposed that, theoretically, at least, if one could block this "conditioning" phase, then one could stop the entire process. Research must be undertaken dealing with the identification and properties of "conditioning" agents. If surfaces could be modified to "reject" these "conditioning" agents or be made unacceptable for their adsorption, then nutrient adsorption and bacterial attachment would be impeded.

Bacteria as Colloidal Particles

Marshall was the first to recognize that bacteria suspended in an electrolyte exhibited properties that were characteristic of colloidal systems. Rutter and Vincent subsequently also suggested that bacteria may act as colloidal particles, whose adsorption kinetics were directed by forces between the bacteria and the surface. The behavior of bacteria and their interaction with a surface can be considered in terms of colloidal chemistry as proposed by Marshall and supported by Mitchell, Corpe, and Winters. Bacteria at pH between 5 and 9 usually possess a net negative charge. Since most solid surfaces possess a negative charge, it is unlikely that bacteria would be attracted to these surfaces by electrostatic interactions.

A negatively charged colloidal surface tends to attract a diffuse layer of cations, while anions are excluded. This is referred to as the electrical double layer. The thickness and extent of this double layer is dependent upon the concentration and valency of the ions. As the concentration and valency increase, the thickness of the double layer decreases. A bacterium and a surface will both possess a double layer and their cation layers will overlap. When the double layers are great, there exists a double layer repulsion energy which is sufficient to overcome attractive Van der Waals forces and no adsorption or surface interaction will occur. While Van der Waals attractive forces remain constant between a particle and a surface, the double layer repulsive forces may vary.

Bacteria can overcome this double layer repulsion energy and become firmly attached to a surface. Does the "conditioning" of the surface affect the double layer repulsion energy? Does "conditioning" of the surface enhance the sorption of the more hydrophobic part of the bacterium? Ridgway has shown that the more hydrophobic bacteria attach more rapidly and to about a 50 fold greater extent than the more hydrophilic bacteria. Does the production of extracellular acidic polysaccharides by primary periphytic bacteria aid in the polymeric interaction in the adhesion process?

The Membrane Surface and the Microfouling Process

All membrane surfaces possess a characteristic charge at appropriate pH which is referred to as the zeta potential value. Ridgway has shown that the rate of bacterial adhesion and fouling is dependent upon the nature of the membrane surface and its zeta potential value. Does the zeta potential of the membrane surface affect the double layer repulsion barrier? Can a membrane be developed which resists "conditioning" and also possesses a zeta potential which would equate into a great enough repulsion barrier to also resist bacterial adhesion?

When the membranes are used for desalination, such as in Reverse Osmosis (RO), other factors and forces must now be considered in dealing with the repulsion energy barrier. In RO desalination, the raw water supply is usually subjected to chemical pretreatment which will modify the surface charge and affect the double diffusion layer of bacterial and organic colloidal particles. Chlorination of the raw water supply is usually performed to kill the existing bacteria and thus inhibit microbial fouling. In many RO applications, because the RO membrane is susceptible to oxidation, the water is then dechlorinated. Numerous instances have been reported where chlorination-dechlorination has promoted microbial fouling, rather than inhibiting it. Is the rate of microbial fouling greater with chlorine-inactivated bacteria than with living bacteria? Does chlorine, a strong oxidizing agent, affect the double layer repulsion energy barrier of the bacterium, thereby promoting adhesion and fouling? Does the addition of acid, antiscalants, coagulation agents, such as iron and organic polyelectolytes, affect the double layer repulsion energy barrier?

Does the desalinization process, in itself, affect the repulsion energy barrier? Permeation flux is a force which brings the organic and bacterial colloidal particle closer to the membrane surface. Do membranes with greater flux rates possess a lower repulsion energy barrier? Does concentration polarization at the membrane surface, which increases the fouling rate by increasing the salt concentration and thus affecting colloidal stability, decrease the repulsion energy barrier between the bacterium and the membrane surface? Does the feedwater/brine velocity across the membrane surface, which plays a very important role in the fouling process, affect the repulsion energy barrier and adhesion?

Characterization and Monitoring of Biofilms

Hans-Curt Flemming
Institute for Water Chemistry and Water Microbiology, University of Duisburg

Early warning of biofilm development is mandatory. An attempt to obtain this information is the practice of sampling the water phase. However, as biofilms contaminate the water phase irregularly, no correlation can be established between water data and location or extent of biofilm growth. Data acquired from the water phase, although costly, usually are of no help to prevent biofouling problems effectively. Thus, only sampling of representative surfaces can provide the information required.

The classical way of biofilm sampling is either to scratch material from a more or less defined area of accessible surface or to expose test surfaces. The best known device for the latter purpose is the so-called "Robbins device" (Ruseska et al., 1982). Basically, the device consists of a screw which fits into a pipe, with contact of the front surface to the water phase. It is removed after a given time. All classical methods operate off line and are genuinely destructive. The deposit is removed and analyzed in the laboratory. The parameters have been compiled earlier (Flemming and Schaule, 1996) and include:

- *Microbiological parameters* such as morphology and number of organisms, heterotrophic plate counts (HPC), number of sulphate reducers, sulfur oxidizers, nitrifiers, acid formers, fungi, protozoa.
- *Chemical parameters* such as water content, total organic carbon, total content of nitrogen and phosphorus.
- *Biochemical parameters* such as the content of protein, carbohydrate, uronic acid, lipid, lipid-bound phosphorus, polyhydroxyalcanoate, ATP, DNA, and respiratory activity (TTC, CTC) or hydrolases.
- *Physical parameters* such as weight, thickness, volume, spectroscopical properties.

The ultimate detection of biofilms is carried out in the laboratory. In Table 1 some parameters suitable for the detection of biofilms are summarized

(Flemming and Schaule, 1996). For screening, the contents of water and organic carbon are indicative; if these are high, there is a high probability that the material is a biofilm. The presence of ATP or respiratory activity indicates living organisms. It is wise to use more than one parameter for the characterization of a biofilm. If the occurrence of a problem can be related to the actual presence of biofilms, the diagnosis "biofouling" is justified. The choice of parameters is, of course, attributed to the given system and the question to be solved. A good overview of these methods is given by Geesey and White (1990). Off line sampling involves some clear advantages. The first is that many methods are available already, that practically all parameters of interest can be determined, that the data is usually reasonably accurate, and that they can be compared to a large body of literature data. However, some disadvantages must be taken in account. The first disadvantage is that the methods operate off line, with sophisticated laboratory analyses, which take usually days until results are obtained. Classical methods all are destructive, preventing the ability to observe the same sample along a period of time. In addition, the methods usually include requirements which exceed average microbiological routine investigations and have to be carried out by specialized personnel.

Table 1: Examples for Biofilm Parameters

Parameter	Detection Method, Reference
Water content	24-h, 110°C
Organic carbon	TOC, COD, incineration loss
Protein	Bradford, 1976
Carbohydrates	Dubois et al., 1956
DNA	Thomanetz et al., 1983
Lipids	Geesey and White, 1990
Muramic acid	Geesey and White, 1990
Polyhydroxybutyrate	Geesey and White, 1990
Total cell number	Meyer-Reil, 1978
Colony forming units	Various standard methods
ATP	Chalut et al., 1995
Hydrolase activity	Obst and Holzapfel-Pschorn, 1990
Respiratory activity	Schaule et al., 1991
Indolacetic acetic acid production	Bric et al., 1991
Catalase activity	Line, 1983

Advanced Methods

An ideal monitoring device should provide the following information about biofilms: *location, quantity, thickness, spatial distribution, matrix stability, kinetics of formation and removal.* The information should be provided *on line, in situ, non-destructive, in real time, continuously and even from inaccessible sites. Continuous acquisition and processing of data should be possible.* Physical methods seem to be the most promising option for these requirements.

Table 2 gives an overview about the principles already in application for biofilm monitoring, although the list is incomplete. It includes electrochemical, optical, spectroscopical, hydrodynamical (fluid friction, filtration resistance) methods, measurements of heat transfer, viscosity, speed of sound, viscosity (quartz crystal microbalance, QMB; surface acoustical waves, SAW) - just to name a few of the possibilities. The criteria for the usefulness of such methods are manyfold and almost impossible to fulfill in full range: the method has to be easily available, easily installed and integrated into existing systems and not too complex, representative, accurate, reliable, sensitive, and the data should be able to be acquired electronically. It should be adaptable to different systems and a wide range of environmental conditions such as temperature, pH, redox potential, pressure, and others. An early warning capacity should be provided. And, of course, it should be as cheap as possible and require no special skills from the personnel. Of course, there is no single method listed which fulfills all these criteria, although a few of them might come close (such as the glass fiber sensor). Some disadvantages have to be taken into account, such as the fact that most of the methods are still in the state of laboratory development and that the correlation among the methods is sometimes relatively poor (Roe et al., 1994).

Table 2: On-line Monitoring Systems

Monitoring Principal	Direct	By-Pass	Sens.	Avail
Optical				
• Flow cell, image analysis (Shakespeare and Verran, 1988)	x	x	I	A
• Glass fiber sensor (Tamachkiarowa and Flemming, 1996)	x	x	II	B
Spectroscopical				
• FTIR, FTIR-ATR (Schmitt and Flemming, 1996; Siebel et al., 1995)		x	I	A/B
• Fluorescence of biomolecules (Angell et al., 1993; Mittelman et al., 1993)		x	II	C
• Photoacoustic spectrometry (Dexter and Lucas, 1983)		x	II	B
Heat Transfer				
• Heat exchanger (Roe et al., 1994)	x	x	III	A
• Hot wire (Hillman et al., 1985		x	III	B
• Microcalorimetry (Sand et al., 1994)		x	II	B
Ultrasonics				
• Sound velocity (Hillman et al., 1985)	x	x	II	B
• Pulse echo (Hillman et al., 1985)	x	x	II	B
• Quartz crystal microbalance (OMB) (Nivens et al., 1994)		x	I-II	B
• Surface acoustic waves (SAW) (Stenberg et al., 1988)		x	I-II	B
Electrochemical				
• Impedance spectrometry (Warwood et al., 1991	x	x	I-II	B
• Dielectric spectrometry (Maky and Kell, 1990)	x	x	I-II	B
• Capacity measurement (Warwood et al., 1991)	x	x	I-II	C
Friction resistance				
• Pressure drop (Roe et al., 1996)	x	x	II-III	A
• Torsiometer (Roe et al., 1996		x	II-III	B

I = high; II = medium; III = low sensitivity

A = commercially available; B = experimental method; C = concept (after Hillman and Anson, completed with more recent methods)

Conclusions

The number of approaches to meet the requirements of an optimal monitoring system is surprisingly high. Physical methods prevail. However, most of the systems have never left the laboratory and the experimental phase. This may be mainly due to the fact that monitoring of biofilms is attempted in very diverse fields, according to the various areas in which biofouling and MIC occur. Thus, joint efforts to develop monitoring units which can be used for a wide range of applications are mandatory. A systematical review is the first step and will be performed in a book, in which as many advanced biofilm monitoring methods as possible are compiled (Flemming, 1997). A very important next step will be to compare the results as obtained by the various methods and to define the degree to accuracy required in different industrial or environmental areas.

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Characterization and Monitoring of Biofilms

H.-C. Flemming and G. Schaule

Why does biofouling hurt?

- **Pressure drop increased**
- **Increase of $\Delta p_{membrane}$**
- **Concentration polarization**
- **Salt passage increased**
- **Permeate quality decreased**

Microbiolocial

- ⇒ Morphology
- ⇒ Architecture
- ⇒ Cell number cm^{-2}
- ⇒ CFU cm^{-2}
- ⇒ Species
- ⇒ ATP, CTC...

Chemical

- ⇒ Water content
- ⇒ TOC
- ⇒ Protein
- ⇒ Polysaccharides
- ⇒ Humic substances
- ⇒ Inorganics

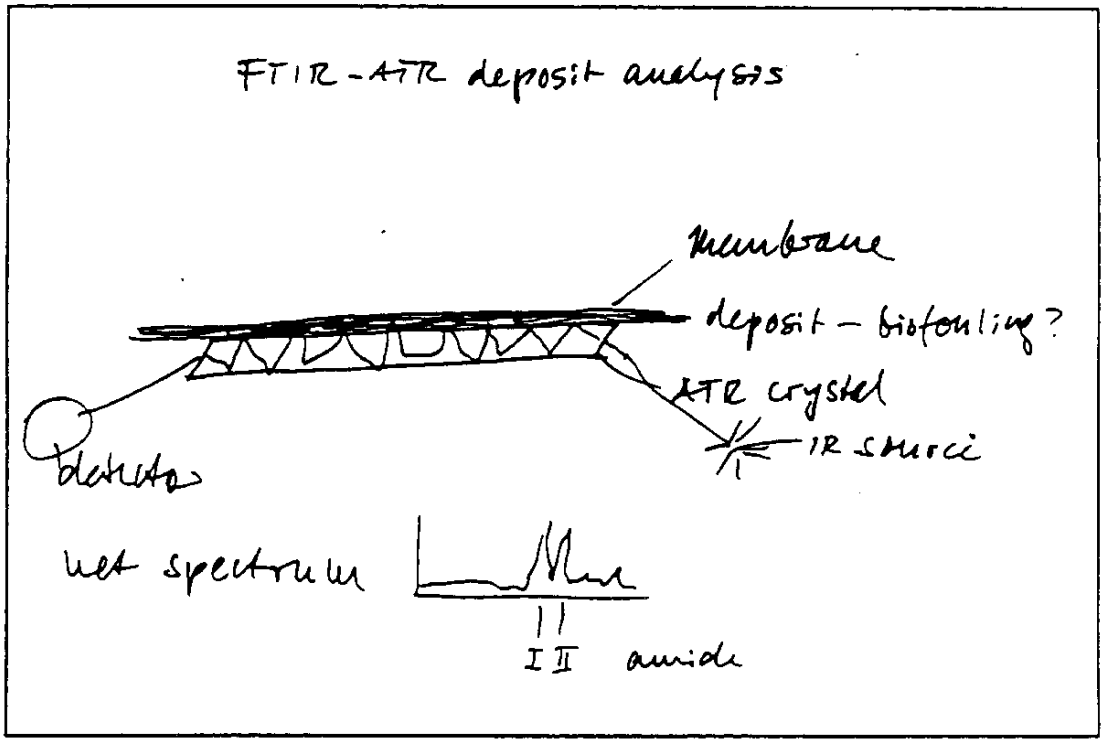
Physical

- ⇒ Thickness
- ⇒ Permeability
- ⇒ IR absorption

Methods

- ⇒ Light microscope, CLSM, SEM
- ⇒ Fluorescent indicators
- ⇒ Classical microbiology
- ⇒ FTIR-ATR
- ⇒ TOC
- ⇒ Fluorescence spectroscopy
- ⇒ Analytical chemistry

Very important: Sampling



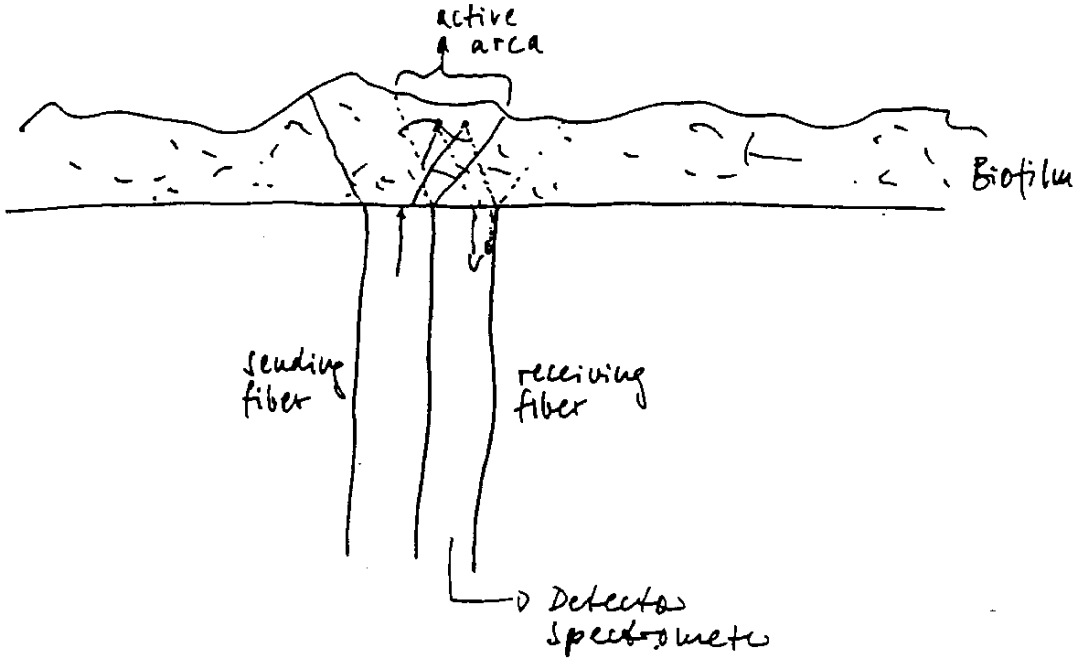
What do we want to know?

- ⇒ **Nature of deposit**
  - ➢ **organic/inorganic**
  - ➢ **biological/abiological**
  - ➢ **chemical composition**
- ⇒ **Quantity**
- ⇒ **Thickness**
- ⇒ **Site**
- ⇒ **Distribution**
- ⇒ **Formation and removal kinetics**

Strategy

a) Installation of sensors at inaccessible sites, central processing of the signals

b) Development of probes for scanning of accessible surfaces; micro-, meso- and macro-scale



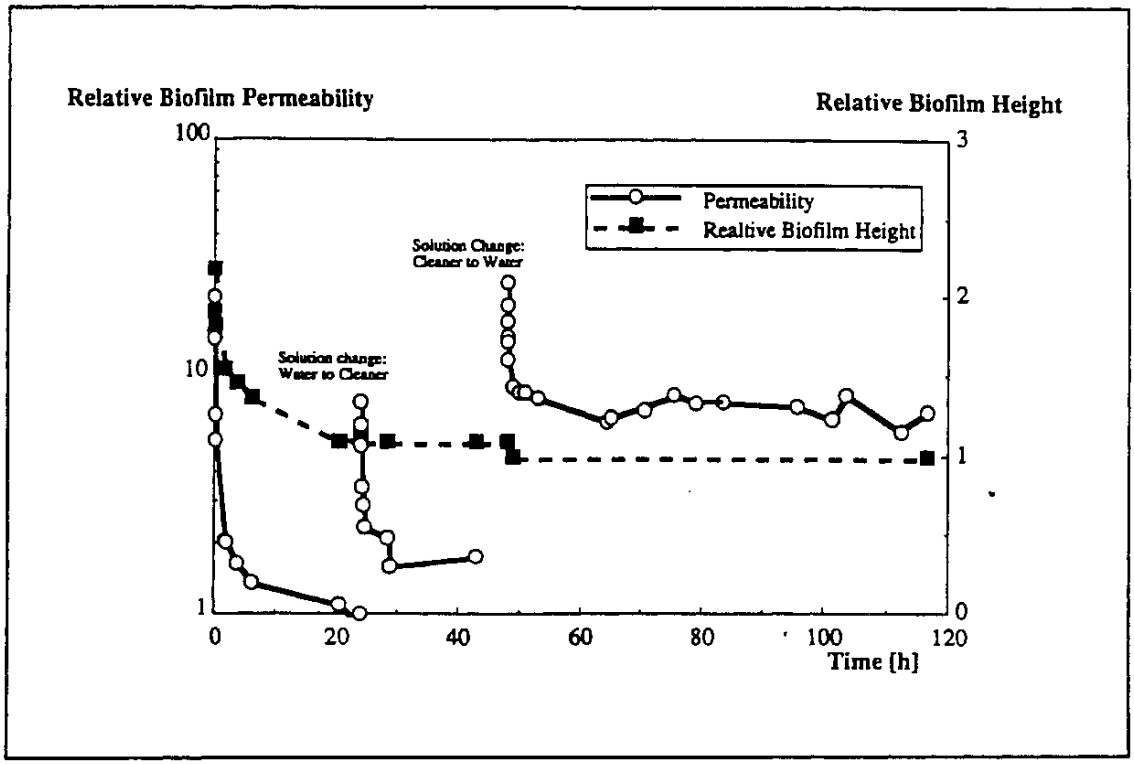
Options for Monitoring

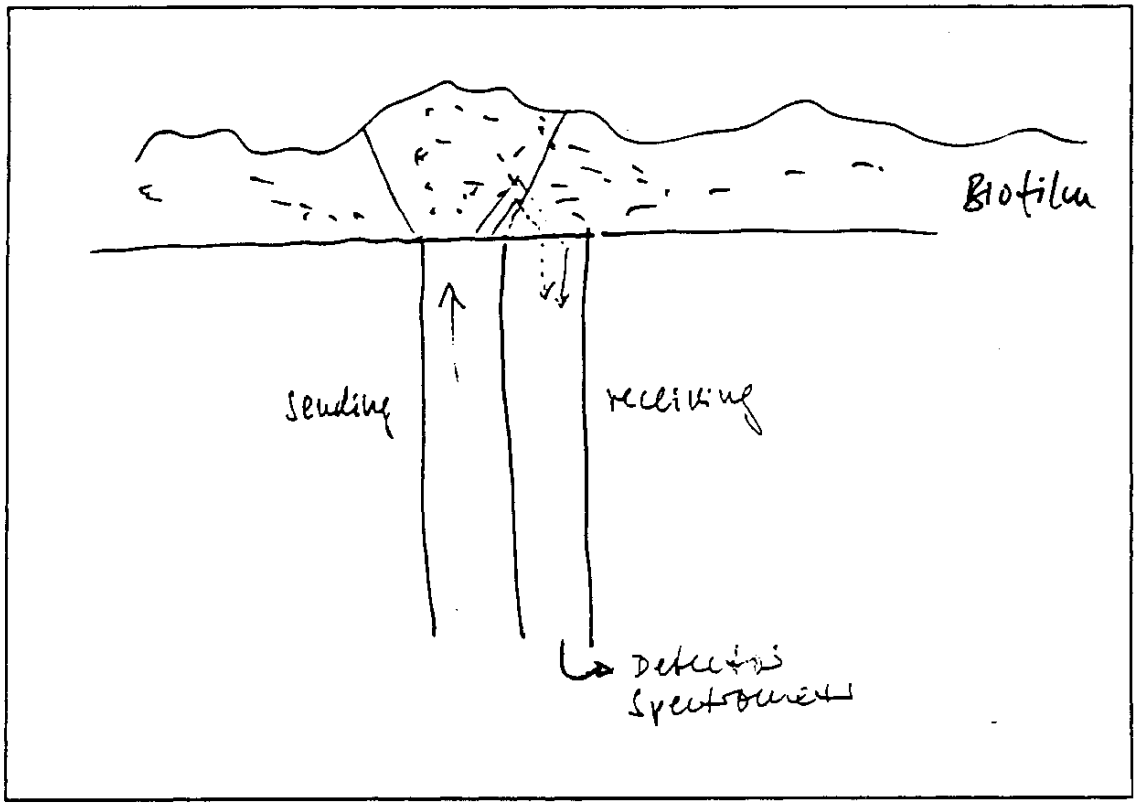
- Sacrificial modules
- Analysis of cartridge filters
- Coupons
- Pressure drop

