BIOFOULING PROCESS PROFILE OF A COMMERCIAL 24 MGD SWRO PLANT, SAUDI ARABIA

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Abstract

A two year biofouling study was conducted throughout pretreatment lines and on membranes of a 24 MGD SWRO facility, located on the Arabian Gulf. Water samples were collected in the intake and throughout process lines to determine the types and distribution of bacterial colonies. Biofilm attachment coupons were fabricated and positioned throughout process lines to determine species responsible for feed line fouling after chemical and physical pretreatment. Lastly, membrane autopsies were conducted to identify and determine the nature of bacteria associated with membrane fouling of commercial SWRO systems.

Throughout the experiment, a total of 106 phenotypically distinct biotypes were isolated. Among these, 82 were deemed unique by virtue of biochemical signals and morphological examinations. Isolates were amplified and subjected to 16S rDNA sequencing using 500 BP cross reference identification protocols. Twenty eight isolates were found to be genetically distinct. Identification revealed that species distribution of source water bacteria was not reflective of biofilm bacteria found on coupons and membranes, thus indicating that surface fouling may be selective. Coupon analysis (reflective of feed line fouling) revealed the presence of numerous chemoorganohetrotrophs indicating possible bacterial tolerance to upstream and downstream residual chlorine. Only 3 of the 28 genetically distinct isolates were associated with SWRO membrane fouling. They are the mixotrophic, chemo-organotroph's *Vibrio alginolyticus, Vibrio parahaemolyticus* and *Shewanella algae*. The presence of *Shewanella algae* on membranes is concerning and raises questions about the effect of upstream ferrous coagulants on membrane fouling.

I. INTRODUCTION

Water scarcity is a global problem that requires the collective cooperation of nations to technically support and financially invest in technologies and solutions to help mitigate its effects. At present, only 30% (12800 billion cubic meters [bcm]) of the estimated 42700 bcm of fresh water can be tapped and processed for human consumption, use and development [1]. According to UNESCO, growing per capita water scarcity, in many parts of the developing world, will force the global water crisis to reach unprecedented highs [2].

Arab countries are the poorest with regards to water accessibility housing only 0.62% of the world's water resources. Furthermore, ESCWA (Economic and Social Commission for Western Asia [Bahrain, Egypt, Iraq, Jordan, Kuwait, Lebanon, Oman, Palestine, Qatar, Saudi Arabia, Syria, United Arab Emirates and Yemen]) nations contain only 0.38 % with an estimated 163 bcm of fresh water. Of this amount, 142.5 bcm is from surface water, 18.5 bcm from ground water and 2.1 bcm from desalination [1].

As a solution to water shortages, Arab countries have and continue to invest heavily in seawater desalination. Multi Stage Flash (MSF) is the dominant seawater desalinating technology used in the Gulf region because of its ability to cogenerate mega quantities of power and potable water. However, Reverse Osmosis is gradually gaining ground because it is effectively cheaper and more diverse in application. Efficient energy recovery systems and high pressure pumps and thermal chemical tolerant membranes are a few technologies that have lowered effective operational costs of RO systems. RO is diverse lending itself to be used as part of water treatment systems for civil, industrial and potable water treatment and production facilities. Though reverse osmosis is effectively cheaper than MSF, one if its operational and costs setbacks comes from process fouling that is mostly of biological origin.

Biofouling "constitutes a major factor in limiting useful membrane lifetime and is responsible for the high cost of SWRO system pretreatment [3]." Biofouling affecting SWRO systems results from seawater bacteria transported throughout pretreatment lines that settle on surfaces producing or becoming part of a biofilm matrix. Biofouling can have direct economic effects on SWRO processes causing membrane flux declines, a rise in pretreatment costs, frequent membrane cleaning and membrane damage and replacement. Over 10% of a SWRO plant's operational cost is consumed by membrane replacement resulting from biological fouling [4-5]. As a rule, if membranes are not replaced, they should be chemically cleaned when product water output reduces by 10-15% and on average, most Nano Filtration (NF) and SWRO membranes are cleaned within 6 to 12 months of operation. There is a strong need to determine the biofouling potential of feed seawater in reverse osmosis desalination processes since a detailed knowledge of biological matter may lead to the development of more cost effective feed water pretreatment, membrane treatment and cleaning and improve plant operation and product water quality.

A biofouling study was conducted in a commercial 24 MGD SWRO facility, located on the Arabian Gulf. Water samples were collected in intake and throughout process lines to determine type and distribution of bacterial colonies. Biofilm attachment coupons were fabricated and positioned throughout process lines to determine species responsible for feed line fouling after chemical and physical pretreatment. Lastly, membrane autopsies were conducted to identify and determine the nature of bacteria associated with membrane fouling of the commercial SWRO system. Bacterial isolates were indentified using 500 BP 16S rDNA sequencing (MIDI Labs, USA).

II. METHODOLOGY

2.1 Planktonic Bacteria

Water samples were collected aseptically from: the intake feed water, before the dual media filter (BDMF), before cartridge filter (BCF), after cartridge filter (ACF) and membrane brine using sterile 100ml polypropylene screw cap bottles. Extinction dilution technique (for seawater bacteria) [6-7], were applied at each sample location for maximization of bacterial isolates as reported by Gonzalez [8]. Aliquots of 1ml sample stocks were diluted to 10^{-1} and 10^{-2} CFU/ml and poured onto Petri dishes containing 5.51% of Difco 2216 Marine Agar fortified with 0.5% Agar (MAA). Samples were incubated for 72h under anti-luminous conditions. Petri dishes were examined for phenotypic variants. Variants were extracted from dishes and streaked for isolation on MAA. Samples dilutions 10^{4} thru 10^{10} were incubated for 8 weeks then subjected to phenotypic screening, isolation and purification. Samples were compared based on their rates of growth (Fast, Medium and Slow) and subject to biochemical analyses of substrate sugar metabolites using Analytical Profile Index reactors (API).

The frequency of prominent samples observed throughout the pretreatment line throughout the project duration was also compared. DNA extractions were performed along with 16S rRNA sequencing to identify bacteria.

2.2 Coupon Studies

Coupon vessels were fabricated from PVC-1 grade material and assembled using WELD-ON 717 PPC. Manual flow regulators were positioned from: the process line source, before coupon chamber and after coupon chamber to regulate flow and flux within vessels. Elbow joints were installed to reduce seawater thrust and enhance continuous flow. Coupon chambers were kept filled to capacity from process line source at a regulated discharge of 15 l/min.

Fiber glass coupons (2.3cm x 2.5cm) were installed and extracted at various locations throughout the 24 MGD SWRO process line.

Coupons were installed before the dual media filter (DMF), before the cartridge filter (BCF) and after the cartridge filter (ACF) of the 24 MGD SWRO Plant (Fig. 1). Procedure for analysis of fiberglass coupons is described [9-10].

2.3 Membrane studies

A SU-822H membrane was extracted from the SWRO plant after 5,000 hours of operation and autopsied in accordance with standard protocols proposed by Leitz [11]. For comparison, a DK 8-8040 Red Sea membrane (in operation for 5 years) was removed and autopsied using the same method. Aseptically sterile plastic spatulas were used to transfer organic slurries from membranes to Petri dishes. Slurries were added to 100 ml sterile pyrex screw cap bottles containing 50ml of sterile filtered After Cartridge Filter pretreated seawater. Bottles were vortexed then serially diluted to 10^{-5}. Aliquots of 1ml were transferred to MAA using pour plate method and incubated for 4 days. Phenotypically unique colonies were re-platted for amplification on MAA using streak plate method. Colonies were subjected to API, Catalase and Oxidase tests. Gram stain, cell shape and locomotion profiling were recorded. Pure isolates were re-amplified on Petri dishes containing MAA and packaged (in accordance with guidelines

provided by United States Department of Agriculture) for 500 BP 16s rDNA analysis (MIDI Labs, USA).

Figure 1. Coupons vessels positioned throughout various pretreatment locations of the 24 MGD Arabian Gulf SWRO Plant

III. RESULTS

A total of 106 phenotypically distinct biotypes were isolated throughout the course of this study, of which, 82 appeared pheotypically unique. Colonies were selected for genetic analysis based on relative distribution throughout the experiment though all isolates from membranes were analyzed. Of the 42 samples slated for rDNA analysis, 28 were found to be genetically unique (Table 1 and Table 2).

	Amount of bacterial Isolates	**Number of Phenotypically unique bacteria**	**Unique isolates after physical/biochemical screening**	**Isolates selected for 16s rDNA Analysis**	**Genetically Unique Isolates**
Bacteria	106	82	58	42	28

Table 1. Distribution profiles of unique isolated colonies after inspection, physical and chemical and genetic screening for Feed Water, Coupon and Membrane isolated bacteria.

Over 46% of phenotypic isolates were found to be redundant colonies based on ether locomotion, Gram stain, sugar metabolisms tests and/or catalase/oxidase activity.