⇒ Glass fiber sensors
⇒ UV and IR spectroscopy

The Optimal Monitoring Method

⇒ In situ
⇒ On line
⇒ In real time
⇒ Representative
⇒ Non destructively
⇒ Fast and accurate
⇒ Integrates over large areas
⇒ Easy to handle and stable
⇒ Cheap


Relative Biofilm Permeability
Relative Biofilm Height
100
10
1
3
2
1
0
0
20
40
60
80
100
120
Time [h]
Permeability
Realtive Biofilm Height
Solution Change: Cleaner to Water
Solution change: Water to Cleaner


Biofilm
sending
receiving
Detection Spectrometer

Effect of various agents on the permeability of a bacterial filter cake

Agent	J/J_0
Cleaner U 53	2,5
Urea 6 M	1,0
Tannin 1%	0,38
Formaldehyde 1%	0,6

Biofilm Prevention and Cleaning of Membrane Systems

Harry F. Ridgway
Orange County Water District, Fountain Valley, CA

1.0 Introduction

Modern water treatment practice is now largely dependent upon membrane separation processes. The types of membrane processes employed today range from (i) microfiltration (MF) for removal of suspended solids and colloids (e.g., particulates and microorganisms) from raw water sources to (ii) ultrafiltration (UF) and nanofiltration (NF) for removal of colloids, microorganisms, and selected high-molecular-weight organics, to (iii) reverse osmosis (RO) demineralization. MF membranes may be operated in a direct filtration mode or in a cross-flow mode whereas NF, UF, and RO membranes are operated only in a cross-flow mode. In cross-flow separations, a substantial portion of the total system flow (typically >75%) is directed tangential to the membrane surface in an attempt to prevent fouling layer accumulation and reduce concentration polarization. However, despite cross-flow operation, colloidal matter and microorganisms often undergo "irreversible" adhesion to the membrane surface. In the presence of ample feedwater nutrients and absence of inhibitory conditions (e.g., a biocide) attached bacteria and other microbes will grow and multiply forming a biofilm. Bacteria comprising surface biofilms typically synthesize extracellular polymeric substances (EPS) which (i) helps mediate and stabilize cell attachment, (ii) imparts resistance to chemical biocides by creating a transport barrier and providing oxidative demand, and (iii) concentrates potential nutrients via ionic and other interactions in proximity to the cell. In their native hydrated state, membrane biofilms generally exhibit a gel-like character which imparts a slippery or slimy texture to the membrane surface. Numerous different kinds of bacteria and fungi have been shown to inhabit membrane biofilms, but to date no general or systematic surveys have been undertaken to identify common membrane biofilm organisms. The EPS of some biofilm bacteria has been shown to consist primarily of acidic

heteropolysaccharides; however, the exact nature of the EPS in most natural biofilms remains poorly, if at all, characterized.

2.0 Effects of membrane biofouling

The membrane biofilm, consisting of attached bacteria and associated EPS, can often provide a substantial barrier to water transport. Consequently, water flux from the feed side of the membrane to the permeate surface is often significantly impeded by biofilm accumulation. This transport impedance is manifested in the need to gradually increase the system operating pressure to overcome flux reduction due to biofouling. The increased pressure (energy) requirements directly translate into higher operating costs. Other detrimental effects caused by biofilm accumulation include (i) an increase in the system (module) differential pressure due to clogging of feed channel voids and elevated frictional drag of water flowing over the typically rough biofilm surfaces, (ii) reduced solute rejection caused by enhanced concentration polarization within the biofilm matrix, and (iii) biodegradation of the synthetic polymer membrane itself or other module components, such as polyurethane glues used to seal membrane leaves together, or rubber O-ring seals used to separate adjacent membrane modules in pressure vessels. A serious problem is the occurrence of biofouling and biodeterioration of membranes and module components during long-term storage. Accumulation of human pathogenic bacteria or viruses in some membrane biofilms (e.g., at wastewater treatment facilities) pose an additional concern and potential hazard to plant operational personnel who may come into frequent contact with discarded membrane modules.

3.0 Current strategies to prevent membrane biofouling

Currently, two principal strategies for preventing or retarding membrane biofouling are in widespread usage. These strategies are (i) physical removal of bacteria from the source water used to feed the membrane system, and (ii) metabolic inactivation of the fouling bacteria by application of a chemical biocide or ultra-violet (UV) irradiation. Standard physical removal methods include multi-media, cartridge, MF, and UF filtration processes, as well as flocculation approaches such as high-pH lime clarification coupled with polymer dosing. Methods commonly used to inactivate fouling bacteria include the use of free

chlorine (as hypochlorous acid) or monochloramine as feedwater biocides. Other chemicals that have been less commonly used as feedwater biocides include sodium bisulfite, chlorine dioxide, iodine, peracetic acid, and hydrogen peroxide. Required properties of feedwater biocides are that they are relatively inexpensive, effective at low doses and short contact times, and do not damage the membrane. Feedwater biocides may be introduced on a continuous or intermittent basis. A distinct disadvantage of feedwater UV irradiation as a biofouling control measure is that it provides no residual at the membrane surface.

4.0 current methods of cleaning biofouled membranes

The purpose of cleaning biofouled membranes is to remove attached biomass (i.e., cells + EPS and associated particulate matter) in an attempt to restore system flux and solute rejection to pre-fouling levels. Unfortunately, because so little is currently known about the underlying molecular mechanisms and forces responsible for maintaining biofilm architecture and bacterial attachment to various polymer membrane surfaces, the "science" of membrane cleaning to remove biofilms is still in its infancy. Complicating the effort to identify effective cleaning agents is the elaboration of diverse forms of EPS by biofouling microorganisms. Thus, it seems likely that any given cleaning agent or formulation may only be effective against a limited range of biofilm types. Today's membrane cleaning formulations often contain one or more detergents to aid in the disruption of adhesive hydrophobic interactions between the bacterial envelope and the synthetic polymer membrane. There are a large variety of anionic, cationic, amphoteric, and netral detergents available commercially. Some detergents, such as quanternary amines (e.g., hexadecyltrimethylammonium bromide), may also exhibit good biocidal activity. Common surfactants for membrane cleaning include sodium dodecyl sulfate, sodium dodecyl benzene sulfonic acid, and sodium tripolyphosphate. Many commercially available detergents will irreversibly destroy membrane performance (e.g., benazlkonium chloride and Triton-X100 will adversely affect cellulose acetate and polyamide membrane flux and solute rejection); thus, caution must be exercised in surfactant selection for cleaner formulation. Other components of membrane cleaning formulations may include (i) divalent cation chelating agents such as ethylenediaminetetraacetic acid, (ii) pH buffers, (iii)

biocides such as isothiazolone, and (iv) enzymes (lipases, proteases, esterases). Extensive bench-scale and pilot testing is often required to determine whether a new cleaning formulation is compatible with one or more membrane types.

5.0 New horizons

There are many novel approaches possible for controlling membrane biofouling. Some of these possibilities are listed below to stimulate discussion.

a. use of alternative biocides (e.g., gamma irradiation)
b. regenerable affinity columns to remove feedwater bacteria
c. incorporation of biocides in membranes or module
d. biological control (e.g., protozoan grazers)
e. particle dispersants to maintain cells in suspension
f. design of more effective feed channel spacers
g. design of more favorable module hydrodynamics
h. design of low-fouling membrane polymers
i. identification of more effective cleaning agents
j. membranes with renewable surfaces

Some of the above possibilities (e.g., new dispersants, polymer surfaces, cleaning agents, etc.) will necessitate development and application of rapid quantitative methods of evaluating anti-biofouling effectiveness. Large numbers of experimental membrane polymers or chemicals will need to be tested for their anti-biofouling activity. Currently, two relatively rapid laboratory-based screening approaches are employed to help identify potential chemical cleaning agents or new synthetic polymers having low biofouling potentials. In one method, microscope flow cells are employed containing early biofilms which can be challenged by experimental cleaning compounds or anti-adhesion agents. The effects of the test agents are directly observed microscopically (via a window in the flow cell) and evaluated by means of confocal microscopy and digital image analysis techniques. The microscope flow cells can also be used to study the dynamics of initial bacterial attachment and early biofilm development. In another approach, the kinetics of attachment of radiolabeled fouling bacteria are determined in the presence and absence (control) of test

anti-fouling compounds. Attenuated total reflection Fourier transform infrared spectrometry (ATR-FTIR) may also be used to rapidly evaluate whether organics or microorganisms will absorb to polymer separation membranes cast onto the surface of internal reflection elements.

6.0 Research needs

The molecular mechanisms that mediate bacterial attachment to membrane surfaces are poorly understood at this time. Research into the molecular mechanisms by which bacteria (and EPS materials) interact with polymer membrane surfaces would provide the necessary knowledge from which rational anti-biofouling strategies could be developed and applied. Techniques such as ATR-FTIR, nuclear magnetic resonance spectrometry, atomic force microscopy, and molecular simulations, when applied in conjunction with one another, could result in a rapid advancement of our current knowledge and understanding of molecular adhesion processes involved in initial bacterial attachment to polymer separation membranes. Of special relevance is the role of adsorbed (interfacial) water in adhesion phenomena. It has been proposed that hydrogen-bonded water molecules organized at a membrane surface are displaced by other adsorbing species (e.g., a surfactant, EPS, or cell surface polypeptide). Transfer of water from the adsorbed state to the bulk fluid is associated with a corresponding increase in system entropy (resulting from decreased molecular order) and a further decrease in potential energy (as displaced water becomes available for hydrogen bonding in the bulk phase), two factors which are believed to drive hydrophobic adsorption processes. The exact role of adsorbed water in bacterial adhesion processes has not been explored and a better understanding in this area would lead to pragmatic applications.

Cleaning and Disinfection of Biofilms Using Peroxygens

Alex Blanchard*, Michael Bird**, John Wright*

*School of Biological Sciences, University of Bath, UK
**School of Chemical Engineering, University of Bath, UK

Presentation Summary

(i) Biofilm studies in our laboratory.

(ii) Modified Robbins Device (MRD) - a protocol for determining cleaning efficacy.

(iii) Transfer of information from hard surface cleaning to membrane cleaning?

Biofilm Establishment (In a non-flow system)

- Bacteria used were *Pseudomonas aeruginosa* and *Staphylococcus aureus*.
- Polished stainless steel disks of dimensions 20 mm x 1 mm were used.
- Disks were inoculated with a 0.4 ml bacterial suspension, and incubated at 30°C for 1 hr. Surfaces were subsequently washed and incubated in sterile nutrient broth at 30°C for 24 hrs. This procedure leads to a biofilm with ca. 10^7 CFU cm^{-2}.

Cleaning Studies (all done with a 5 minute biocide contact time)

- Biofilm viability shows an exponential decay with increasing peracetic acid (PAA) concentration (See Figure 1).
- Biofilms are more resistant to attack than cells in suspension. A concentration of 100 ppm PAA was needed for a 99% kill of the biofilm. Only 5 ppm PAA was needed for a 99% kill of cells in suspension. (Compare Figure 1 to Figure 2).

- Older biofilms are more resistant to biocide action than younger ones. 150 ppm PAA was needed for a 3 log order reduction in viability of a 24 hr *P. aeruginosa* biofilm. 250 ppm PAA was needed for a similar reduction in viability of a 48 hr *P. aeruginosa* biofilm (Figure 3).

- Interfering organics seriously reduce the efficacy of the biocide (Figure 4).

Current Studies and Future Work

- An apparatus and protocol now exists to develop biofilms in a flow system using a MRD constructed from PTFE (Figure 5).

- The biofilm is developed on plugs (Figure 6) which are then removed from the MRD and inserted into a cleaning rig containing a second MRD.

- Complete control of biofilm establishment and removal conditions is therefore possible.

Points to Consider

- How relevant are biofilm cleaning studies carried out on stainless steel surface to membrane biofilm removal?

- How important is the distinction between removed cells and attached nonviable cells?

- What is the relationship between biofilm cleaning and sanitation?