3.1 Planktonic Bacteria

Plankton colonies were categorized based on their rates of growth and presence throughout the experiment. Fourteen isolates were consistently present throughout the experiment (Colonies A-I, K-L, N-O). They were found not to be affected by seasonal changes or operational conditions. Colonies were categorized in accordance to colony growth rates (categorized as Fast Growing, Medium Growing and Slow Growing) and biochemically compared (Table 3).

Aspergillus versicolor	Pseudoalteromonas ruthenica
Bacillus cereus	Ralstonia eutropha
Bacillus fastidious	Rhodobacter bacterium
Bacillus silvertris	Rhodovalum imhoffii
Bacterium bacteroidetes	Rosebacter sp.
Bacterium rhodospirillace	Ruegeria atlantica
Erythrobacter longus	Shewanella algae
Idiomarina sp.	Sporosacina sp.
Marinobacter hydrocarbonoclasticus	Staphylococcus warneri
Microbacterium schleiferi	Stappia sp.
Micrococcus luteus	Vibrio alginolyticus
Oceanobacillus iheyensis	Vibio parahaemolyticus
Ochrobactrum grignonense	Virgibacillus pantothenticus
Planococcus kocurii	Vibrio tubiashii

Table 2. Bacterial Species Isolated and Genetically Identified using 16S rDNA Sequencing and analysis of samples from Intake Waters, Process Lines and membranes of a 24 MGD SWRO Plant

Rate	Colony	GEL	GLU	MAN	INO	SOR	RHA	SAC	MEL	AMY	ARA	OX
Fast Growth	B	0	0	0	0	0	0	0	0	0	0	2
	C	0	0	0	0	0	0	0	0	0	0	2
	K	1	0	0	0	0	0	1	0	0	1	2
	L	1	0	0	0	0	0	0	0	0	0	2
	N	0	0	0	0	0	0	0	0	0	0	2
Medium Growth	D	1	0	0	0	2	0	0	0	0	0	0
	E	0	0	0	0	0	0	0	0	0	0	2
	I	0	0	0	0	0	0	0	0	0	0	2
	O	0	0	0	0	0	0	0	0	0	0	2
Slow Growth	A	1	2	0	0	0	0	0	0	0	1	2
	F	1	0	0	0	2	0	0	2	0	0	2
	G	0	0	0	0	0	0	0	0	0	0	2
	H	1	0	0	0	0	0	0	0	0	0	2
	P	0	0	0	0	0	0	0	0	0	0	2

Table 3. Biochemical Profiles of Planktonic Baceteria based on Rates of Growth

In general, the fast, medium and slow growing groups exhibit similar biochemical profiles though there are some salient differences (Table 3). Rapidly growing colonies include *Bacillus silvertris*, *Oceanobacillus iheyensis* and *Pseudomonas Stutzeri.*

These bacteria are mixotrophic, chemo-organotrophs with methylotrophic properties. They can degrade and metabolize sugars, proteins, aromatics, petrochemicals and are candidates for bio-remediation

projects, xenobiotic degradation and industrial enzyme manufacture and application. Medium and slow growth isolates include *Bacillus cereus* and *Idiomarina*.

These organisms are also mixotrophic. *B. cereus* is involved in methane, nitrogen, sulfur and carbohydrate metabolism in addition to caprolactam, gamma-hexachlorcyclohaxane, styrene, ethybenzen xenobiotic biodegradation and metabolism.

3.1.1 Identification Based on Colony Abundance throughout pretreatment stations - Colony F was most abundant at all stations. In the intake, colony F represents 85% of the population. At station BDMF, it increases by 10% to a dominating 95%.

In the BCF station, colony F dominates the population by 40% and at the ACF station it represents 50% of the bacterial population grown on Petri dishes. Unfortunately this colony could not be identified by 16S rRNA and 500 BP genetic analysis and molecular comparison techniques. It is a unique possibly unknown species.

Colony B is the second most abundant representative colony. It constitutes 7% of the total bacterial population in the intake and 3% at the BDMF station. However, it losses ground at the BCF station to be the least representative colony at 10% but picks up at the ACF station to attain a 50:50 ratio with another dominant colony. Genetic analysis (16S rRNA and 500 BP identification) reveal that this colony is *Oceanobacillus iheyensis*.

Pseudomonas stutzeri (colony L) is represented throughout most of the stations. In the intake, it is the third most represented colony at 6% and the least most represented colony at BDMF with 2%. However, it shares second place at the BCF station at 25% but is not represented at the ACF station (Fig. 6).

Bacillus cereus (colony A) and *Pseudomonas stutzeri* (colony L) appear as representative colonies but only at point locations. In the intake, colony A is the least most representative colony at 2% and another Pseudomonas sp. (colony type I) shares second place dominance with colony L at 25% and is only observed at BCF. *Bacillus cereus* is a gram (+) bacteria mostly found in soil which is also present in seawater but has not been found to be involved in fouling.

The unknown species, colony F, considered to be of the category of slow growing bacteria, was the dominant species from the intake throughout the process line up until ACF. At ACF it shared dominance with *O. iheyensis* a fast growing species. It is interesting that one of the least robust species (in growth rate) survived physical and chemical treatments to be of the dominate colonies at all stations.

3.2 Coupon Studies

Attachment studies were carried out on coupons positioned throughout various locations of the 24 MGD SWRO plant's process line. Multiple coupons were extracted at 2 day, 4 day, 8 day and 15 day durations. Analyses were carried out at the Intake, BDMF, ADMF and ACF locations (Table 4).

3.2.1 Intake - The intake did not experience much variation in bacterial content between days 2 through 8. Bacterial concentrations remain within the same order of magnitude (Table 4) even-though a 9.92%

decline in bacterial content is recorded (from day 2 to 4). From day 4 to 8 bacterial concentration increases 66.7%. From day 8 to 15 bacterial density is enhanced by 1 order or magnitude.

Station	Incubation Time-d	Species	Percentage of Species/100	Total Concentration CFU/cm^2
Intake				
	2	*Micrococcus luteus*	0.05	1965
	4	UC/UI	1	3540
	8	*Rhodobacterace bacterium*	0.9	5310
	15	*Erythrobacter longus*	0.02	1360
	15	UC/UI	0.98	66640
BDMF				
	2	UC/UI	1	5510
	4	*Vibrio tubiashii*	0.2	19640
	4	*Pseudoalteromonas ruthenica*	0.2	19640
	4	UC/UI	0.6	58920
	8	*Marinobacter hydrocarbonoclasticus*	1	15600
	15	*Ruegeria atlantica*	0.25	15800
	15	*Bacterium rhodospirillaceae*	0.1	6320
	15	*Marinobacter hydrocarbonoclasticus*	0.3	18960
BCF				
	2	*Bacterium bacteroidetes*	0.5	120
	2	*Microbacterium schleiferi*	0.5	120
	4	UC/UI	1	34500
	8	*Vibrio sp.*	0.1	579
	8	UC/UI	0.9	5211
	15	*Ruegeria atlantica*	1	33300
ACF				
	2	*Planococcus kocurii*	0.1	4
	2	*Sporosacina sp.*	0.1	4
	2	*Idiomarina sp.*	0.7	28
	4	*Rhodovalum imhoffii*	0.3	177300
	4	*Ruegeria atlantica*	0.6	354600
	4	*Ochrobactrum grignonense*	0.1	59100
	8	*Ruegeria atlantica*	0.1	473
	8	*Rhodobacterace bacterium*	0.25	1182.5
	8	*Bacillus fastidious*	0.25	1182.5
	8	UC/UI	0.4	1892
	15	*Ruegeria atlantica*	0.5	219000
	15	*Stappia Sp.*	0.05	21900
	15	*Rosebacter Sp.*	0.25	109500
	15	*Aspergillus versicolor*	0.2	87600

Table 4. Average Bacterial Percentage and Concentration of Identified Species from Biofilm Study

In the intake on day 2 there are six bacterial types. Of the six, five were culturable on MAA plates. They constitute 25% of the 3930 CFU's observed. Only 5% of the total ambient population, represented as a single bacterium, succeeded at polymerase chain reaction (PCR) analysis and nucleotide sequencing. Genetic analysis indicates that this bacterium is *Micrococcus luteus* (Fig. 2).

Bacteria of coupons extracted on day 4 did not survive the second generation isolation on MAA. Consequently, these bacteria were not only non-culturable but also non-identifiable.