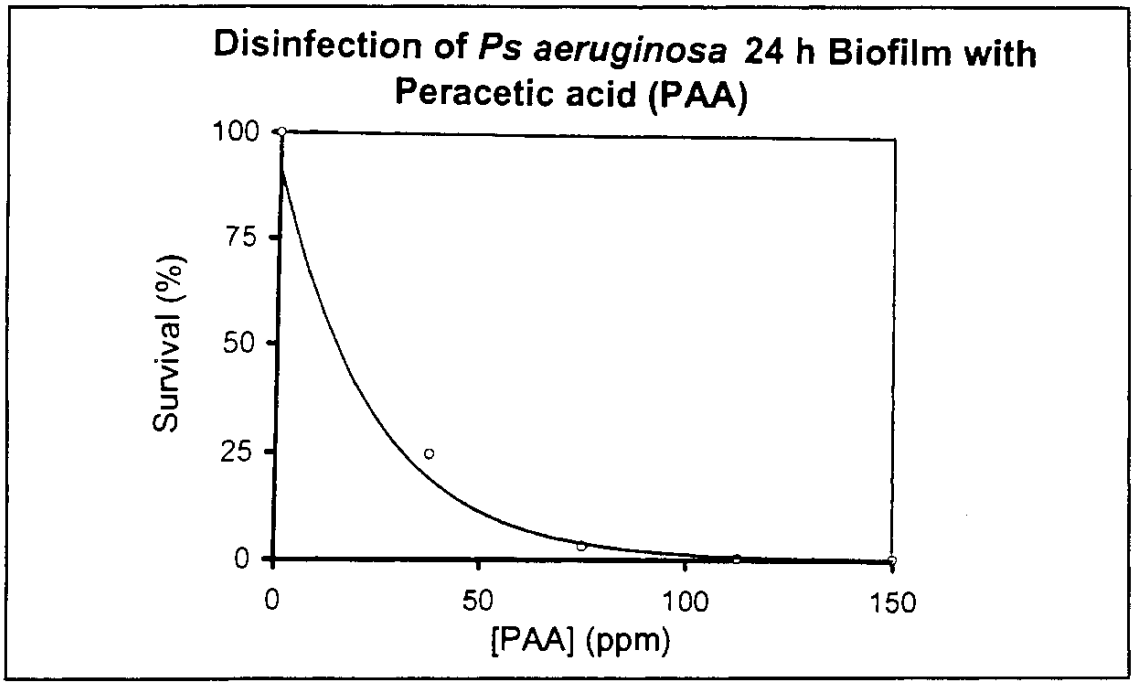


Figure 1

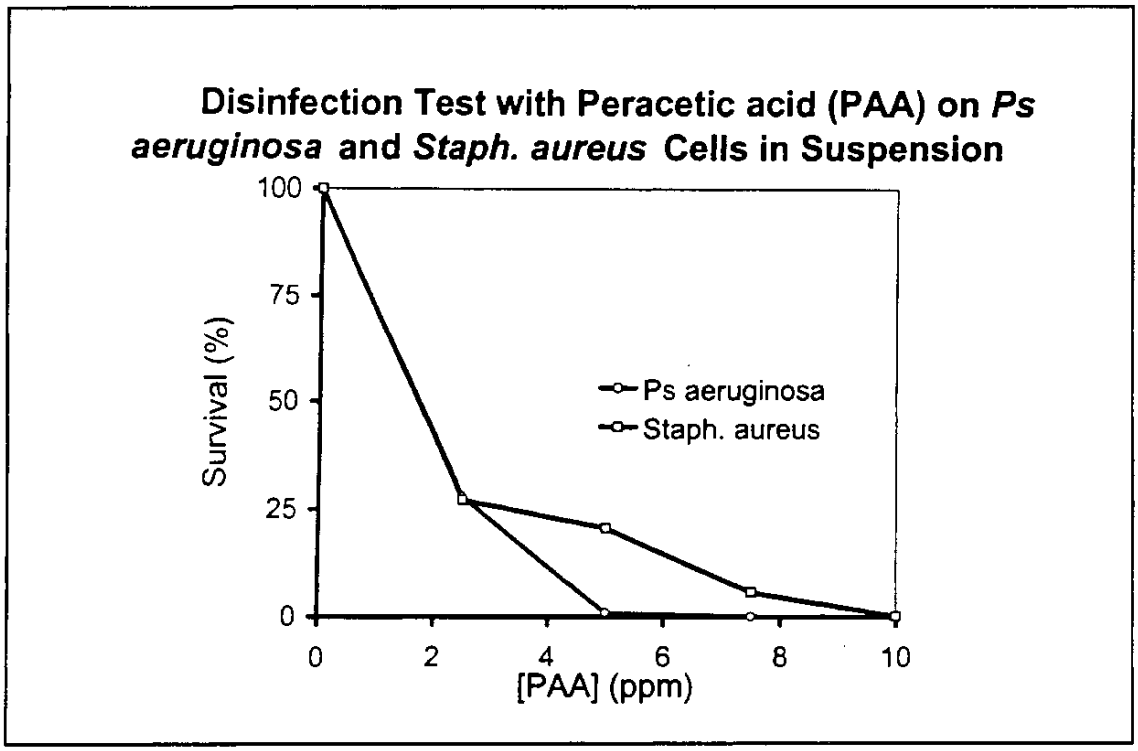


Figure 2

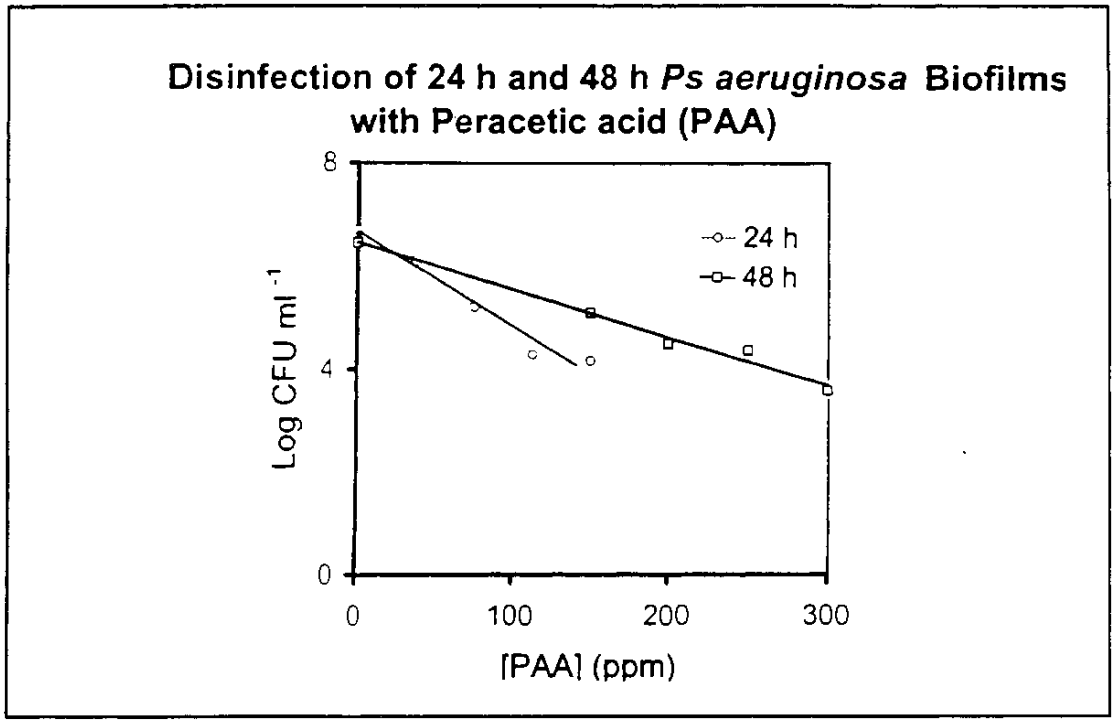


Figure 3

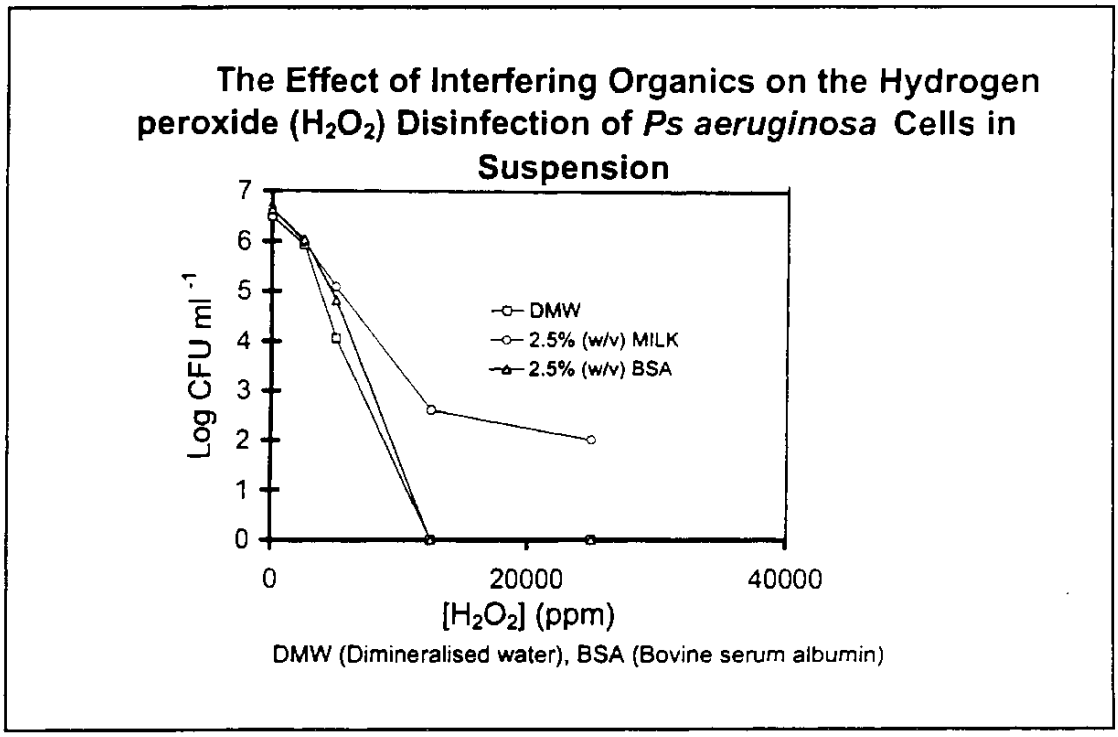
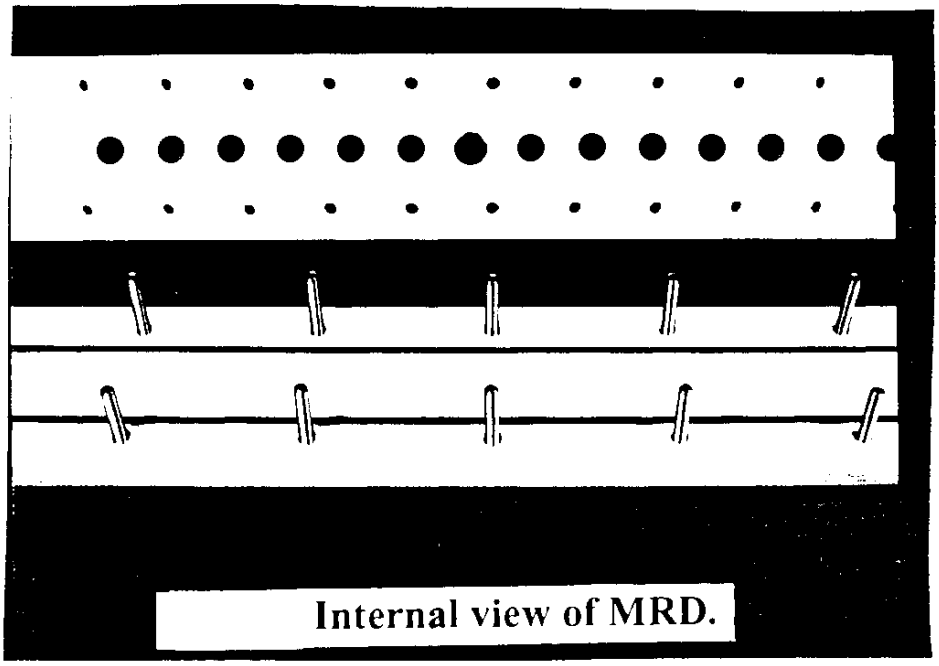
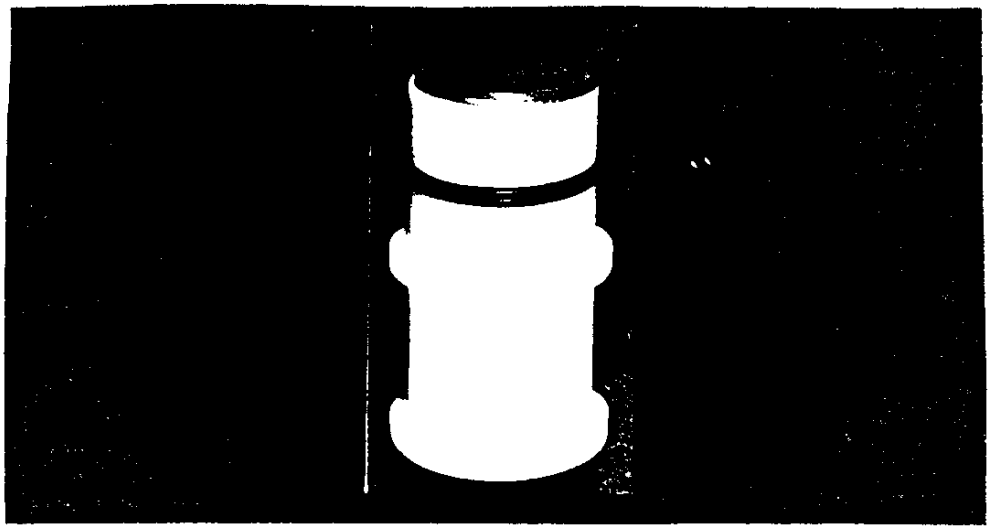


Figure 4



Internal view of MRD.

Figure 5



A sample stud with attached stainless steel surface.

Figure 6

Biofouling Workshop
November 1996
Sydney

Surface Interactions

and

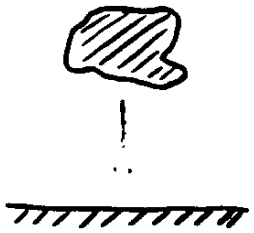
Polymer Properties

by

Hans J. Griesser

Division of Chemicals and Polymers, CSIRO
Clayton, Vic.
Australia

Or : Subtitle :



A Molecular Look at How and Why Polymeric Surfaces Acquire Biological Material

Study of:

- the First Monolayer of Adsorbed Biological Material
- the Forces involved in Adsorption

Why ? - - Once the first monolayer is adsorbed, we've lost the game - further biological materials piles on top

- adhesion of bacteria
- complement activations

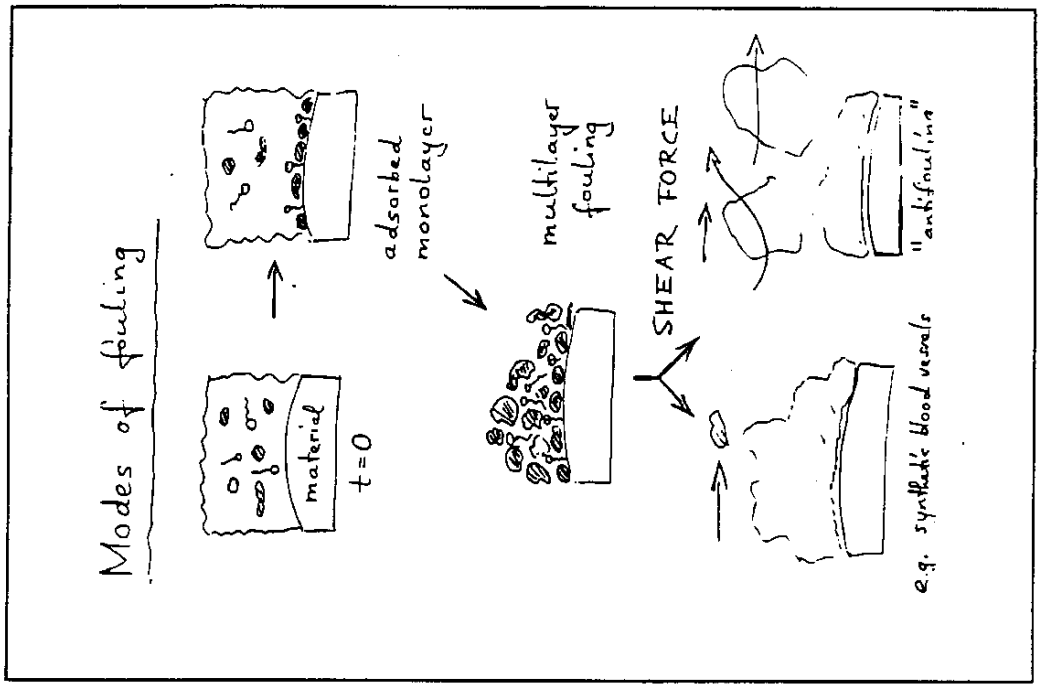
Premise:

To understand biofouling, we need to understand:

- our polymer surface
- our protein mixture
- the forces between them at the molecular level

"Know thy enemy"

- Fundamental polymer surface science
- Theoretical considerations in protein adsorption
- Experimental studies



Definitions:

Truly <u>nonfouling</u> or <u>antifouling</u>:

- **nothing - not even a monolayer - adsorbs**

Operationally <u>nonfouling</u> or <u>antifouling</u>:

- **it does not interfere too much with your process**

Can we ever get a truly nonfouling surface?

- **a matter of time**
- **biological variability (e.g., contact lenses)**

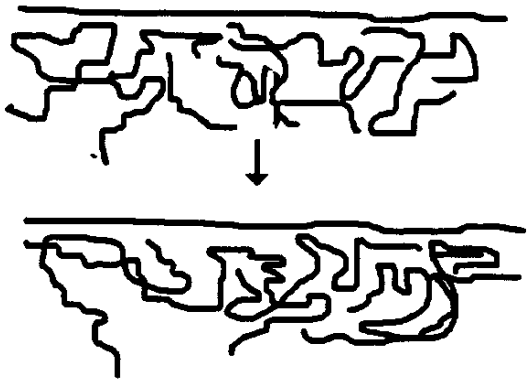
Polymer Surfaces

are:

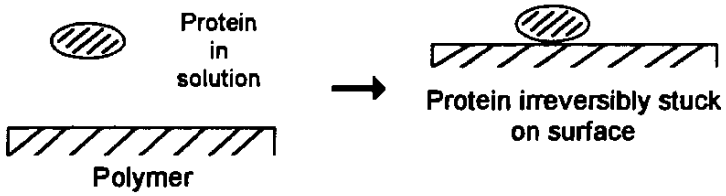
- **mobile**
- **usually unpredictable**
- **contaminated!!!!!**

Mobility:

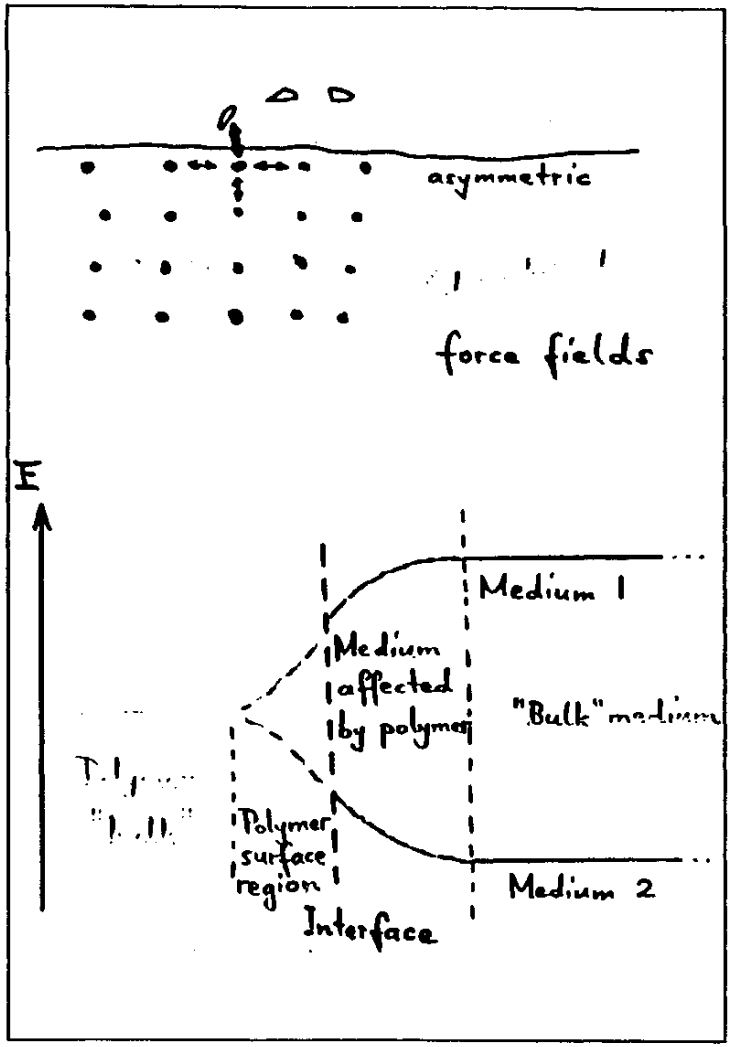
- **thermally driven, random motions of chain segments**



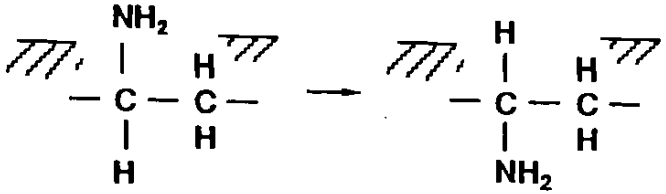
Protein/Polymer Interactions



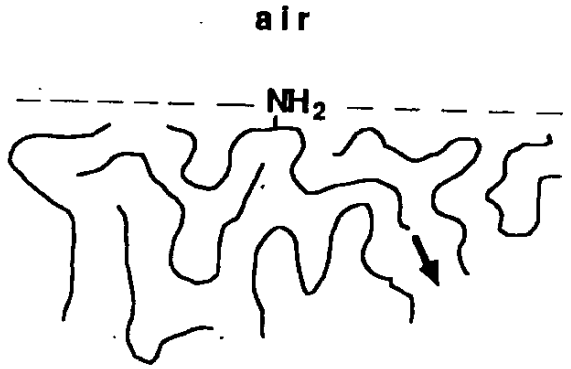
- **Membrane fouling**
- **Fouling of contact lenses**
- **Clogging of artificial blood vessels**
- **Deposits on synthetic heart valves**
- **Attachment of bacteria to**
  - **contact lenses**
  - **biomedical implants**



Rotation of chain segments



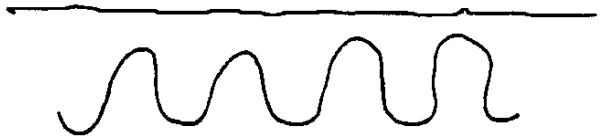
Reptation :



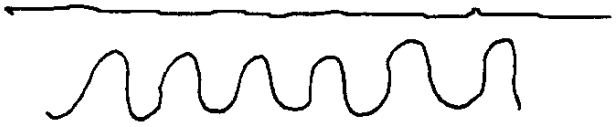
Add Non-Random Motions (e.g. Polyurethanes)

non polar polyether segments — polar polyurethane segments

in air, vacuum



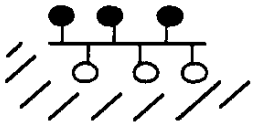
in water, body



Surface analysis?!

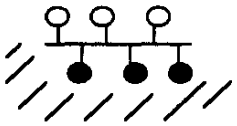
Surface Mobility

In water



Polar groups up

In air/vacuum

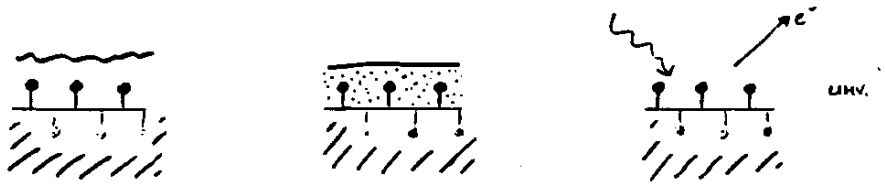


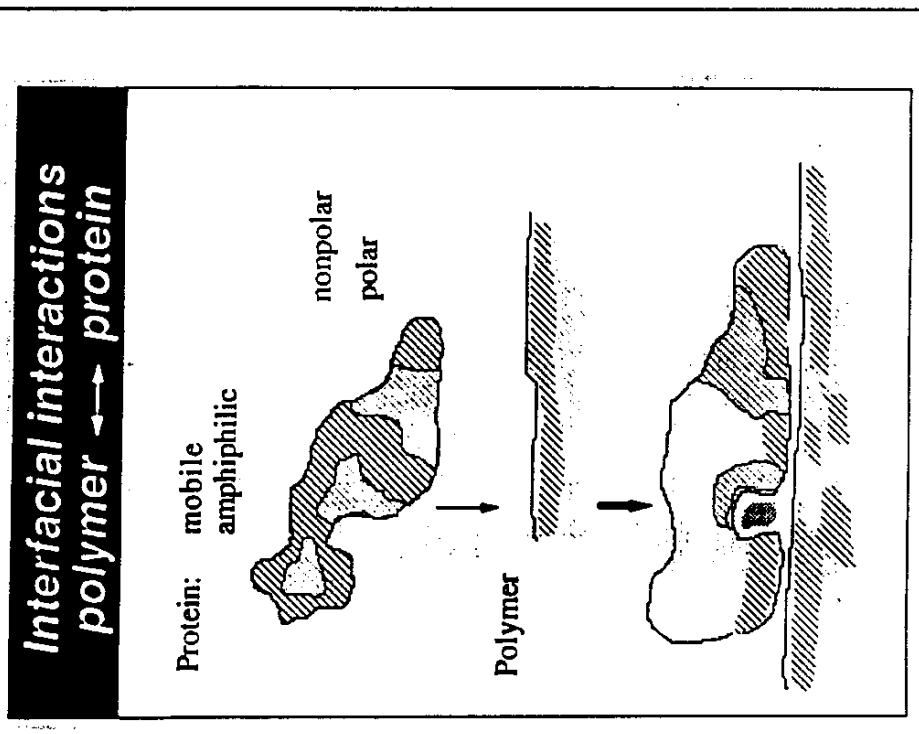
Polar groups buried

Reorientation

Freeze-Hydration XPS

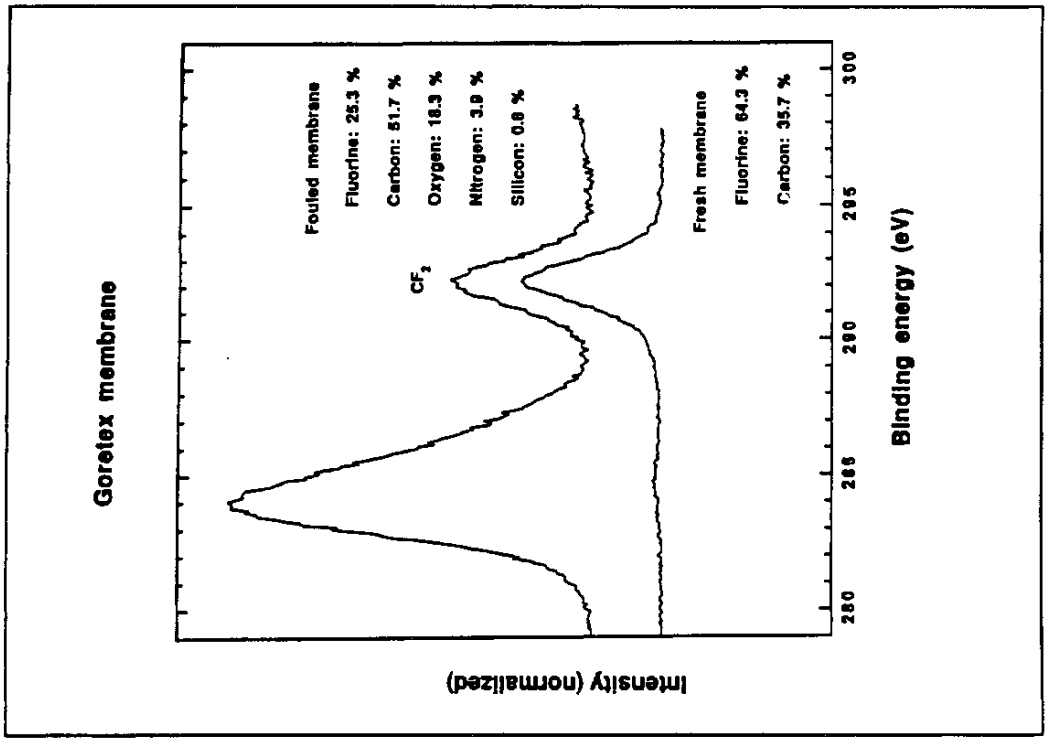
1. Hydrate
2. Freeze
3. Pump off Ice
   Analyse
   at low T