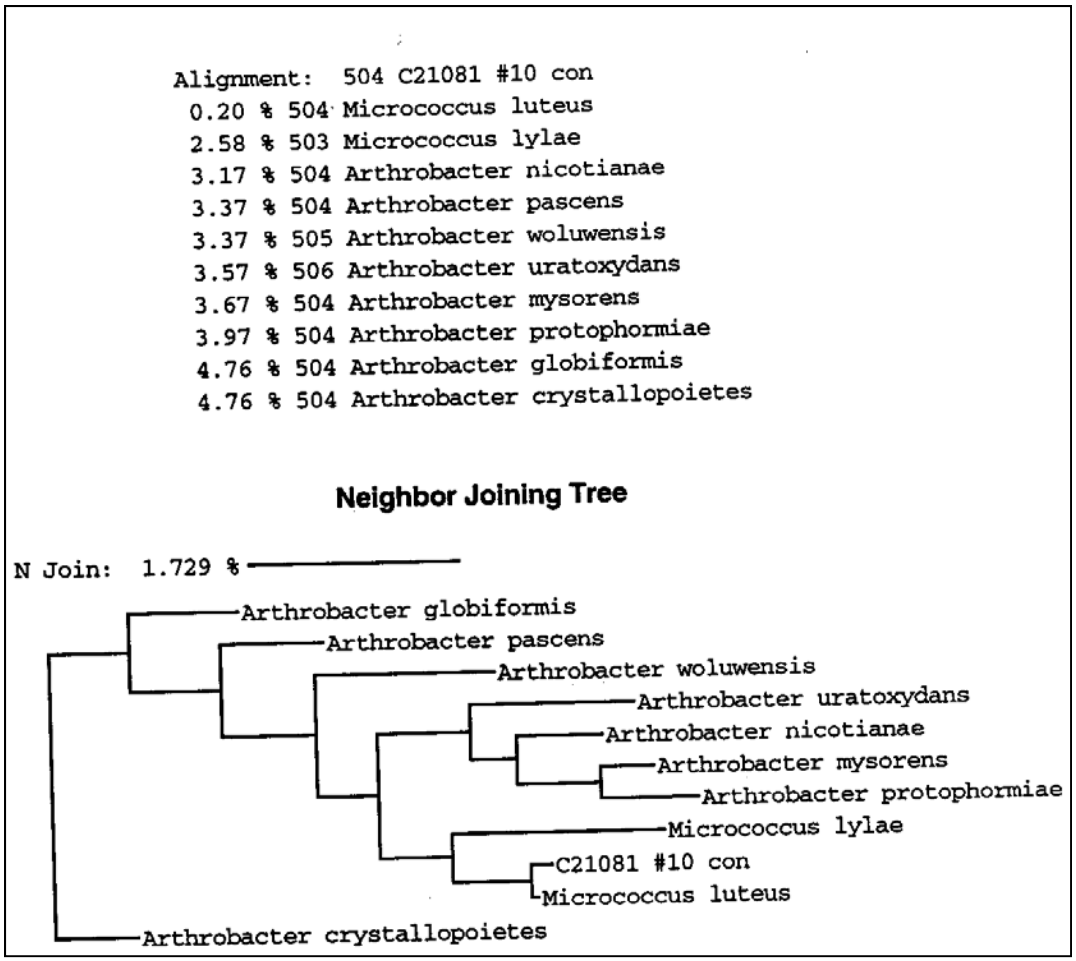

Figure 2. 16s rDNA Genetic Sequence Identification of *Micrococcus luteus*.

In the 8th day coupon exposure, three genera of bacteria are present. *Rhodobacter bacterium*, comprise 90% of the total population. The remaining 10% of bacteria contained one type that did not survive second generation identification and the other that did not withstand genetic analysis due to a lack of available information on this species. It could be the case that this species is new or a genetic hybrid which has not been identified or recorded.

The 15th day of coupon exposure reveals three bacterial species. One species did not survive second generation purification and amplification on MAA. Another, comprising 30% of the 6.80 x 10^4 CFU's isolated, did not withstand PCR and sequencing and was thus unidentifiable. The third was identified as *Erythrobacter longus* which comprised 2% (1360 CFU/cm^2) of the population.

3.2.2 BDMF - Bacterial densities from coupons positioned BDMF experience an increase from day 2 to day 4 by one order of magnitude (1.68x10^2 %) then decreases by 84.1% from days 4 to 8. In days 8 through 15 a rapid increase by 305% is observed. Though observable differences in bacterial density are present, from days 4 through 15 bacterial concentrations associated with coupons of at BDMF remain within the same thee orders of magnitude.

Day 2 extraction of coupons reveals 4 phenotypes. Three of the four are non-culturable in 2nd generation isolation. The fourth culture did not withstand genetic testing and therefore was also unidentifiable. Day 4 extractions revealed 3 bacterial colony types. Nearly 60% of this sample is one type is un-culturable during 2nd generation isolation. There are only two culturable bacteria each represents up to 20% (19640 CFU/cm^2) of the total bacterial density of biofilm on day 4 coupons. These bacteria are genetically identified as *Vibrio tubiashii* and *Pseudoalteromonas ruthenica.*

Day 8 coupons contain one bacterial species identified as *Marinobacter hydrocarbonoclasticus*. The density of this isolate on coupons is 1.56 $x10^4$ CFU/cm^2. The increase in bacterial concentration (day 2 to day 4) in addition to the decline in phenotypically diversified species (day 2 to day 4) coupled with rapid population decrease on day 8 with one exclusively dominate species is indicative of ammenalism. This results from antagonism between species which leads to the excretion of antibiotics, organic acids and or lytic enzymes from dominant species [causing death to competitive bacteria].

At day 15, the *Marinobacter hydrocarbonoclastica* isolate (observed in day 8) is still present at 1.89 $x10^4$ CFU/cm^2 (30% of the bacterial population on coupons) along with 3 other genetically different bacteria and one fungal colony type. Of the three bacterial types one is not genetically identifiable. The other two are *Ruegeria atlantica* and *Bacterium rhodospirillaceae* at 1.58 $x10^4$ CFU/cm $x10^4$ and 6.32 $x10^3$ CFU/cm^2, respectively. The fungal colony present is *Penicillum acthiopicum* at 1.89 $x10^4$ CFU/cm^2. The abundance of diversified microbiological colonies up until day 8 and their complete exclusion at day 8 gives credence to the possibility of ammenalism (in 8th day extractions of BDMF coupons). However, the increase in representative bacterial populations at day 15 challenges the conclusion of absolute ammenalism and may be indicative of a change in the biofilm that promotes the growth of other species.

3.2.3 BCF - Bacterial densities from coupons positioned BCF experience an increase from day 2 to day 4 by two orders of magnitude (14300%). From day 4 through 15 bacterial concentrations associated with coupons at BCF varied considerably although they remain within the same order of magnitude. After 2-day exposure, there are two prevailing bacterial species present. *Bacterium bacteroidetes* and *Microbacterium schleiferi* each constitute 50% of the sample population.

On day 4, there are three phenotypically distinct colonies. These colonies comprise 2.07 x 10^4 CFU/cm^2, 6.90 x 10^4 CFU/cm^2 and 3.45 x 10^4 CFU/cm^2. However, each colony type did not survive second generation isolation and therefore were unable to undergo genetic identification.

Coupons extracted after 8 days contain several colony types. However, all with the exception of 1 did not survive second generation isolation. The single colony that survived second generation purification and genetic sequencing was of *Vibrio* species.

Day 15 coupon extraction reveals four phenotypically distinct colonies. However, when genetic analysis is performed, three of the four colonies comprising 3.00 x 10^4 CFU/cm^2 are *Ruegeria atlantica*. The other colony was not able to withstand second generation isolation and could not be subjected to genetic identification.

3.2.4 ACF - Initial bacterial density of ACF coupons after 2 days exposure is 4.0 x 10^1 CFU/cm^2. During day 4 extraction, bacterial density increases by 4 orders of magnitude representing an overall

gain in population density by 1.48 x 10^6 CFU/cm^2. Between days 4 to 8 bacterial density drops by 83.2% to give an overall density of 4.73 x 10^3 CFU/cm^2. A rapid increase by 2 orders of magnitude is observed at day 15 bringing the overall bacterial concentration to 4.38 x 10^5 CFU/cm^2.

In day 2 coupons, only three phenotypically distinct colonies could be isolated and sent for genetic identification. *Planococcus kocurii, Sporosarcina sp., and Idiomarina sp* comprising 10%, 10% and 70% of the sample volume, respectively, were identified.

Day 4 coupons contain four bacterial types. They are *Rhodovalum imhoffii, Ruegeria atlantica* and *Paracoccus denitrificans, Halomonas halophila* with bio-densities of 1.77 x10^5 CFU/cm^2, 2.96 x10^5 CFU/cm^2 and 5.91 x10^4 CFU/cm^2 (for both *P. denitrificans and H. halophila).* These bacterial species represent 30%, 50%, 10% and 10% of the bacterial biofilm population on day 4 coupons.

Day 8 contains 5 phenotypically distinct colonies. Of the five, two could not be genetically identified. The remaining are identified as *Ruegeria atlantica, Rhodobacterace bacterium* and B*acillus fastidious*.
Day 15 coupon isolates contained 5 phenotypic colony types that were subjected to genetic identification. Four of the 5 comprise *Ruegeria atlantica* (2.19 x10^5 CFU/cm^2 and approximately 50% of the total bacterial density), followed by *Roseobacter sp.* at 1.10 x10^5 CFU/cm^2 (25%) then *Stappia* sp. 2.19 x10^5 CFU/cm^2 (5%). The 4th identifiable isolate is the fungus *Aspergillus versicolor* at 8.76 x10^5 CFU/cm^2 (20%).