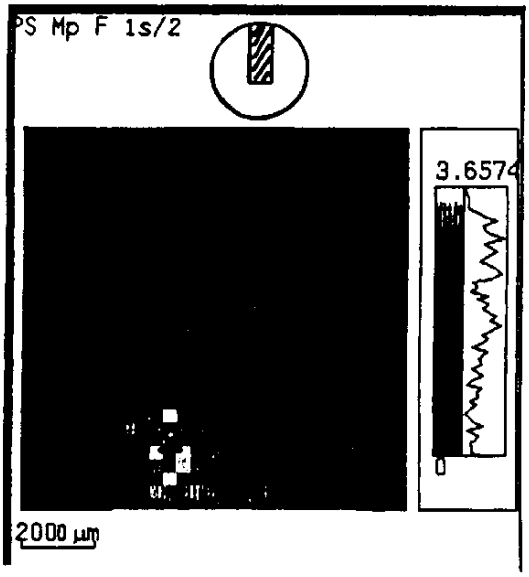


Quantification of Adsorbed Proteins

- ^{125}I Labeling
- ELISA
- XPS/ESCA
- Ellipsometry
- Surface plasmon resonance
- Quartz crystal microbalance



Variation of Coating Thickness as Determined by XPS Mapping

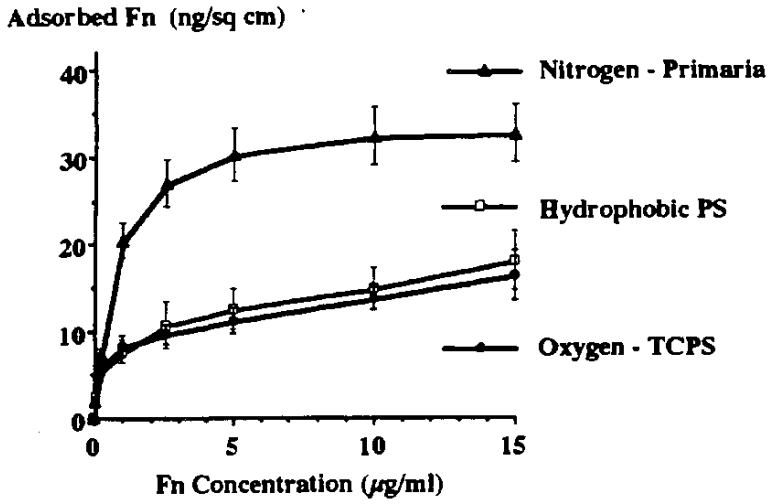


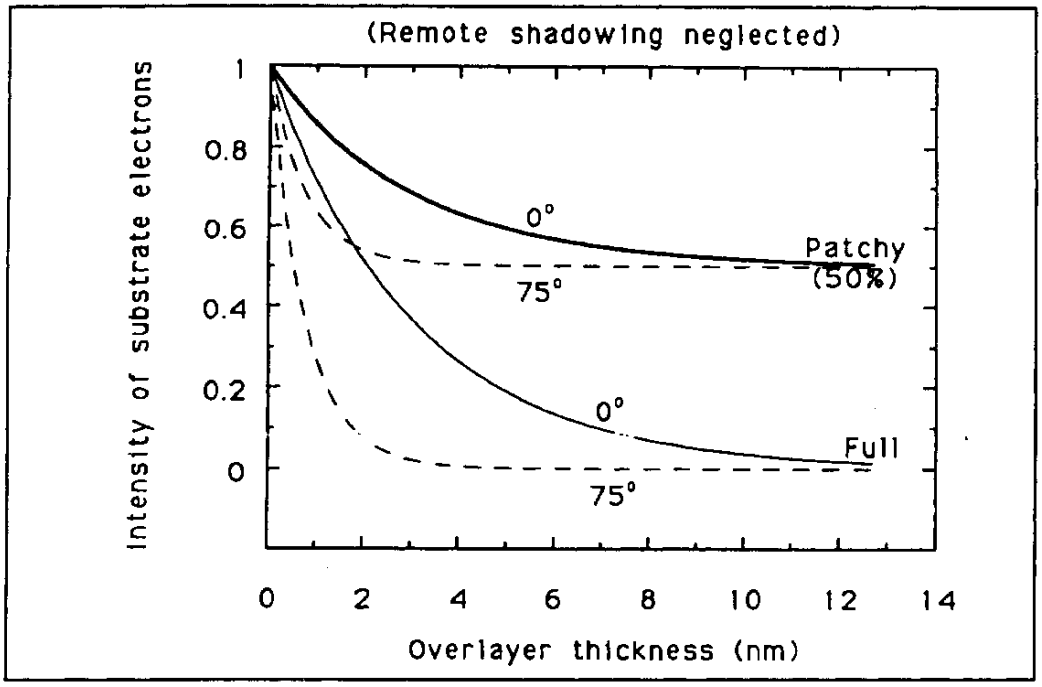
*Fluorine signal indicative of substrate

increases towards centre of lens

Coating thins towards centre of lens

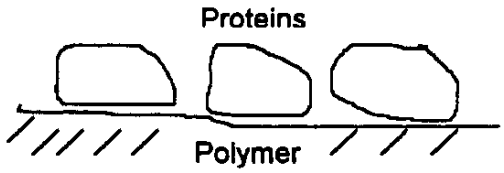
Adsorption of Fn from medium which contains serum





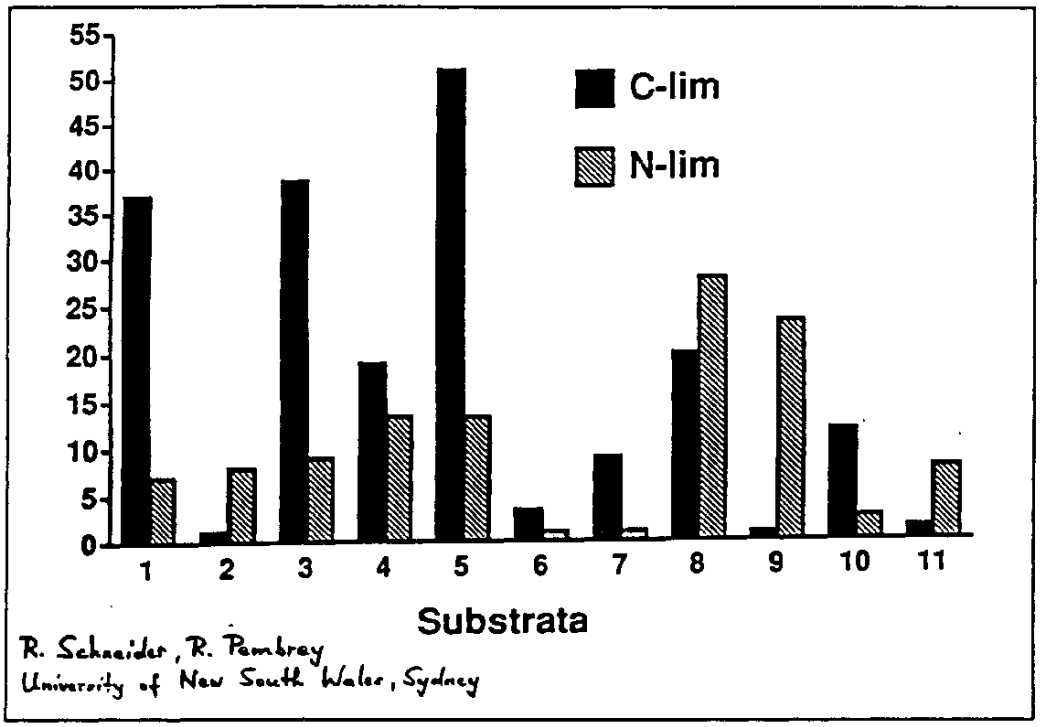
XPS can tell us

- **how much protein is present (ng/cm^2)**



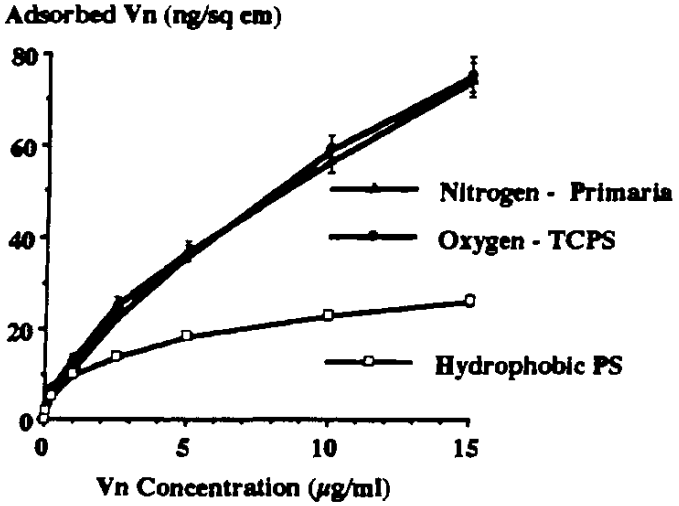
by:

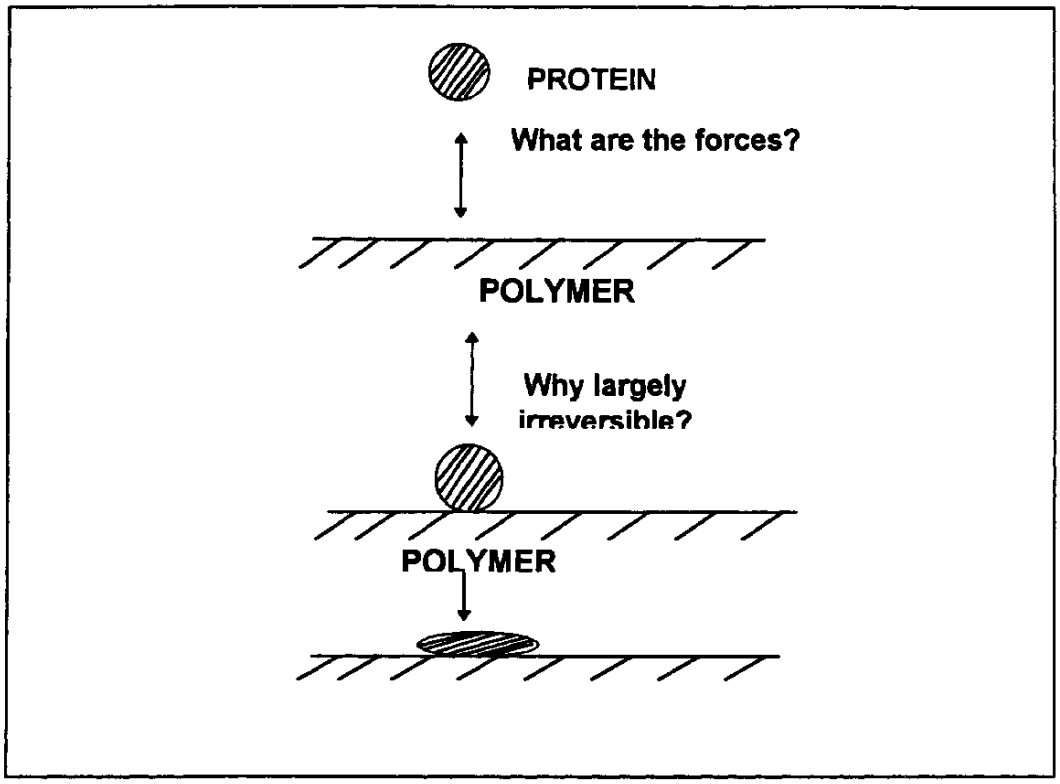
- **N intensity**
- **attenuation of signals from polymer**
- **whether patchy or confluent**



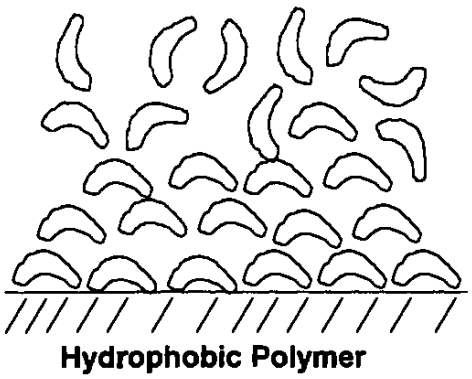
R. Schneider, R. Pembrey
University of New South Wales, Sydney

Adsorption of Vn from medium which contains serum





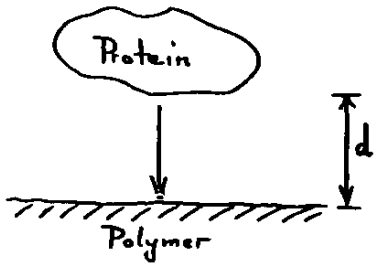
Hydrophobic Structuring of Water

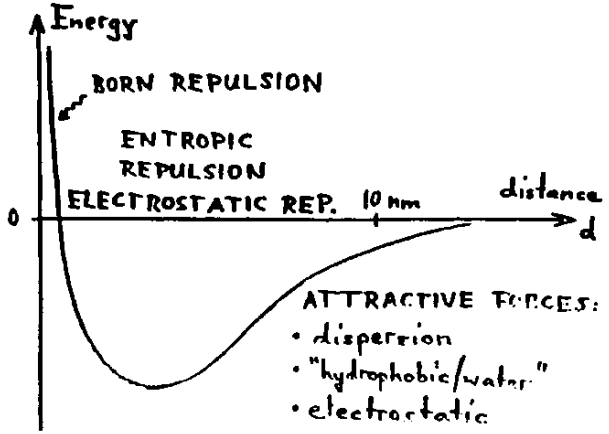


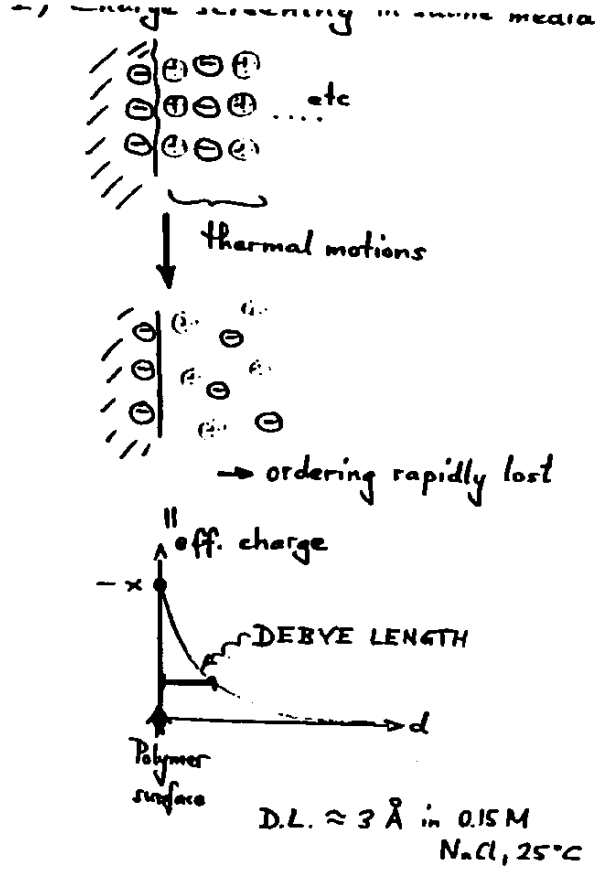
bulk water

"ice-like" water

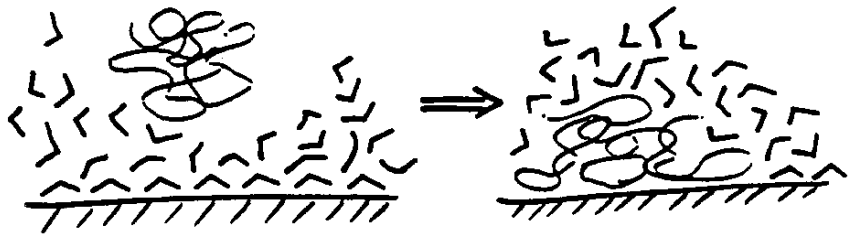
Protein adsorption







On rigid surfaces:



$\Delta S_{H_2O} > \Delta S_{Protein}$

important for irreversible adsorption:

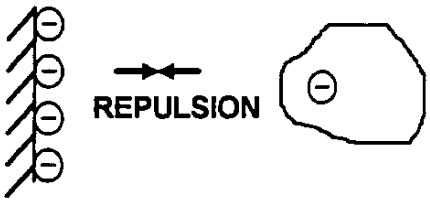
ΔS_{H_2O}, charge interaction terms

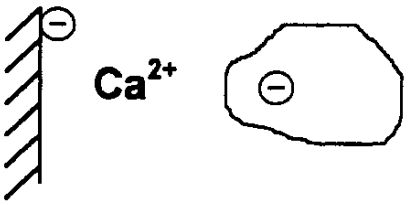
What is the role of surface charge/electrostatic interactions?

Often considered important, but recent doubts (Norde, ...)

Needs care in interpretations!

Counterion effects?

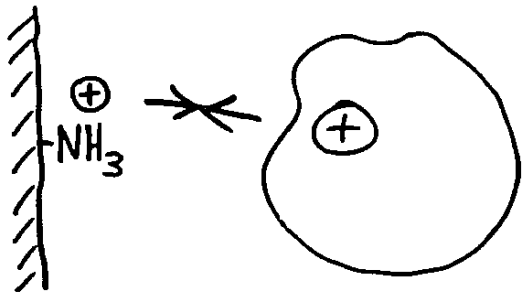




(or: Beware of assumptions !)

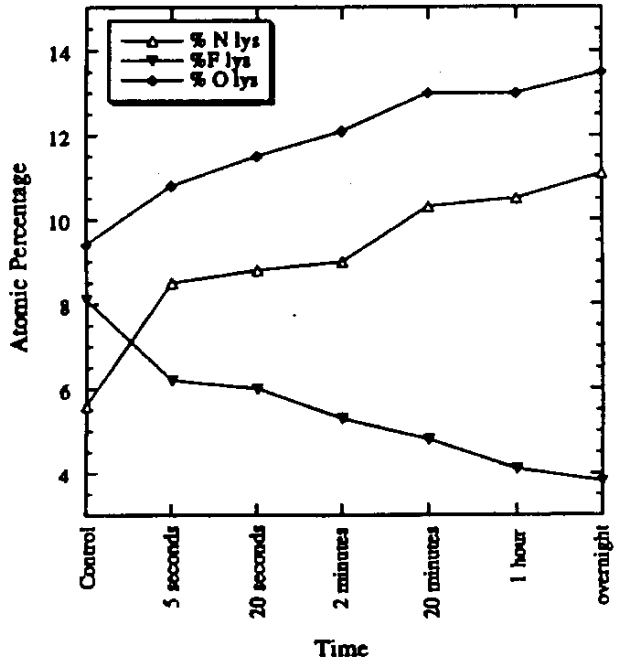
Idea:

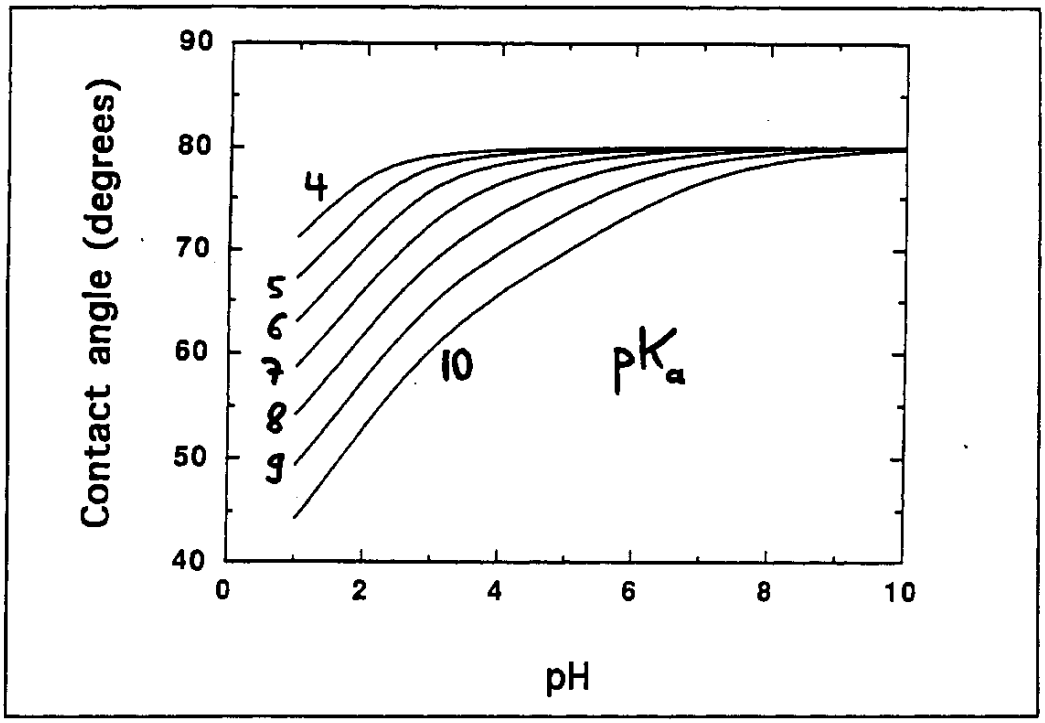
repel lysozyme by $-NH_3^+$

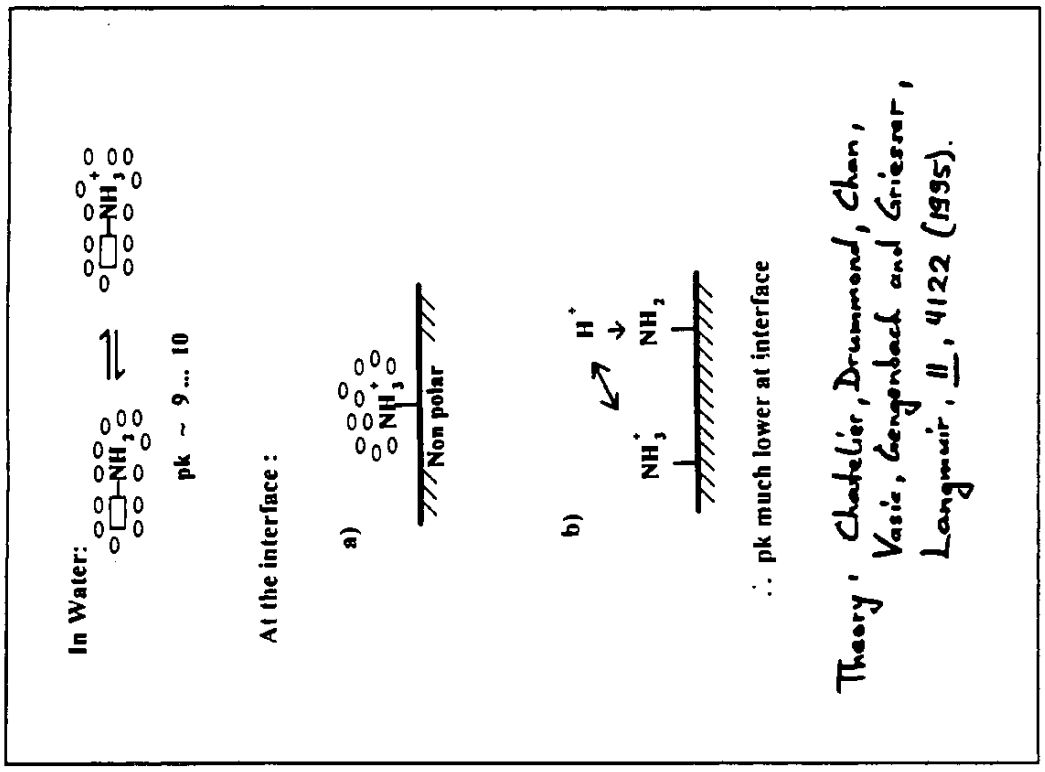


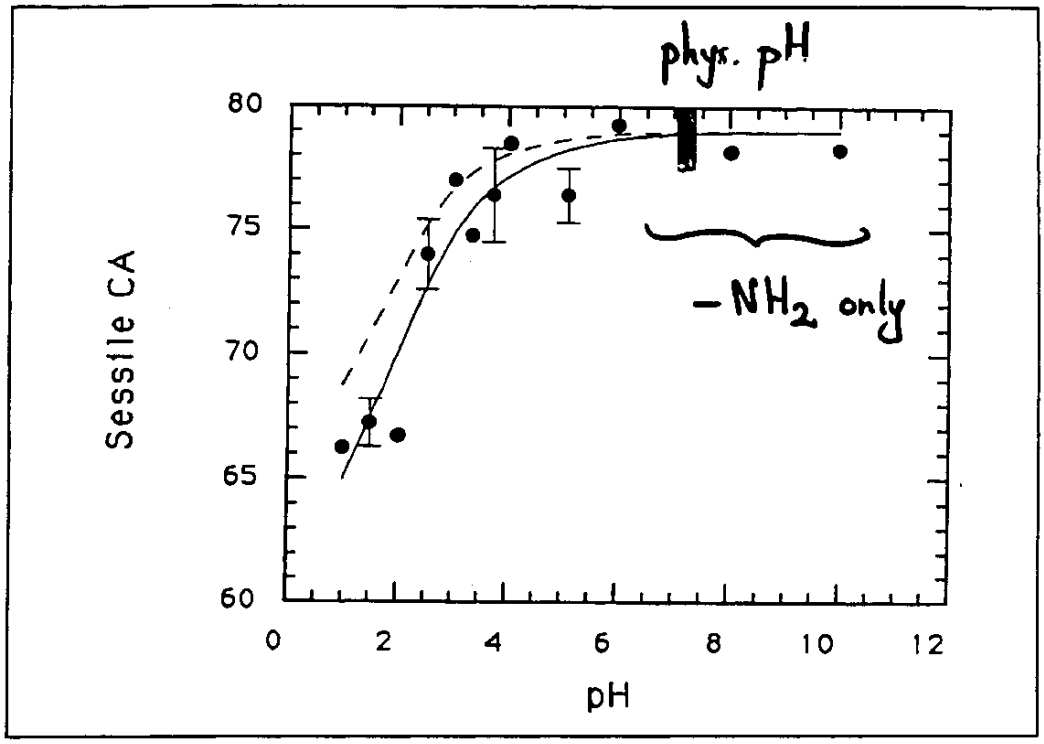
Coat contact lens with thin amine polymer

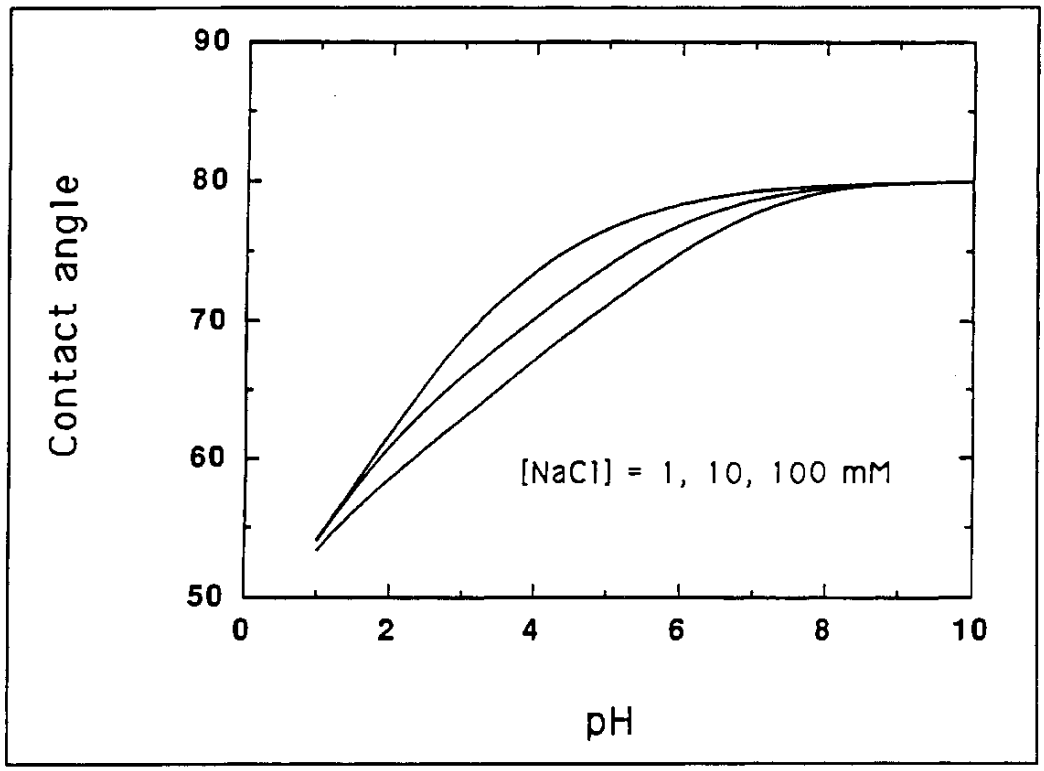
Change in Fluorine, Nitrogen and Oxygen XPS Signals for Lysozyme Adsorption to FEP/Heptylamine plasma polymer. 1mg/mL Lysozyme, pH-7.4.

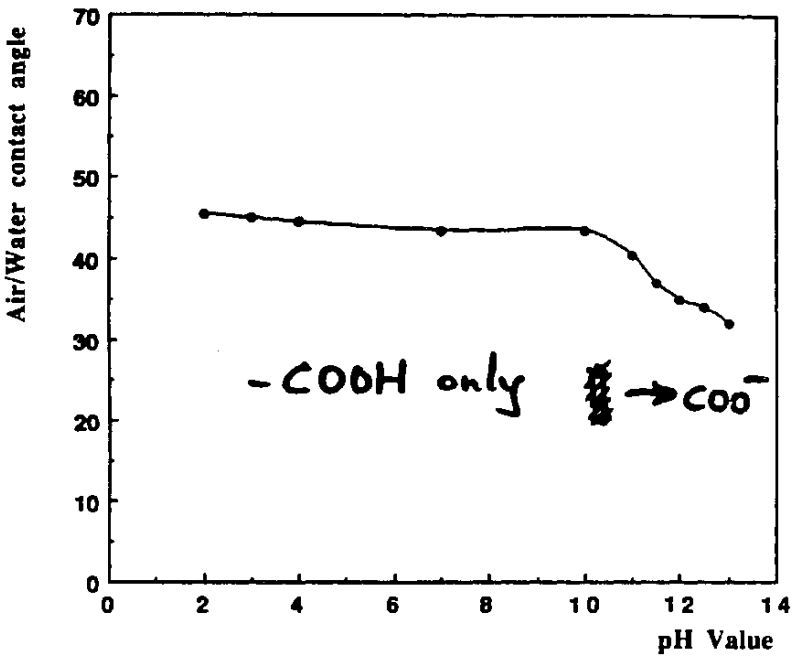



Contact angle (degrees)
90
80
70
60
50
40
0
2
4
6
8
10
pH
4
5
6
7
8
9
10
pK$_a$

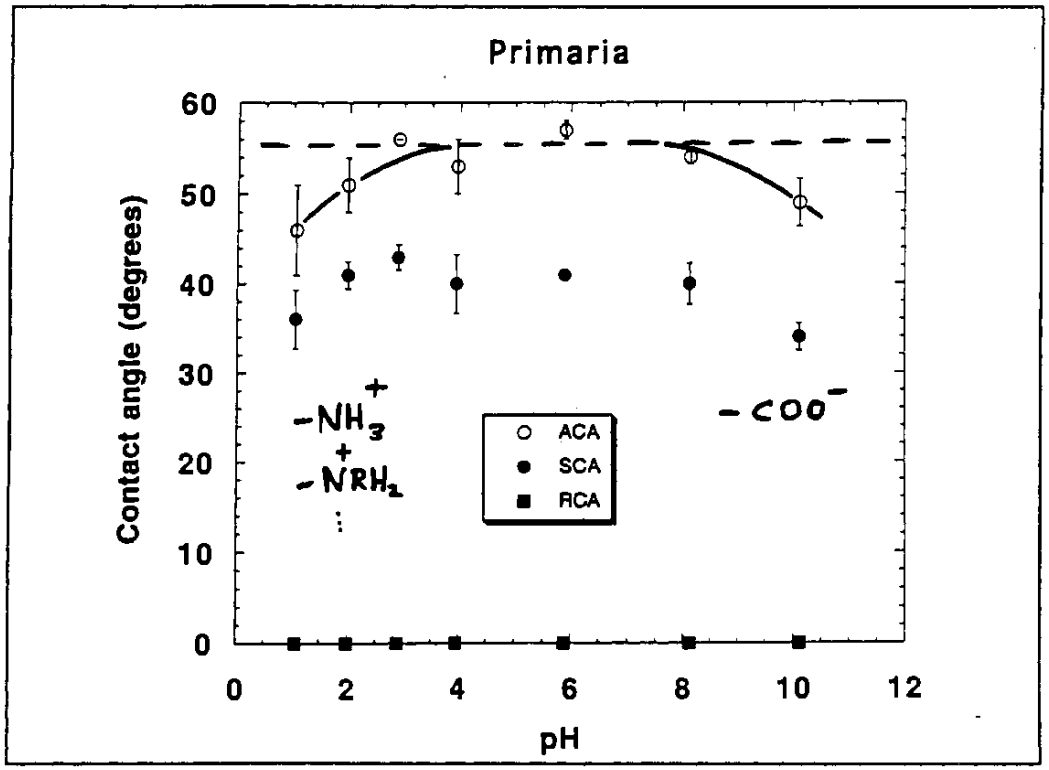

In Water:
pk ~ 9 ... 10
At the interface :
a)
Non polar
b)
H$^+$
NH$_3^+$
NH$_2$
∴ pk much lower at interface
Theory: Chatelier, Drummond, Chan, Vasic, Lengnback and Grieser, Langmuir, 11, 4122 (1995).


phys. pH
Sessile CA
80
75
70
65
60
0
2
4
6
8
10
12
pH
– NH_2 only


Contact angle
90
80
70
60
50
0
2
4
6
8
10
pH
[NaCl] = 1, 10, 100 mM



Air/water contact angle ofcorona treated HDPE as a function of pH value of aqueous solution.



The induction of dipoles is not instantaneous:

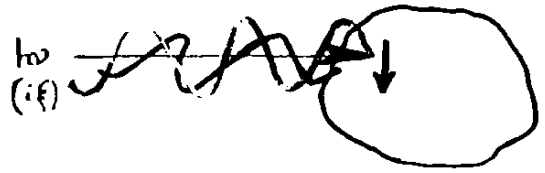
time lag = phase shift

phase-shifted dipoles

⇒ ATTRACTIVE FORCE

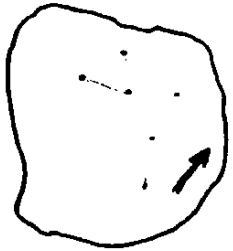
model: retarded Hamaker description (Lifshitz theory)

key: dielectric permittivity of molecule $\varepsilon(if)$

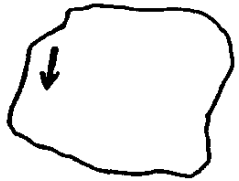


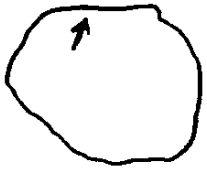
[illegible] forces

Random thermal motions of electron density distributions create temporary dipoles



they induce dipoles:





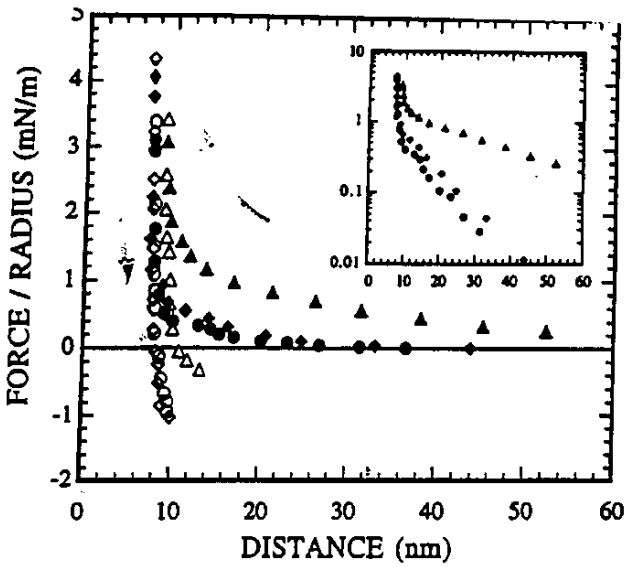


Fig. 18. Comparison of the forces obtained between proteoheparan sulphate layer adsorbed on hydrophobized mica (mica coated with a monolayer of dimethyldioctadecylammonium bromide) as a function of surface separation at different excess electrolyte concentrations. The forces were measured in 0.1 mM NaCl before addition of $CaCl_2$ (▲,△) and after addition of 1.25 mM $CaCl_2$ (♦,◊) and 2.5 mM $CaCl_2$ (●,○). Filled and unfilled symbols represent the force measured on compression and decompression, respectively. The *inset* shows the forces measured on compression on a logarithmic scale.

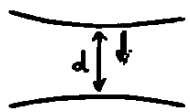
* Polymer surfaces are mobile
* Proteins can denature on contact
* Proteins may affect polymer surface
* Dynamic adsorption - desorption

⟹ Interfacial interactions
- very complex
- time dependent

Designed approaches require support by:
- sensitive analytical techniques
- fundamental studies

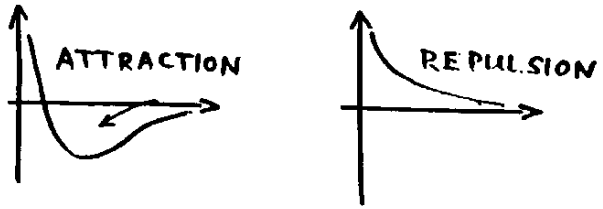
materials science
biology

Surface force apparatus

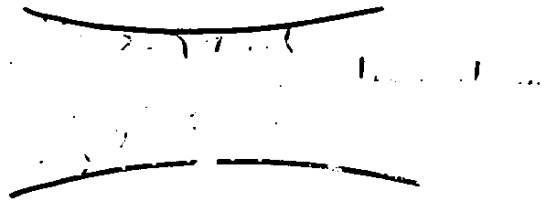


curved mica sheets

measure Force vs. distance



Coat mica:



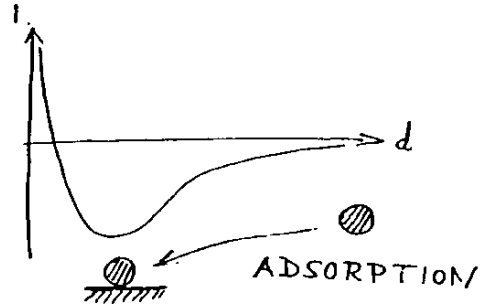
∴ No rigid polymer surface will ever be antifouling with proteins / H_2O.

∴ We need to add entropic repulsion.

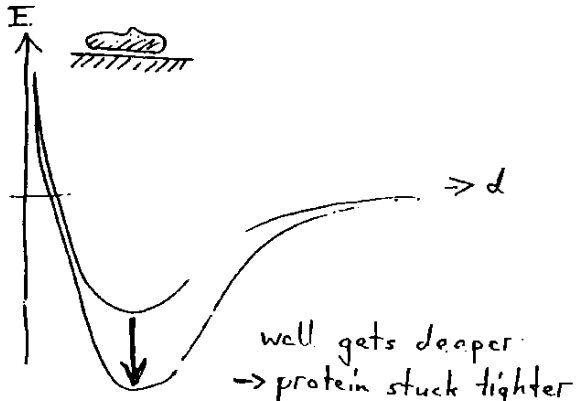
Loose, "fluffy" surface

PEG, PC, ...