3.3 Membrane Studies

Two SWRO membranes were obtained from two SWCC RO plants. A Nano-Filtration (NF-DK-8040) membrane (operated for 5 years) from a Red Sea SWRO Plant and a SWRO (SU-822H) membrane (operated for 5,000 hours) from an Arabian Gulf 24 MGD SWRO plant membranes were autopsied. Only three bacterial species were isolated and identified.

The genus Vibrio was observed in both membranes. The NF element contained *Vibrio alginolyticus* whereas the RO element contained *Vibrio parahaemolyticus*. Both elements contained the bacterial species *Shewanella algae*.

IV. DISCUSSION

4.1 Plankton Bacteria

It is clear that identification programs compiled from biochemical tests, that are useful in medical laboratories, are of limited use in identification of environmental bacteria. API tests were useful in making relative distinctions between aquatic bacteria but were overall unsuccessful as a taxonomic tool. Comparing relative rates of colony formation and distribution throughout physical and chemical pretreatment processes yielded interesting results. Fast growing colonies were not dominantly represented throughout the pretreatment process line. A slow growing, unidentifiably (possibly new hybrid or new colony)- colony F was found to be dominant.

Dominance of the slow growing microbe (colony F) though-out physical and chemical pretreatment zones, indicates the effectiveness of pretreatment in reducing the presence of sessile more robust species throughout the process line. Prominence of colony F throughout the treatment indicates its resistance to

chemical and physical pretreatments and its ability to successfully metabolize end products produced by oxidative waters.

The examination of 1500 Petri dishes through the course of a year, yield only five predominant plankton colonies (A.- *B. cereus,* B.- *O. iheyensis,* F.-*(Unknown),* I.- *Pseudomonas species and* L.- *P. stutzeri)* that may lead one to suggest their culpability in pipeline fouling, however, this is not the case. Likewise the predominance of the unknown colony (F) and *B. cereus* (A) did not show up in autopsied membrane analysis.

4.2. Coupon Studies

Generally, intake coupons have lower bacterial concentrations than those before the dual media filter (BDF). Exposure to ferric chloride produces coupons with higher particulate attachments and greater bacterial concentrations than those found in the intake. This is to be expected as the effect of ferric chloride not only acts to concentrate and induce agglomeration of silica sand and other cationic particulates but also bacterial appendages mutually attracted to them due to their effective anionic charge. In fact, it is the matrimonial charge differential between bacteria and silica sand that promotes cohesion and threatens long term RO membrane performance (during repeated episodes of high SDI). Though colloidal fouling may be significantly removed from membranes by physical and chemical treatment, Extracellular Polymeric Substances (EPS) secreted by bacteria are more difficult to remove and may permanently affect RO membranes [12].

The appearance of auburn slime was observed on all coupons extracts from BDMF and ADMF. The stain is from the ferric coagulant. Although the ADMF stain is not as intense as that of BMF, it indicates that the dual media filter is not capable of fully retaining iron particles that provide additional surface area for microbial growth and production of slime that impedes water flow. This phenomenon leads to downstream seeding of bacteria that develops biofilm on micron cartridge filters and RO membranes. The suitability of the DMF particle size and its effect on SDI of pre-filtered water needs to be investigated.

One of the primary colonizers of intake coupons is *Micrococcus luteus*. *M. luteus's* role in biofouling is known. It acts to congregate bacteria forming biofilm through lectin-like appendages that bind bacteria to biofilm cementing them to the bacterial consortium [13]. It forms yellow colonies that are gram (+) and appear as staphylo, diplo and tetrad configurations. It is found in water, soil, fish, meat and dairy products. It is also found on mammalian skin but is generally non-pathogenic except in immune compromised individuals where conditions of bacteraemia, endocarditis and septic arthritis may be observed [14]. It is Catalase and Oxidase positive and liquefies gelatin. *M. luteus* has heat-stable enzymes that not only liquefy gelatin but digest EPS thus resulting in biofilm dispersion [15]. Commercial manufacture of these enzymes through genetic means could be useful for membrane and pipeline cleaning and or treatment.