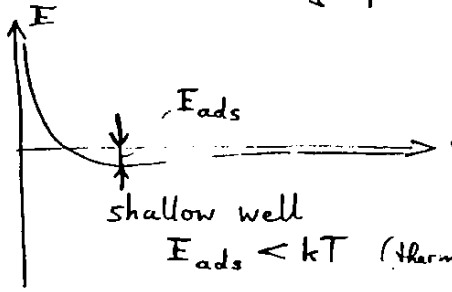
Compounding the problem:
proteins are mobile



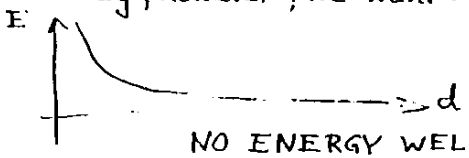
DENATURATION



Partial reversibility of adsorption:



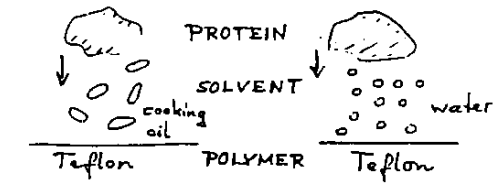
Ideally, however, we want:



There is one (and only one) truly antifouling surface:
Teflon frying pan

but Teflon artificial blood vessels clog badly (Atrium fiasco)

Compare:



$$E(d) \sim -\frac{A_{123}}{d}$$

A_{123} is

NEGATIVE POSITIVE

because

$\mathcal{E}(i\mathcal{E})$ of

Protein > oil > Teflon Protein > Teflon > water

(Drummond & Chan, Langmuir 12, 3356 (1996))

HOW CAN WE KEEP PROTEINS AWAY FROM POLYMER SURFACES?

(and lipids, mucins, .. too!)

The non-fouling surface = the holy grail of biomaterials

> 20 years of experiments

APPENDIX E

Additional Material from Breakout Groups 2 and 4

Members from Groups 2 and 4 felt that some of the material they discussed were of value but were not incorporated into the issue worksheets and therefore appear as a separate appendix.

Group 2

(Comments provided by other group members are italicized and initialed)

- **L. Dudley**
  - Drinking water RO autopsies at Houseman Ltd. Include:
    - bacterial and yeast/mold counts (cfu per cm^2) - typically 10^6 to 10^8
    - chemical content - notably Fe (from acid induced precipitation); total organic content (>80% dry weight, humics/fulvics are 30-40% of this by HPLC); water content 85-95%
    - flat sheet testing for permeability and porosity, effects of cleaning
  - The major "plant health" symptom in diagnosing biofouling is transverse pressure drop.
  - In future, looking at installing Robbin-type devices and detecting fouling as change in electrical resistance.
  - Most interested in:
    - the recruitment of particulates (such as Fe salts) from the feed to the fouling deposit caused by biofilms
    - the manipulation of feed composition to prevent fouling

- **P. Aimar**

  - Biofilms as additional semi-permeable layers, i.e., how they alter membrane feed processes by imposing their own permeability factors and selectivities for particular species on top of the membrane.

  - Prediction of biofilm evolution - in what ways does fouling modify membrane properties, in particular:

    - roughness and topography

    - surface charge (streaming potential)

    - hydrophobicity (contact angle)

  - *Contact angle measurement gives an "average" result; cannot reveal important small-scale variations in hydrophobicity across a surface. (ZL)*

  - *Surface imperfections can also change the local chemistry as well as enhancing roughness. Can these effects be separated? (ZL)*

  - Internal biofilms - conditions within the membrane matrix may be quite different, more sheltered from shear and cleaning chemicals.

- **C. H. Lee**

  - Influence of the feed biota - in MF or UF of activated sludge the fouling problem depends on the floc type - bulking sludge fouls worst. Why?

  - *"Packing" of flocs on membrane? Quantity and type of EPS? (HCF)*

  - *Bulking sludge has filaments protruding from flocs. If these are forced to intertwine in a pressurized cake, perhaps they will stabilize the fouling layer - like H. Griesser's high entropy surface polymers. (PB)*

  - The contribution of inorganics to fouling should not be overlooked, activated sludge filtration there was struvite depositing within the membrane.

  - *Could this be microbially induced? (HCF)*

- **R. Sheikholeslami**
  - Roughness:
    - fouling deposits do not fill "valleys" in a surface; they exaggerate existing roughness
    - cleaning efficacy could be quantified in terms of decreased roughness
    - aging effects on surface conditions and propensity to fouling
    - fouling studies should be done in dynamic rather than static systems
    - Using fiber optic reflectance to measure roughness
  - *Reflectance at different incident angles will give gradient distribution of surface features - better measure of "true" roughness than peak-to-trough amplitudes. (PB)*
- **G. Leslie**
  - Getting the best from membrane "autopsies," what do autopsies tell you that you don't already know from the module's on-line operation?
    - changes in permeability/rejection will be known from the permeate quality
    - damage can be detected as increased bacterial/viral counts in the permeate
  - *But what about post-membrane regrowth? (HCF)*
  - Autopsies can tell you:
    - If the deposit is biological

    Does the feed have enough BDOC to account for the size of the deposit?

- **Z. Lewandowski**

  - Minimize effort in fouling diagnosis - in the literal sense, "biofouling monitors" are not feasible. The key parameters for routine detection of fouling are:

    - pressure drop (along the modules)

    - permeate quality

  - For understanding a biofouling deposit the following analyses are required:

    - *Structural/Chemical* (thickness and porosity [CSLM]; density; polymer content; viscoelastic properties [how?])

    - *Microbiological* (total cell count; physiological groups, their response to environmental changes, e.g., feed composition; activity [microelectrodes; use/uptake of substrates; CTC vs. DAPI counts])

- **N. Ashbolt**

  - Must understand how the type and quantity of electron donors BDOC) in the feed affect the nature of resultant biofouling on membranes.

  - Cryosectioning of deposit on an "autopsied" membrane can give you:

    - thickness of deposit (-> volume per unit membrane area)

    - number of cells per unit volume

    - cell growth per unit volume (labeled substrates)

    - cell activity per unit volume (CTC staining)

    - identification of physiological groups

    - identification of phylogenetic groups

  - All of this information can be related back to the composition of the feed. Perhaps it will be possible to predict development of fouling given particular conditions and to reduce fouling by manipulating the feed.

- Breakthrough of pathogens - improvements in module integrity/reliability required for public health assurance (e.g., sewage reclamation). Testing by viral probes

- **H-C. Flemming**

  - Measurement of rigidity, viscosity, and viscoelasticity will give important information about biofilm structure.

  - These are parameters linked to transverse pressure drop, the key plant diagnostic for biofouling.

  - *Viscosity measurement - biofilm could be infused with fluorescently labeled magnetic microbeads. Their movement rate under an applied electromagnetic field of adjustable strength could be tracked at various depths in the biofilm by CSLM. (PB)*

- **"Wish List" of Future Methods for Biofouling Analysis**

  - roughness - physical, hydrodynamic (from transverse pressure drop)
  - viscosity
  - viscoelasticity
  - surface charge
  - "aging"
  - extracellular Polymeric Substances (EPS)

- **"Ideal" Monitoring Device for Membrane Plants**

  Will comprise both the following:

  - surface probe integrated into module, even into membrane surface.
  - feedwater monitor of bacterial load (flow cytometry?).

The monitors are on-line, providing continuous data, and able to alert the operator to a developing biofouling problem. This enables the operator to take remedial action and then check the results using the monitors.

Example of a surface probe - FTIR crystal appressed to fouling layer. Gives information on thickness (how much is there?) and chemical composition (what is it?) of the fouling layer. (HCF)

Example of a water probe - "Total Activity probe." Feed the module a labeled nutrient containing heavy and light carbon (or sulfur, etc.) isotopes in known proportion. Analyze organics in reject and permeate; measure change in isotopic proportions due to selective uptake of light isotopes by living systems. (NA)

Less ideal, but probably more practical, is to incorporate testing surfaces into the plant. These can be entire sacrificial membrane elements, accessible non-membrane surfaces, or removable membrane coupons (Robbins device).

Laboratory systems must incorporate uniform adjustable shear to simulate crossflow (ala ROTOTORQUE™), as well as transmembrane flows. Should be designed to allow continuous analysis of fouling during operation, rather than stop/start autopsying.

Group 4 - Issues Identification

- **BIOLOGICAL**

Antifouling Mechanisms

- signaling:

What molecules do bacteria use to talk to each other and how can these substances be employed to control biofilms?

- response to surfaces:

What new proteins, polysaccharides and other types of biomolecules do cells produce when they adhere to surfaces? Basically, what are the physiological differences between attached (biofilm) bacteria and suspended (planktonic) organisms?

Biological Indicators

- "reporter genes".
- bioluminescence
- lipid shifts
- specific genes - heat shock, mRNA
- morphologic changes
- physiological indicators
- stains - live/dead
- scanning microelectrodes
- 4-dimensional mapping (time and space).

Practical Responses

- RO - self-clean coatings

  - polyamide coatings: release ↑, resistance to fouling ↑

- flow characteristics
  - laminar vs. turbulent
  - effects of shear on biomass
- indicators of biofouling potential: marine desalination, waste water

- **POLYMER / SURFACE SCIENCE**

Accurate Measurement of Heterogeneity of Surface

- over time
- under water
- under biofilm !!

Particularly in relation to:

- topography
- chemical functionality
- chemical activity
- structure
  - mobility
  - stability
- surface energetics

Measurement of Interfacial Adhesion

Interfacial Dynamics in Response to Biofilms (Not Average, Molecular Resolution at Å Level Required!

Advanced Theoretical Modeling of Interfacial Interactions

- advanced modeling of interfacial interaction

  advanced modeling of surface chemistries for testing hypotheses, e.g., mixed SAMS

- development of new methodologies/instrumentation

Controlled Release

- reservoirs
- polymers

Surface Design

- desquamation (controlled shedding of polymer layers).
- passivation - non stick surface chemistries (requires control of the entropy of the surface)
- alternatively: termination of biofilm growth at acceptable levels
- use of biological polymers as membrane materials and as membrane coatings
- design of controlled mobility surface
- design of polymers with specific properties (or exchange of knowledge)
- surface immobilized natural or synthetic antifoulants and other bioactive species
- controlled chemical heterogeneity of surfaces
- stabilized "wobbles", e.g. make sure that the flexibility of polymer chains immobilized on substratum surfaces is not lost by immobilization of these polymers in "energy troughs"

APPENDIX F

CONSOLIDATED ISSUES

Following the last group presentation on Sunday, the issue title sheets were posted on around the walls of the meeting room and numbered in sequence. The Chair then drew the delegates' attention to each issue sheet in turn, asking them to identify other sheets with similar or subordinate concepts. Each issue originator was assured that they would retain the right to either merge their issue into a group of similar issues, or to insist that their issue stand alone. Sheets that had already been subsumed were skipped when they were encountered later in the sequence.

The authors that originated each of the issues thus clustered were asked to meet and combine their ideas into consolidated concepts, which were entered onto a fresh Working Form. Issues subsumed under a major issue group were included in their entirety in the final report.

ISSUE 1

Improve Communication

Originators: Schaule on behalf of herself, Fergus, Flemming, Leslie, Lewandowski, and March.

Significance:

Create mechanisms for enhanced communication within the worldwide membrane community - researchers, designers, membrane manufacturers, users.

Suggested Approach:

- Workshops/meetings/joint ventures.
- Establish network supported by database on biofouling control and membrane fouling monitoring.
- Develop expert system to evaluate and standardize biofilm methods relevant to membrane biofouling.

THE FOLLOWING ISSUES WERE SUBSUMED UNDER THE ABOVE TITLE:

Sub-Issue: **Biofouling Control Database & Expert System.**

Originator: Fergus

Significance:

Knowledge gained worldwide by system users/designers/researchers on system design/membrane selection, operating procedures and experiences (including short- term and long-term shutdown, disinfection techniques) should be accessed to optimize system effectiveness.

Suggested Approach:

Establish database (and expert center/network) of operating/maintenance strategies for control of biofouling for reclamation and desalination applications.

Sub-Issue: **Expert System to Evaluate Optimal Parameter Selection in Module Autopsy**

Originator: Flemming

Significance:

Module autopsy gives information about reasons of failure. It is important to correctly identify the reason in order to design effective countermeasures.

Suggested Approach:

- Statistically correlate data of parameters with problem case histories.
- Identify significant correlations.
- Evaluate "tool-box" appropriate to different characteristic situations.

Sub-Issue: **Obtaining Best Information from Membrane Autopsy**

Originator: Leslie

Significance

Detection of microbial phylogeny and physiology. Determine what species are selected for on the membrane surface.

Suggested Approach:

Develop a suite of methods/techniques for characterization of phylogenetic groups and physiological states of bacteria in biofilms.

Sub-Issue: **Create a Database of Membrane Fouling and Membrane Fouling Monitoring**

Originator: Lewandowski

Significance:

Organizing information relevant to membrane fouling.

Suggested Approach:

Use existing published papers to organize a system where membrane operators could refer the problem they experience to other known situations.

Sub-Issue: **Worldwide Database of the Biology of Membrane Biofouling Organisms Should be Constructed**

Originator: March

Significance:

It is important to know what is present in systems with problems and what is present in systems that work. Determine a baseline and characterization of tolerable biofilm fouling.

Suggested Approach:

- A committee should select reference sites around the world which routinely determine what organisms & other biofilm parameters, are present in the membrane systems.
- The committee should also select standard methods to be employed at the sites. The methods should be modern, rapid, cost effective, molecular probing and should be correlated with membrane performance.

Sub-Issue: **Enhance the Possibility of Communication Between Microbiologists and Engineers**

Originator: Schaule

Significance:

Reduce redundancy, improve cooperation, and maximize financial resources.

Suggested Approach:

- Include microbiology in the study (curriculum?) of engineers.
- Workshops and meetings between experts.

ISSUE 2

How can Feed Water be Pretreated in Order to Minimize Biofilm Formation or Enhance Biofilm Removal in Membrane Systems?

Originators: March on behalf of himself, Ben Aïm, Fane, Fergus, and MacLean.

Significance:

If microbial populations could be minimized and/or inactivated in feed water, then biofouling may be reduced. These treatments may be applicable and may be effective within the environment of the membrane itself.

Suggested Approach:

- Nutrient removal.
- Create an "unfriendly" environment by preconditioning feed.
- Promote the aggregation/adhesion of bacteria in feed water.

THE FOLLOWING ISSUES WERE SUBSUMED UNDER THE ABOVE TITLE:

Sub-Issue: **Promoting the Aggregation of Bacteria for Preventing the Biofouling or Modifying the Nature of the Biofilm**

Originator: Ben Aïm

Significance:

Probably not possible with spiral modules but with tubular or hollow fiber. Would concern mainly UF/MF more than RO. Could be focused on combined processes like flocculation/MF or PAC/UF used for water treatment.

Suggested Approach:

Look to the best conditions for the use of flocculants and PAC addition when used in combination with membrane process for enhancing bacterial adhesion/aggregation; consequences on biofilm formation and characteristics.

Sub-Issue: **Controls of Membrane Properties by Tailoring pH and Ionic Environment**

Originator: Fane

Significance:

Hans Griesser's observations that lower pH lowers contact angle implies the possibility of less fouling at lower pH. Surface charge will vary with pH.

Suggested Approach:

Evaluate lower pH operating on biofouling. (Possible economic penalty here - need optimization).

Sub-Issue: **Environment Creation to Minimise/Avoid Fouling. Modification of Feedwater and Membrane Environment to Minimize Biofouling**

Originator: Fergus

Significance:

Hypothesis: Biofouling mechanisms/morphology is known (!) so an environment can be created to avoid or control unacceptable performance caused by biofouling.

Suggested Approach:

Create an environment (e.g., preconditioning feed stream temperature control, pH, and maintain optimum conditions within membrane) which avoids biofouling.

Sub-Issue: **Can Nutrient Removal be an Effective Means of Inhibiting Bio-fouling in Seawater RO Plants?**

Originator: March

Significance:

If nutrient removal can be accomplished, then bacteria will not grow.

Suggested Approach:

Introduce an organism that can actively accumulate a limiting nutrient. Investigations should be performed to select an ideal organism. Then remove the organism in a pre-filtration step prior to the osmosis step. Carry out RO on nutrient-depleted water.

Sub-Issue: **Mechanism of Fouling Layer Formation: Effect of Nutrient Status on Biofilm Growth**

Originator: MacLean

Significance:

Presence of assimilable organic carbon or other nutrients (e.g., nitrogen and phosphorus) will have a marked influence on growth of attached organisms. The removal of any of these elements limits the potential for any microbial growth.

Suggested Approach:

Development of techniques to limit the availability of nitrogen, phosphorus or a trace element. Nitrogen and/or phosphorus could be removed by traditional physicochemical processes prior to water being fed to the membrane system.

ISSUE 3

Relationship of Polymer Properties to Molecular Mechanisms of Attachment and Interactions Within the Biofilm

Originators: Kingshott on behalf of himself, Ridgway, St. John and White.

Significance:

Systematically generate polymers with defined chemical and physical properties to test responses for attachment and interactions of the biofilm at the molecular level.

Suggested Approach:

Requires a multidisciplinary research approach involving *de novo* polymer design, synthesis, and molecular scale characterization. Relationships would be

explored between microbial attachment and interactions with the defined polymers surfaces.

THE FOLLOWING ISSUES WERE SUBSUMED UNDER THE ABOVE TITLE:

Sub-Issue: **Understanding Biofilm Formation at the Fundamental Level in Terms of the Exact Chemistry Involved in Cellular Responses**

Originator: Kingshott

Significance:

Understanding what triggers a bacterium to change its phenotype as it adheres to the surface, i.e, what surface triggers the production of particular extracellular proteins or polysaccharides.

Suggested Approach:

Use surfaces with well defined chemistry and structure. In particular, self-assembled monolayers (SAMs) on gold to particularly control the exact chemistry of the surface. Then study the effect of changing the chemistry on cellular adhesion. For example, mixed monolayers of carboxy-, methyl- and amine-terminated SAM.

Sub-Issue: **Molecular Characterization and Modeling of Polymer Surfaces in Hydrated Conditions and Under Biofilms**

Originator: Ridgway

Significance:

There is inadequate information concerning the surface molecular conformation of polymer membranes. Knowledge of surface conformations is essential to understanding foulant-polymer interactions and would greatly assist in the design of new polymer membranes with reduced biofouling potentials.

Suggested Approach:

- Application of modern surface analytical techniques to characterize the molecular structure of hydrated polymer membranes.

- Correlation of polymer surface chemistry and molecular structure with organics adsorption and bacterial attachment.
- Molecular modeling of polymer conformations would represent an important part of this study.
- Development of techniques which could characterize surface heterogeneity on a molecular scale, e.g., AFM mapping techniques.

Sub-Issue: **Analysis of Foulant-Polymer Molecular Interactions**

Originator: Ridgway

Significance:

Molecular interactions between foulants (e.g., organics, bacteria, etc.) and separation membranes are poorly understood. Theses molecular interactions form the basis of bacterial adhesion and initial biofouling. Knowledge of foulant-polymer molecular interactions is needed for the rational design of new cleaning agents or feedwater additives to retard bacterial attachment.

Suggested Approach:

- A multi-disciplinary approach is proposed involving various surface analytical techniques, such as ATIR-FTIR spectrometry, NMR, and molecular modeling.
- Initial studies should probably be limited to no more than a few foulants (e.g., several EPS types or proteins) and membrane types and could benefit from model studies on well-defined surface chemistries.
- Include effects of water on foulant-membrane molecular interactions.

Sub-Issue: **Identify Primary Trigger Foulant(s) in Each Particular System, and Understand How it Interacts with Membrane Surfaces in Order to Design Strategies to Prevent Fouling**

Originator: St. John

Significance:

Different bio-species, different environment (e.g., pH, ionic content), and different polymer surfaces lead to different fouling mechanisms. An anti fouling

approach may be very effective in one system (e.g., RO) but not in another (e.g., biomedical membrane).

Suggested Approach:

- Nominate systems of interest and conditions.
- Sample appropriately.
- Identify primary foulant.
- Characterize chemistry and structure of polymer surface prior to and during adsorption of biological species.
- Determine adhesive forces between species and polymers.

Sub-Issue: **Polymer Properties vs Antifouling and Fouling Release Properties of Microbial Biofouling Biofilms**

Originator: White

Significance:

What polymer properties including heterogeneity, affect antifouling and fouling-release effectiveness. Systematically generate polymers with defined physical and chemical properties and test antifouling and fouling-release.

Suggested Approach:

Make polymers with a gradient of properties including mobility effects over time and in the water. Measure antifouling and fouling-release rates in a quantitative way using a bioluminescent bacteria in a laminar flow apparatus as a quantitative initial screening system.

ISSUE 4

Non-destructive Monitoring of Membrane Biofouling

Originators: Ashbolt on behalf of himself, Aimar, Flemming, and Lewandowski.

Significance:

- Quartz crystal microbalance ~ 1 -2 cm^2 gold oscillating surface - calibrate with varying thickness of fouling.
- Tomography/ X-ray analysis, etc. unit tomography or *in* plant analysis including in situ AEM, Raman spectra and ellipsometry.
- Streaming current potential: relationship of charge change and fouling.
- Fiber optics reflectance: top and bottom analysis of fouling on optical fiber *in situ* (several per unit).

Suggested Approach:

All methods will be calibrated against autopsy and analysis of fouling thickness and composition, plus *in situ* measurement of Dy/Dp of membrane. Also, follow dampening of oscillation at optimal frequency of quartz crystal microbalance; try Raman spectroscopy, ellipsometry, X-ray with different additives to enhance signal from fouled layer and NMR tomography; calibrate charge changes by varying distances between 2 electrodes up and across the whole membrane unit; vary light frequency and angle to calibrate optical fiber sensor.

THE FOLLOWING ISSUES WERE SUBSUMED UNDER THE ABOVE TITLE:

Sub-Issue: ***In-situ* Measurement Device for Measuring the Coverage of the Surface by Fouling (Streaming Potential)**

Originator: Aimar

Significance:

- Identifies which fraction of the whole surface is covered by at least one monolayer.
- Global information on a whole module.

Suggested Approach:

Measure streaming potential along module and monitor during use; compare with pressure drop.

Sub-Issue: **Tomography of Whole Modules using NMR/X-RAY Dense Materials**

Originator: Ashbolt

Significance:

Non-destructive *in situ* method to assay total fouling of a membrane unit may not even have to move the unit, (i.e., external X-ray assay).

Suggested Approach:

Feed a membrane a "barium meal" and then detect the spatial distribution of X-ray dense material. Alternatively, use NMR to detect H3 / C13 ratios in the biofilm.

Sub-Issue: ***In-situ*** **Measurement of Changes in Optical Density at the Biofilm / Bulk Water Phase Interface**

Originator: Flemming

Significance:

The transition between the bulk phase and the biofilm is important for pressure loss in membrane systems.

Suggested Approach:

Develop sensors, (lasers, optical fibers, etc.) that can be used to make non-destructive measurements of biofilm surface roughness.

Sub-Issue: ***In situ*** **Measurement of the Degree of Fouling**

Originator: Lewandowski

Significance:

Monitoring the progress of Biofouling.

Suggested Approach:

Use quartz microbalance, which is known to respond to the amount of material deposited on the crystal surface, to monitor the amount of fouling.

ISSUE 5

What are the Molecular Mechanisms that Keep the Biofilm Structure Together?

Originators: Schneider on behalf of himself, Dudley, Flemming, and Lewandowski

Significance:

Knowledge of the structural properties of the molecules which confer stability to a biofilm is essential because:

- These compounds determine the structure of the biofilm and thus its elasticity, visco-elasticity, local hydrodynamics and rigidity.
- These compounds determine how abiotic particles (humic acids, iron) get incorporated into biofilms.

Suggested Approach:

- Improve autopsy characterization techniques.
- Link laboratory studies with onsite studies.
- Identify the chemical components of the biofilm matrix.
- Develop means to measure biofilm matrix composition, elasticity, and other mechanical properties *in situ*.

THE FOLLOWING ISSUES WERE SUBSUMED UNDER THE ABOVE TITLE:

Sub-Issue: **Effect of Abiotic Particles (in Particular Humic and Iron Colloids) on the Stability of Biofilms in Membrane Systems**

Originator: Dudley

Significance:

Improvements in surfactants and dispersants are contingent upon an understanding of the materials that stabilize the biofilm. Knowledge in this area may influence the most suitable chemical cleaning practices and pre-treatment selection.

Suggested Approach:

- Characterize the effect/role of abiotics on biofilm stability.
- Assess the effects of cleaning solutions on the removal and dispersal of biofilms.
- Correlate cleaning efficiency with abiotic material in the presence of biofilms.
- Determine the mechanisms of abiotic fouling with biological fouling also present.

Sub-Issue: **Measurement of Biofilm Rigidity / Viscosity**

Originator: Flemming

Significance:

Rigidity controls the ease of dispersion, the hydrodynamic resistance and possibly the permeability of the fouling layer. Rigidity measurements would be useful to characterize the biofilm "wickedness."

Suggested Approach:

- Measure the force necessary to cause deformation of the biofilm.
- Measure the resistance the biofilm applies to magnetic beads "dancing in the slime."

Sub-Issue: **Characterization of Biofilm Viscoelasticity and Internal Structure, and the Effect of these Properties on Local Hydrodynamics**

Originator: Lewandowski

Significance:

- Fouling
- Economic performance

Suggested Approach:

- Relate the visco-elastic properties to nutrient composition; polymer concentration; inorganic composition, and bacterial physiology.
- Correlate these biofilm properties with pressure drop.
- Result - to define a critical yield stress / shear stress for biofilms.

Sub-Issue: **What are the Molecular Mechanisms that keep the Biofilm Structure Together ?**

Originator: Schneider

Significance:

Knowledge of these mechanisms will help us to:

- Develop antifouling compounds on a rational basis.
- Develop membrane surface chemistries which produce minimal biofilm stability.

Suggested Approach:

Characterize the EPS in biofilms.

ISSUE 6

Determination of Optimal Cleaning Strategies to Remove Biofilms

Originators: Bird on behalf of himself and Fane

Significance:

- Essential for performance.
- Suboptimal cleaning has implications for chemical and water use, energy consumption, membrane and module life time and increased environmental impact.

Suggested Approach:

- Examination of the effect of multiple deposition/cleaning cycles on the lifetime and the characteristics of the membrane.
- Investigation of the relationship between sanitation and cleaning: defining the criterion for cleanliness required.
- Examine use of low ionic strength water and *in situ* generation of acid and base as cleaning agents.

THE FOLLOWING ISSUES WERE SUBSUMED UNDER THE ABOVE TITLE:

Sub-Issue: **The Effect of Multiple Deposition / Disinfection Cycles upon the Lifetime and Characteristics of the Membrane**

Originator: Bird

Significance:

A "clean" membrane surface that has been fouled and disinfected may give a good flux recovery. However, the surface may be conditioned to accept adhesion on subsequent use.

Suggested Approach:

Evaluate the real life situation of the effect of multiple fouling and cleaning cycles on membrane performance and lifetime.

Sub-Issue: **The Relationship Between Disinfection and Cleaning Performance**

Originator: Bird

Significance:

Effective disinfection can still create large numbers of nonviable cells on a membrane surface. What is the criterion for a successful cleaning/disinfection cycle?

Suggested Approach:

- Define the criterion for cleanliness needed with each system. Until the "end point" or goal of cleaning and disinfection is defined, it is impossible to develop a cleaning strategy.
- How clean is clean?

Sub-Issue: **Design a Simple Cleaner - Low Ionic Strength and Acid / Base Cycling by *in situ* Electrolysis**

Originator: Fane

Significance:

Low ionic strength water appears to remove some biofilms. Acid/base cycling encourages removal of biopolymers.

Suggested Approach:

- Evaluate sequencing of low ionic, pH varying aqueous solutions for cleaning.
- Evaluate the application of *in situ* generation of acid/base.

ISSUE 7

Improved Module Design with Low Fouling Propensities and Enhanced Cleanability

Originators: Henthorne on behalf of herself, Fane, and Schaule.

Significance:

Currently used module designs do not consider the biofouling issue.

Suggested Approach:

- Develop alternatives to spiral-wound or hollow-fiber configurations.
- Develop alternative spacers for spiral-wound elements to avoid dead spots and provide open flow path.
- Develop "active" spacers that provide agents that inhibit biofouling.

THE FOLLOWING ISSUES WERE SUBSUMED UNDER THE ABOVE TITLE:

Sub-Issue: **Enhanced Control of Concentration Polarization by Critical Flux and Module Design**

Originator: Fane

Significance:

- Bacteria can move up salt concentration gradient by diffusiophoresis. This mechanism would be mitigated by enhanced control of concentration polarization.
- Different scenarios for SW desalination and low TDS water reclamation.
- The observation of critical flux for biofouling may relate to this. Could be controlled by module (spacer) design.

Suggested Approach:

- Evaluate effect of flux on biofouling, in terms of salt, organics and biosolids polarization.
- Evaluate effect of module (spacer) design on biofilms in terms of salt polarization.

Sub-Issue: **Development of New Membrane Configurations with Low Fouling Propensity and Enhanced Cleanability**

Originator: Henthorne

Significance:

If module design could be improved to provide better hydrodynamics, significant reduction in biofouling could be realized. In addition, present module hydrodynamics makes cleaning ineffective. Existing infrastructure further restricts making drastic changes in module design >> must develop a module that can use existing infrastructure.

Suggested Approach:

Transverse flow hollow fiber configuration may provide a low-fouling environment. Terrific hydrodynamics can be achieved, but high surface area to volume ratios must be optimized. Fiber-potting techniques must be developed for high OR pressures (MF, UF and NF presently achievable). Importantly - the configuration can be fitted into existing vessels.

Sub-Issue: **Limit the Growth of the Biofilms by Changing the Design of the Spacer**

Originator: Schaule

Significance:

How do the hydrodynamics interfere with the biofilm growth in RO systems?

Suggested Approach:

Change the spacer design to enhance shear forces and reduce the area where biofilm can grow.

ISSUE 8

Tailored Surface Modification/Immobilization of Membrane Functional Sites to Prevent Attachment/Adsorption of Biospecific Functional Groups

Originators: Kingshotton behalf of himself, Y.M. Lee, and Nyström.

Significance:

Characterization of the bonds which occur between foulants and the membrane polymer surface to design the modification of the surface with specific functional groups.

Suggested Approach:

- Characterize the fouled membrane and modify it in order to prevent the process, e.g., chelating agents like humic acids need a charged metal ion to chelate with, if that is covered with reducing agents, fouling results.
- Attempt to mimic the biological environment by surface immobilization of natural molecules which are known to interact in a specific manner, e.g., zosteric acid of seaweeds prevent protein adsorption and subsequent bacterial adhesion.

THE FOLLOWING ISSUES WERE SUBSUMED UNDER THE ABOVE TITLE:

Sub-Issue: **Surface Immobilization vs Biospecificity**

Originator: Kingshott

Significance:

Mimic the mechanisms of bacterial non-adhesion, e.g., Zosteric acid of seaweeds.

Suggested Approach:

Immobilize these molecules on polymer surfaces so that they closely mimic the role that happens in the natural environment. Modify these molecules before surface immobilizing them. Play around with molecular architecture and adapt it to your specific system.

Sub-Issue: **Surface Modification of Graft Copolymer to Prevent Biofouling**

Originator: Y. M. Lee

Significance:

Entropic repulsion, enthalpic repulsion of graft material on the membrane surface to avoid adsorption of bacteria.

Suggested Approach:

- Pluronicmaterial (LCST, pH, emp.....), PEO-PPO copolymer .
- PEO-SO_3H to CA, PA in laboratory scale.
- NIPAM to commercial membranes.
- LB membrane approach to understand interactions between protein monolayer and membrane.

Sub-Issue: **Tailored Modification of Membrane Functional Sites to Prevent Attachment of Specific Foulants**

Originator: Nyström

Significance:

Characterization of bonds between foulants and membrane to tell what part of membrane chemical groups should be modified.

Suggested Approach:

- Characterize the fouled condition.
- Modify in order to prevent that.
- Examples: Chelating agents like humic acid need a charged metal ion to chelate with, if that is covered - no fouling. (Inorganic membranes sputtered with neutral gold.) Water should be retained at the membrane surface in order to prevent proteins from fouling.

ISSUE 9

Identification of Critical Operating Conditions to Control Biofilm Formation on Membranes

Originators: Winters on behalf of himself, Ben Aïm, and Bird.

Significance:

- Will involve operation of existing modules.
- Will explain the interplay of hydrodynamics of flow, permitted flux velocity, and feed composition on biofilm formation on membranes.
- Will explain the influence of operating parameters on the kinetics of biofilm formation on membranes.
- Will develop membrane process controls to reflect changing feed conditions.
- Will explain how pretreatment chemicals will affect the critical operating conditions.

Suggested Approach:

- Use RO membranes and calculate their critical flux rates and energy repulsion barriers to organisms and organics that are known to cause biofouling.
- Using "couette" or similar device (perfectly defined shear stress) for studying the influence of hydrodynamics in actual conditions of membrane operations, focusing on transient period where the forces between the bacteria and the membrane are increasing.
- Investigate the thermo-hydraulic conditions which lead to a constant biofilm thickness which is acceptable in an operating environment.

THE FOLLOWING ISSUES WERE SUBSUMED UNDER THE ABOVE TITLE:

Sub-Issue: **Influence of Hydrodynamics on the Characteristics and Kinetics of Bacteria Attachment and Detachment.**

Originator: Ben Aïm

Significance:

- Optimal design and operating conditions of modules.
- Evaluation of the forces existing between the biofilm and the membrane.
- Influence of the physiological state of the bacteria (stresses).
- Characterize the kinetics of attachment.

Suggested Approach:

Using "couette" or similar device (perfectly defined shear stress) for studying the influence of hydrodynamics in actual conditions of membrane operations, focusing on transient periods where the forces between the bacteria and the membrane are increasing.

Sub-Issue: **Selection of Thermo-Hydraulic Parameters Affecting the Temporal Development of Acceptable Asymptotic Biofilm Properties**

Originator: Bird

Significance:

All fouling processes will be at a constant rate, increasing rate, or asymptotic in nature. If we can develop an asymptotic biofilm thickness which is acceptable, we have made an advance.

Suggested Approach:

Investigate the thermo-hydraulic conditions which lead to a constant biofilm thickness which is acceptable in an operating environment.

Sub-Issue: **Adhesion of Bacteria to Membrane by Regulation of Flow Rates. Critical Flux Rates and Velocity of Feed / Brine**

Originator: Winters

Significance:

If we knew what the critical flux rates are for RO membranes in relationship to the bacteria and organic concentration, we could design membrane systems that would have low fouling potential and high repulsion barriers.

Suggested Approach:

Use RO membranes and calculate their critical flux rates and energy repulsion barriers to organisms and organics that are known to cause biofouling. These critical operating rates must be determined at different flux rates, different concentrations of bacteria and concentrations of organics. The effect of temperature and salinity of feedwater must be studied.

ISSUE 10

Which Biofilm Parameters are Strongly Correlated to which Aspects of Membrane Performance (Flux Decline, Transverse Pressure Drop, Permeate Quality), and How do They Coevolve over the Lifetime of a Membrane?

Originators: Nyström on behalf of herself and Beatson

Significance:

- To make an informed decision on which biofilm parameters are useful to monitor a real system.
- The early stage biofilm is not the same as the biofilm at a later stage that causes unacceptable performance degradation. Need to pinpoint what changes have taken place in the nature of the biofilm.

Suggested Approach:

- Laboratory, then field trials.
- Sacrificial elements, autopsies or replicate "healthy" modules over time.
- Combine with monitoring of plant performance.
- Statistical comparison of trends in variables.

THE FOLLOWING ISSUES WERE SUBSUMED UNDER THE ABOVE TITLE:

Sub-Issue: **Which Biofilm Parameters are Strongly Correlated to Which Aspects of Membrane Performance (Transverse Pressure Drop, Flux Decline, Permeate Quality)?**

Originator: Beatson

Significance:

To make an informed decision on which biofilm parameters are useful to monitor a real system.

Suggested Approach:

Measure a whole slather of variables and use statistical methods to find the correlations.

Sub-Issue: **Life-cycle Characterization of Biofilm Development vs. Performance Under Industrial Conditions**

Originator: Nyström

Significance:

- Enables the characterization of short-time and long-time fouling.
- Fouling occurs in different steps; Initial foulants not identical with later stage foulants.
- When and why is there a threshold when fouling comes to "the point of no return" >> collapse of module.

Suggested Approach:

- Sacrifice of one module every week to follow the fouling process.
- Needs cooperation with the site management and membrane manufacturers.

ISSUE 11

Understanding How Different Conditions Regulate Specific Genes in Membrane Biofilms

Originators: Kjelleberg on behalf of himself, March, and White.

Significance:

- Biological responses invoked by bacteria during the initial stage of colonization.
- Biological responses to conditions in the mature biofilm.

Suggested Approach:

Invest in molecular microbiology tools generally available.

THE FOLLOWING ISSUES WERE SUBSUMED UNDER THE ABOVE TITLE:

Sub-Issue: **Understanding Gene Expression / the Biology of the Biofilm, Particularly How Different Condition (e.g., Flow Rate, Temp., etc.) Regulate Specific Gene Expression via a Signal Transduction Pathway. For Example it is Known that Viscosity (Structured Water) Induces the Surface Mobility of Swarming Phenotype in Bacteria that Leads to Rapid Colonization**

Originator: Kjelleberg

Significance:

Not provided by originator.

Suggested Approach:

- Invest in molecular microbiology tools generally available.
- Set up a model system and understand genes expressed in a pure culture of an appropriate model organism. When specific expression has been identified, develop probes to see whether the same genes are expressed in more complex systems. The next step would be to determine the function of gene products with a long-term goal of blocking function.

- Generate membrane system - tandem bioreactor + known manipulations of membranes + bulk fluid chemistry + physics - to correlate biofilm nutritional status to membrane parameters.

Sub-Issue: **There Needs to be More Research into the Biological Responses Invoked by Bacteria During Membrane Colonization**

Originator: March

Significance:

During RO, bacteria move from the planktonic environment into a non-planktonic. It is known that there is a surface-specific expression of physiological traits (e.g., exopolysaccharide); but the mechanisms regulating such expression are not known. The scientific significance is that it is necessary to understand biological responses that are essential for successful surface colonization.

Suggested Approach:

Set up a model system and understand genes expressed in a pure culture of an appropriate model organism. When specific expression has been identified, develop probes to see whether the same genes are expressed in more complex systems. The next step would be to determine the function of gene products with a long term goal of blocking function.

Sub-Issue: **Biological Responses to Conditions on Membrane Biofilm Surface**

Originator: White

Significance:

Correlate biological response in biofilm to satisfactory membrane function - determine markers for unbalanced growth, toxicity responses, growth phase, cell lysis and determine responses that correlate with satisfactory membrane response.

Suggested Approach:

Generate membrane system - tandem bioreactor + known manipulations of membranes + bulk fluid chemistry + physics - to correlate biofilm nutritional status to membrane parameters.

ISSUE 12

Study the Ecology of Membrane Biofilm Communities

Originators: Kjelleberg on behalf of himself, and Lewandowski.

Significance:

- Use probitotic bacteria to establish a biofilm that remains thin and deters other bacteria from colonizing.
- Biofouling control by grazing.

Suggested Approach:

- Inoculation of the water tank, or immobilization beads, or immobilization onto spacers.
- Use protozoa.

THE FOLLOWING ISSUES WERE SUBSUMED UNDER THE ABOVE TITLE:

Sub-Issue: **Add "Probiotic" Bacteria to Establish a Biofilm that Remains Thin and Deters Other Bacteria from Colonizing**

Originator: Kjelleberg

Significance:

- Biological control rather than chemical control.
- Isolation of bacteria inhibitory toward other bacteria is relatively straight forward. Probiotics have been proved very useful in other systems.

Suggested Approach:

Inoculation of the water tank, or immobilization beads, or immobilization onto spacers.

Sub-Issue: **Biological Control of Biofouling Using Grazing Organisms to Increase Biofilm Permeability**

Originator: Lewandowski

Significance:

Biological control of biofouling.

Suggested Approach:

Use protozoa.

ISSUE 13

Studies of Signaling Molecules, Their Receptors and the Development of Specific Antagonists.

Originators: Kjelleberg on behalf of himself, and White

Significance:

It is clear that signaling molecules in the biofilm mediate the expression of many adhesion colonization phenotypes. The interference with these systems can be done in a nontoxic fashion. It is also clear that such signaling systems are wide spread, e.g., the homoserine lactone system.

Suggested Approach:

- Identify environmental conditions that regulate signaling mediated responses
- Use bio-mimics or antagonists of homoserine lactones or homoserine lactone like signals.

THE FOLLOWING ISSUES WERE SUBSUMED UNDER THE ABOVE TITLE:

Sub-Issue: **Understanding the Use of Signaling Molecules in Biofilm Development**

Originator: Kjelleberg

Significance:

It is clear that signaling molecules in the biofilm mediate the expression of many adhesion colonization phenotypes. The interference with these systems can be done in a nontoxic fashion. It is also clear that such signaling systems are wide spread, e.g., the homoserine lactone system.

Suggested Approach:

- Identify environmental conditions the regulate signaling mediated responses.
- Use bio-mimics or antagonists of homoserine lactones or homoserine lactone like signals.

Sub-Issue: **To Define the Receptors, Signals and Blocking Agents Useful in Confusing the Sequential Reactions Necessary for Biofilm Formation and Maturation**

Originator: White

Significance:

To stop biofouling before onset with biodegradable molecule(s) that target receptors using coating polymer reservoirs with slow release properties.

Suggested Approach:

Use bioluminescence in a biofilm organism - to identify the response on-line and non-destructively, and follow molecular responses in a biofilm system that controls the inoculum, the substratum and the bulk fluid.

ISSUE 14

What is the Mechanism of Action of Natural Antifoulants?

Originator: Schneider on behalf of himself.

Significance:

- Natural antifouling compounds would be safer to use because nature would have evolved manners of safely disposing of them.
- The molecular mechanism of how these antifoulants interfere with biofilm formation needs to be understood to formulate suitable antifouling mixtures.

Suggested Approach:

- Analyze the effects of antifoulants on the molecular composition of biofilms.
- Identify the function of the biomolecules which are affected by antifoulants.