Attached also to coupons were *Rhodobacter*. This purple, ubiquitous non-sulfur, anoxygenic bacteria is metabolically diverse. It has one of the most complex biochemical pathways enabling it to grow in the presence or absence of light metabolizing a variety of components that are deadly to other bacterial species. Its high tolerance to chlorine is one reason that it appears in copious concentrations in ACF coupons. *Rhodobacter* is mixotrophic because it degrades not only monohalomethanes and polyhalomethanes that may result from in-situ metabolic activities of aquatic life or chlorination of

seawater. It also has pathways for nitrobenzene, styrene, methane, pyruvate, toluene and xylene degradation. Its potential for bioremediation processes as well and industrial enzyme extraction and use is known.

There are 4 other types of mixotrophic bacteria isolated from coupons in this experiment. *Marinobacter hydrocarbonoclasticus* is a ubiquitous, denitrifying and facultative aerobe. It is halotolerant and produces osmo-protectant ectoin. This bacteria may be sessile or as part of biofilm. It produces extracellular phosphatase which may play a role in enzyme function and regulation (by removal of phosphate cofactors from protein kinases). *M. hydrocarbonoclasticus* was isolated from biofilm coupons at BDMF near a location for chlorine dosing. It comprises 100% and 30% of total isolatable biofilm bacteria at days 8 and 15, respectively. At BDMF, the range of representative bacterial isolates decrease from $\geq$ 4 to 1 indicating selectivity that more likely results from effective chlorination and *Marinobacter's* ability to metabolize mono and polyhalomethanes (and other chlorine derivatives).

Paracoccus denitrificans is another mixotrophic bacteria observed in this experiment. It appears after 4 day coupon exposure in the ACF position. It comprises only 10% of the population. However, due to the abundance of bacteria within ACF coupons, *Paracoccus denitrificans* is the second more abundant mixotrophic species observed (59100 CFU/cm^2).

Roseobacter is the third species of mixotrophic bacteria observed. It was found after 15 day exposure also in the ACF position. It comprised 25% of the population and has a close genetic relationship to *Ruegeria atlantica*.

Ruegeria atlantica is the more abundant mixotrophic bacteria. It appears in many locations after 2 day coupon exposure. At 4 day exposure, it dominates the population at 60% (354600 CFU/ cm^2) in coupons positioned ACF. At 8 day exposure, it appears in both the BCF and ACF coupon positions comprising 10% of the population. At 15 day exposure, it appears in all locations except the intake. Although most species of methylotrophic bacteria appear in the ACF location, *Ruegeria* expresses itself throughout different coupon locations within the plant and is by far the most dominant organism present among all bacterial species found.

Halomethanes are manufactured by "Cyanobacteria, marine alga, higher plants and fungi [16]." They also result from chlorination of natural components occurring in water whose formation is pH dependant. Chlorination of seawater with high pH for either thermal or SWRO disinfection produces trihalomethanes (THM's) that are metabolites for many mixotrophic chemo-organotrophic bacteria.

4.3 Membrane Studies

The genus Vibrio was observed in both membranes. The NF element contained *Vibrio alginolyticus* whereas the RO element contained *Vibrio parahaemolyticus*. Both elements contained the bacterial species *Shewanella algae*. All three species are mixotrophic, chemo-organotrophs. Their biochemical pathways allow them to degrade and metabolize a wide range of organic and petrochemical byproducts making them strong candidates for bioremediation and xenobiotic metabolism. S. algae is also of unique interest and bas been studied at Department of Materials and Science and Engineering of the University of Southern California for its iron reducing -corrosion prevention properties [17-18]. Further investigation to determine the use of these bacteria in pipeline and equipment preservation of desalination processes may also be considered.

V. CONCLUSIONS

1. When dealing with bacteria, initial concentrations in relation to the reality of biological foul of pipeline and membrane structures exist outside of the realm of conventional statistical probabilities and models.
2. A new approach for gauging biofouling that involves genetic analysis of species and available micronutrients (online) to establish algorithmic models is required.
3. Not all sessile bacterial can be implicated in biological fouling of membranes and process lines. This study reveals that there is no link between the concentration of bacteria in sea water and throughout feed water lines and their effect on biofouling of process lines and membranes.
4. Though bacteria may exist in trace amounts, its affinity towards surface attachment may be more than those that exist in copious quantities.
5. Reconsideration with regards to the understanding of biofouling and biofilm production using conventional techniques such as Most Probable Number (MPN) and Generation Time (GT) must be reviewed.
6. The dynamics of biofouling complex and should be given serious evaluation into its cause, analysis and proposal of solutions.
7. This study indicates occurrence process line fouling necessitating feed line cleaning as part of the general maintenance strategy of SWRO Plants.

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