THE FOLLOWING ISSUES WERE SUBSUMED UNDER THE ABOVE TITLE:

Sub-Issue: **Identification of the Mechanisms of Action of Natural Antifoulants**

Originator: Schneider

Significance:

Membrane systems are used on a large variety of waters. Knowledge of the action mechanisms of natural antifoulants would assist in targeting and optimizing the use of specific compounds or mixtures thereof for specific applications.

Suggested Approach:

- Identify the changes in molecular composition of biofilms produced by the action of natural antifoulants.
- Characterize modifications of biofilm structure due to the action of natural antifoulants.
- Evaluate the effect of natural antifoulants from biofilm species composition and how this relates to biofilm matrix EPS composition.

Sub-Issue: **How do Natural Antifoulants Behave in the Target Environment ?**

Originator: Schneider

Significance:

- Knowledge of whether the compounds are lost through non-specific actions (absorption, etc.)
- Definition of the rate of supply of the compound to ensure optimum biofilm control at minimum cost.
- Assess the persistence of these compounds in product water.

Suggested Approach:

Add target compounds to real matrices and find out how they partition; how active they are in the natural systems.

APPENDIX G

Additional Issues Contributed After the Workshop

ISSUE: **Change the Chemical Composition of the Spacer to Deliver Antifouling Agents**

Originator: Schaule

Significance:

Limit the growth of the biofilm.

Suggested Approach:

Modification of the spacer by including "signaling molecules" or inhibitors in a coating or matrix of the spacer surface: controlled release of agents.

ISSUE: **Critical Flux Versus Membrane Properties and Fluid Physical Chemistry**

Originator: Aimar

Significance:

Predict operating conditions to reduce tendency for fouling.

Suggested Approach:

Measure critical flux as a function of modified polymers properties in different fluid conditions.

ISSUE: ***In situ*** **Biological Cleaning of the Biofouling Layer**

Originator: Ashbolt

Significance:

Induction of shear plane in biofilm by acyl homoserine analogues.

Suggested Approach:

Model biofilm; demonstrate proof of concept. Assay a range of AHL's and apply to native biofilms. Compare to other methods of shear plane induction, e.g., disinfectants, etc.

ISSUE: **Repulsion of Biogrowth**

Originator: Chida

Significance:

Minimize membrane biogrowth/Dp.

Suggested Approach:

Create environment by addition of affordable quantities of biodegradable chemicals (e.g., tannic acid, etc.) which discourage biogrowth and keep it at a minimum.

ISSUE: **Process and System Hydraulics**

Originator: Chida

Significance:

Present classical pre-treatment creates colloidal and biological instability.

Suggested Approach:

- Value analysis to eliminate pre-treatment chemicals and equipment which destabilize process and enhance fouling/biogrowth.
- Improve system velocities in a cost-effective manner.

- Improve device hydraulics and minimize dead ends/very low velocity areas, etc.

ISSUE: **Study of the Relationship Between Membrane Autopsy Results and Plant Fouling/Performance Characteristics**

Originator: Dudley

Significance:

To enable prediction of performance and relate foulant composition to flux decline/Dp

Suggested Approach:

- Database of autopsy results vs. plant performance.
- Determine 'biofilm threshold level."
- Data correlation.

ISSUE: **Biological Scavenger to Clean Biofilm**

Originator: Fane

Significance:

Protozoa (?) and scavengers could be used to graze the surface of the biofouled membrane.

Suggested Approach:

- Need to evaluate efficacy of the scavengers (types and times required).
- Need to develop systems to deliver scavengers.

ISSUE: ***In situ*** **Measurement of Biofilm Dielectric Properties (Impedance)**

Originator: Leslie

Significance:

A method for monitoring biofilm growth.

Suggested Approach:

Use platinum electrodes to measure changes in the dielectric constant of the bulk solution/biofilm (region between electrodes).

ISSUE: **Development of Useful *In Situ* Detection Technique to Study the Mechanism of Biofouling and Interaction Between Membrane Surface and Solution**

Originator: Mansouri

Significance:

To extend/expand this knowledge (mechanism of biofouling/interaction) can lead to the design of new systems more resistant to biofouling.

Suggested Approach:

- Identification of present methods to understand their abilities and limitations.
- Modify those techniques to be used in *in-situ* scheme (*in-situ* Raman spectroscopy, Ellipsometry, optical microscopy, AFM).
- Polymer design with desired surface properties.

ISSUE: **Identification and Evaluation of Chemical Dispersants to Maintain Bacteria in Suspension and Prevent their Attachment to Membrane Surfaces**

Originator: Ridgway

Significance:

Polymeric dispersants have been shown to stabilize colloid suspensions. It may be possible to use such dispersants to retard bacterial attachment to polymer separation membranes. Currently, very little is known about the interaction of high-molecular-weight polymeric dispersants with bacterial cells.

Suggested Approach:

Screen commercial and experimental dispersants for their ability to inhibit bacterial attachment to separation membranes. Microscope flow cells or coupon assays using radio-labeled fouling bacteria could be used to rapidly evaluate whether selected dispersants act effectively as inhibitors of bacterial attachment to membranes.

ISSUE: **Alternative Non-Chemical Biocides**

Originators: Ridgway, Henthorne

Significance:

Traditional chemical biocides have been evaluated at length, yet none have been found to be ideal and most pose significant handling hazards. Non-chemical biocides could potentially be more effective than traditional biocides and offer handling and disposal advantages.

Suggested Approach:
A number of non-chemical biocides are known. Efficacy of the method should be evaluated in addition to membrane compatibility. Economic evaluation of the method should also be compared to presently used traditional biocides, while compensating for environmental, disposal and handling advantages.

ISSUE: **Establishment of a Biofouling Index as a New Index to Replace SDI (Silt Density Index)**

Originator: Sadr Ghayeni

Significance:

All feeds that have biofouling ability result in non-relevant SDI (silt density index). Therefore a biofouling index could be very important.

Suggested Approach:

Research project.

ISSUE: **Study of the Molecular Structure of the Biofilm at the Interfaces (Water and Substratum)**

Originator: Sadr Ghayeni

Significance:

Lack of the knowledge on this issue keep the biofilm as a "black box."

Suggested Approach:

Chemistry of the water, organics in biofilm, inorganics in biofilm, chemistry at surface. Instrumentations are available. Finally research project.

ISSUE: **Design of Self-Cleaning Membrane and Membrane Modules**

Originator: Sadr Ghayeni

Significance:

Based on manipulation of module geometry and spacer configuration and material.

Suggested Approach:

Research project.

ISSUE: **Study of the Bacterial Supernatant.**

Originator: Sadr Ghayeni

Significance:

Some culture supernatants can prevent bacterial adhesion.

Suggested Approach:

Research project.

ISSUE: **Is the Adhesion of the Biofilm to the Membrane Surface Responsible for the Integrity of the Biofilm or is this Determined by the Interactions of the Components Within the Biofilm?**

Originator: Sadr Ghayeni

Significance:

Energy balance.

Suggested Approach:

Modeling (research project).

ISSUE: **Development of Dynamic Monitoring Methods for Fouling as Opposed to Static Ones Currently Being Used**

Originator: Sheikholeslami

Significance:

Investigate the effects of hydrodynamics of flow, the thickness growth, the effect of cleaning.

Suggested Approach:

Modification of current ellipsometry technique.

ISSUE: **Determine the Effect of Biofouling on Inorganic Fouling and Vice-Versa**

Originator: Sheikholeslami

Significance:

Affects control of fouling and cleaning procedures.

Suggested Approach:

Run controlled tests and monitor the factors affecting each type of fouling.

ISSUE: **Focus on the Bioactivity of the Water Sources that Cause the Greatest Fouling of RO Membranes, i.e., Seawater, Municipal Wastewater**

Originator: Stefanic

Significance:

These are the two major sources of feedwater to the RO membrane systems that cause the greatest biofouling and reduction of efficiency. Eliminating 30% of the irreversible fouling would have significant economic effects on the capital cost and operating cost of the RO system.

Suggested Approach:

Joint involvement of a research group like UNESCO, the R&D group of the membrane manufacturer with pilot testing at a seawater, or water facility with known severe fouling.

ISSUE: **In Membrane Elements Study the Relationship of Shear Effects on the Adhesion of Biofilm to the Velocity as well as the Laminar/Turbulent Flow of the Feedwater in Spiral Wound Element Brine Channels. The Effects of the Shape, Form and Thickness of the Brine Channel Spacer Should be a Major Inclusion in the Study**

Originator: Stefanic

Significance:

By reducing the biofouling tendency and the severity of the fouling and improving the cleaning/removal of the formed biofilm, there would be an increase in the operational effectiveness of the RO membrane element. This would result in capital and operational cost savings which could simplify pre-treatment and system design. It would also increase acceptance of membrane technology in situations in which it had been considered uneconomic or unfeasible.

Suggested Approach:

Research testing of the operation of membrane elements in an 8" diameter, 6-element pressure tube. Research to be executed jointly by a research group and membrane company.

ISSUE: **More Intensive Studies on the Relationship of Bacteriocides and Pre-Treatment Chemicals with Microbiological Matter which Create Biofilms and Biofouling**

Originator: Stefanic

Significance:

The reverse osmosis industry uses many chemicals to pretreat the feedwater to RO systems. The chemicals are injected to treat both organic and inorganic substances in the feedwater. The amount of these chemicals injected varies from one to many, yet very little is known about the collective effects on the microbiological substances causing biofilms and biofouling.

Suggested Approach:

Joint research between a research organization and chemical suppliers at a pilot plant operating at various sites using RO with complex pre-treatment and pre-treatment chemicals experiencing high incidences of biofouling.

ISSUE: **Utilize Microbial Interactions in Biofilm for Biofouling Control**

Originator: White

Significance:

- Use microbial ecology -> predation (ciliates, amoeba, cytophagas, flavobacteria, bacteriophage, *Bdellovibrio*, bacteriocin formation) and probiotic organisms.

- Inoculate with species biofilm (*Lactobacillus* concept) co-aggreation sequences.

Suggested Approach:

Comprehensive examination with biomarkers - lipids, nucleic acids and on-line methods - receptors, signals.

APPENDIX H

INFORMAL PRESENTATIONS

New and Emerging Techniques for Microbial Biofouling Biofilm Characterization and Monitoring

by

David C. White
Center for Environmental Biotechnology, the University of Tennessee, Knoxville, Tennessee, USA

Monitoring and Characterization of Membrane Biofilms

by

Linda Dudley
Houseman, Limited, UK

Membrane Biofouling in Membrane Bioreactor

by

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New and Emerging Techniques for Microbial Biofouling Biofilm Characterization and Monitoring

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METHODS FOR BIOFOULING BIOFILM ANALYSIS

Generation of reproducible microbial biofilms -- To examine the microbial ecology of biofouling biofilms, a system for the development and reproducible microbial biofilms required the control of the: Inoculum (each microbial species is inoculated in sequence from individual continuous culture); Substratum - the chemistry, surface topology, and heterogeneity of these properties on the substratum coupon must be controlled; and, Bulk Fluid - the chemistry which utilizes suboptimal nutrients composition to promote biofilm formation, the pH, the Eh, and the physics (shear forces), temperature must be controlled.

With this system it proved possible to study effects of the three components on the formation, succession, stability, and senescence of microbial biofouling biofilms.

Quantitative assessment of the microbial biofouling biofilm by signature lipid biomarker (SLB) analysis -- Microbial biofilms can be quantitatively monitored without the necessity for isolation and subsequent culturing by utilizing SLB. SLB provides the viable and total microbial biomass, the community composition, and the physiological status. SLB also allows the concurrent recovery of DNA for gene probing for specific analysis as well as the detection of immune potentiating antigens released from bacteria.[1] SLB has proved important in the detection of infectious but not culturable emerging pathogens

from drinking water including the ultrasensitive detection/identification of *Cryptosporidium* species and the simultaneous determination of the infectivity of the oocysts for neonatal mice. SLB also provides "exposure" biomarkers for oxidative biocide treatment as specifically modified lipid biomarkers. SLB analysis has been utilized to quantitatively define the marine microfouling community and to follow succession from a specific initial microfouling community through its maturity on a variety of substrata exposed to seawater[2].

Studies of biofilm ecology of drinking water pathogens in a biofilm formed from *Acidovorax* sp., *Bacillus* sp. and *Pseudomonas* sp. Isolated from drinking water distribution systems were studied. The biofilm was developed in a laminar flow apparatus and the biofilm biomass monitored on-line, non-destructively by fluorescence of tryptophane in the microbial proteins. Once the steady state biofilm formed, the system was inoculated with either *E. coli*, or *Mycobacterium smegmatus* (each engineered to contain green fluorescent protein, GFP) and the effects of 0, 1 and 5 ppm chorine in the bulk phase tested. *E. coli* showed a much greater resistance to chlorine if it was nurtured in the tri-species microbial biofilm when compared to exposure during pelagic growth or in a monospecies *E. coli* biofilm. The *M. smegmatis* was essentially unaffected by the chlorine but could not associate in a biofilm unless the other microbes were present as a basic biofilm to infect. The pathogens showed considerably less indications of metabolic stress as indicated by the following analysis of their SLBs: 1) exposure to toxins induced increased ratio of *trans* monoenoic phospholipids ester-linked fatty acids (PLFA)/*cis monoenoic* (PLFA); 2) slowed growth with induction of stationary phase by an elevated ratio of cyclopropane PLFA/monoenoic PLFA; and 3) evidence of cellular lysis by increased ratio of diglyceride fatty acids/PLFA. Similar nurturing of *Legionella bozmanii* in the tri-species drinking water biofilm and capture of oocysts of *Cryptosporidium parvum* have been detected in this test system [3,4].

Tests of the response of a biofouling biofilm to different substratum chemistry and bulk fluid physics have been performed in a laminar flow apparatus in which the relative fouling rate by the bioluminescent *Vibrio harveyi* has been determined for a series of coatings. The system is a once-through seawater system in which the biofilm is monitored non-destructively on coupons which are compared to stainless steel coupons placed just proximal and distal to the test coasting. Rate of biofouling was monitored by tryptophane fluorescence and relative toxicity by the bioluminescence response relative to the biomass[5]. Antifouling (AF) activity was measured by increase in tryptophane fluorescence with a bulk fluid seawater flow rate of 3 dynes/cm^2. Once established after 4-5 days, the *V. Harveyi* biofilm was subjected to a flow of 300 dynes/cm^2 to test fouling release (FR) properties. Effectiveness of silicone-based FR coatings was readily demonstrated and the results of this 5 day test shown to accurately predict results of 1 year panel testing for AF and FR at sea using an artificial neural network analysis[6].

Tests of substratum interaction and specific polymer structure properties--Analysis of adhesion of four *Pseudomonas aeuginosa* mutants each expressing a different structure of the externallipopolysaccharide have shown that loss of the B-band charged polysaccharide and specific modification in the polysaccharide core resulted in a much greater hydrophobicity. This increased adhesiveness to glass and stainless steel substrata is in contrast to the hydrophilic wild-type with both the compete A and B bands of the O antigen plus a complete core. Hydrophobicity was measured by hydrophobic interaction chromotography, attachment to germanium, and cell aggregation. Surface charge was estimated by detection of cationized ferritin sorption and transmission electron microscopy. Hydrophilicity showed a negative correlation with adhesion. Hydrophobicity showed a positive correlation in the four mutants with different lipopolysaccharide structures and different surface polymer properties.

Biofouling biofilms are characterized by heterogeneous distribution of bacterial species and metabolic activities. To study the interaction of extracellular polymers and biofilm ecology a bioluminescent "reporter" was constructed by fusing the *lux* gene cassette to the promoter for *algD*, the committed step in the extracellular polymer alginate biosynthesis[7]. With this strain conditions for maximal expression were explored[8]. This and other *lux* and GPF reporter engineered strains were then utilized in a scanning vibrating electrode microscope[9] to explore congruence between microbial localization, microbial metabolic activity (indicated by bioluminescence) and the localized pitting corrosion associated with anodic activity detected with the scanning vibrating electrode as peaks in the charge density profile[10]. This apparatus was fitted with a photon counting imaging CCD camera, and the detection of bioluminescence activity by individual bacterial cells were mapped[11]. This system has been combined with confocal laser microscopy to map the heterogeneity in metabolic activities in time and space in biofouling biofilms.

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Monitoring and Characterization of Membrane Biofilms

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SUMMARY

Reduced plant efficiency due to membrane biofouling is unfortunately a common occurrence in reverse osmosis (RO) plant used to desalinate natural water sources for potable and industrial applications.

The performance of RO plant is characterized by the production rate of treated water and the differential pressure across the membrane elements. Plant operators routinely monitor changes in flux and delta P to evaluate system performance. It is normal practice to monitor planktonic microbiological activity and conduct chemical analysis of feedwater samples to determine the potential for fouling. However, analysis of sessile organisms gives the most accurate information about the nature and degree of microbiological fouling.

Biofilms can develop rapidly and cause significant performance decline. The design of spiral wound membrane systems provides ideal micro-environments for microbial attachment and growth.

Biofouling can be characterized by a number of techniques including chemical analyses, microbiological enumeration and identification, surface analysis and mircoscopy techniques.

The nature of Houseman's business of providing practical advice and assistance on chemical products to solve on-site problems initially requires only a broad characterization of the major foulants. This information is used to make suitable

practical and cost effective recommendations for optimizing pretreatment, cleaning and sanitizing procedures.

Autopsy analysis of foulant taken from a number of biofouled spiral elements obtained from severely fouled plant has revealed in some cases both bacterial and fungal contaminants. The main foulant characteristics of immediate significance when troubleshooting are:

- Moisture content
- % organics
- Microbiological counts and identifications (sessile micro-organisms)

Our investigations of a number of biofouled RO plants have found the following genera regularly identified in the sessile form:

- Bacteria: *Aeromonas, Actinomyces, Arthrobacter, Bacillus, Corynebacterium, Enterobacter, Flavobacterium, Klebsiella, Micrcoccus and Pseudomonas.*
- Fungi: *Aspergillus, Fusarium, Mucor, Penicillium, Trichoderm.a*
- Yeasts: sometimes identified.

Bacterial counts of typically between 10^5 - 10^8 cfu/cm^2 membrane have been found. Microbial attachment and biofilm growth on the plastic spacer material can also contribute significantly to restricted flow in spiral wound systems. Although theoretically micro-organisms should not pass through to the product water side of the membrane, evidence suggests that this occurs in many systems. The reasons for this are unclear; it may be attributed to poor sanitization on the product water side of the membrane or to slight imperfections of the membrane surface.

Corynebacterium has been found in the foulant from over 50% of the biofouled membranes autopsied. Gram-negative rods including *Pseudomonas* and *Aeromonas* have been identified in most of the samples. *Pseudomonas* is known to be a major producer of biofilm in water systems. The fungal species *Penicillium* has been identified in over 70% of membrane foulant samples.

The nature and structure of biofilms vary greatly. In addition to the organic and microbiological composition, iron oxide and humic acids content is of particular interest. Some biofilms are predominantly colonized with bacteria, but in some cases fungal species are the major fouling organisms. The quantity of polysaccharide material to number of micro-organisms also varies, as doe the porosity and density of the biofilm itself. These characteristics influence the biocidal effectiveness, penetration ability and dispersing properties of chemicals used to recover membrane performance.

All membrane systems will suffer biofouling, but it is necessary to quantify and maintain an acceptable controllable level of fouling which will not significantly affect plant economics.

The water treatment industry could benefit from the development of new non-oxidizing biocides compatible with polyamide membranes.

The design of new techniques or novel instrumentation to detect and quantify the formation of biofilms on membrane surfaces would be another interesting research area. This may involve measurement of electrical resistance, ATP levels or hydraulic resistance.

MEMBRANE BIOFOULING IN MEMBRANE BIOREACTOR

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A series of ultrafiltration were performed to assess the extent of biofouling according to the floc structures of physiological states of activated sludges. The bulking, pin-point and foaming sludges showed greater fouling tendencies than the normal activated sludge regardless of membrane materials. As the solid retention time increases, the membrane filtration resistance decreases. The activated sludge under nitrogen deficient condition showed less fouling tendency than the normal one. The amount of Extra Cellular Polymer and other Organics (ECPOs) surrounding the microorganisms is closely related to the physiological states of activated sludges and consequently to the extent of membrane fouling. The more ECPOs activated sludge had, the greater membrane fouling was observed.

The floc break-up during crossflow ultrafiltration also affected the physiological state and activity of microorganism. As the floc break-up proceeded, the floc settlability became poorer and the oxygen uptake rate as well as COD removal efficiency was reduced. At the same time the content of ECPOs surrounding the flocs increased and more ECP was released from the flocs into bulk solutions. This led to greater cake and internal fouling resistances.

Activated carbon was incorporated into the membrane bioreactor to reduce biofouling as well as to improve organics removal efficiency. Under the same operating conditions, the biological activated carbon (BAC) sludge showed substantial flux improvement and much better COD removal than the normal sludge although the BAC sludge had higher total solids. The activated carbon

added seemed to absorb and/or coagulate ECPOs and find colloids and thus to reduce the overall specific resistance of mixed liquor of activated sludge.