RESEARCH

RMS Titanic and the Emergence of New Concepts on Consortial Nature of Microbial Events

D. Roy Cullimore, Charles Pellegrino, and Lori Johnston

Contents

I. Introduction

The *RMS Titanic* sank in the early-morning hours of April 15, 1912, and created a storm of controversy that still causes ripples around the world today. Her discovery as a shattered hull in 1985 by Dr. Robert D. Ballard revealed that the stem and the stern were torn apart, with the bow section looking as though it were docked on the ocean floor while the stern lay torn, twisted, and folded a kilometer away. One of the discoveries seen at that time was the growth of rusticles all over the hull. The name 'rusticle' was coined by Ballard because these growths appeared to resemble rust-covered icicles hanging downward all around and inside the ship (Ballard 1987).

Investigation of rusticles revealed that they were complex in structure, were formed as microbially induced concretions (a form of living concrete), and were not composed of a single species of either an animal or plant but were a complex network of microbial consortia. Taxonomists have traditionally classified all living entities as separate species and envisage that these would all normally live as distinct entities reproducing only within their own species. The microbial world, however, does not commonly follow such a simplistic scheme, and most growths of living masses involve a multiplicity of species functioning within a durable

D.R. Cullimore (✉)·C. Pellegrino·L. Johnston
Droycon Bioconcepts Inc., 3303 Grant Rd., Regina, Saskatchewan, Canada S4S 5H4

consortium as the social group. By analogy, Marcus Aurelius (1969a) summarized the advantages of functioning in a coordinated manner in his meditations:

> The intelligence of the universe is social. Accordingly it has made inferior things for the sake of the superior, and it has fitted the superior to one another. Thou seest how it is subordinated, co-ordinated and assigned to everything its proper proportion, and has brought together into concord with one another the things which are the best.

Our understanding of life has long been based on the concept of individual species of animals or plants, each being composed of cells having a common hereditary origin from a single zygote. These organisms, commonly function independently as living entities, each possessing a definable individual space but sharing a common and usually interdependent reproductive process. With the establishment of microorganisms over the past two centuries as a separate and definable group distinctly different from plants and animals, the concept of species, already well established for these higher organisms, was automatically adopted as the primary mechanism for the systematic differentiation of microorganisms. In so doing, the concepts intrinsic to systematics in microbiology became established around the need to isolate, describe, culture, and classify organisms that had a completely common set of characteristics and could be defined as consisting of a single strain of microorganism. To achieve this, the source habitat would have routinely been subjected to intense laboratory-based practices in which all other species had been eliminated so that only a single selected strain survived in any given culture. Thus, the whole classification of microorganisms has been built around the single-species concept.

During the past decade, there has been a growing realization that the rigid application of this concept has been a factor limiting the effective classification of all microbial species. For example, Sly (1995) prepared a report, on behalf of the International Union of Microbiological Societies and International Union of Biological Societies International Committee on Microbial Diversity, that expressed some of the inherent weaknesses in the present application of systematics in microbiology. In stating that "Less than 5% of these species (of microorganisms) have so far been described," there would clearly be a concern that the bulk of microbial species remain enigmas. Like other enigmas, understanding this difficult and confusing problem will require significant thought and investigation. Sly (1995) further indicated that "very little is known about the occurrence of uniqueness of most groups of microbial species." These statements reinforce concerns that the species concept, as it has been applied in microbiology, may not be functionally effective for the understanding of most members of the microbial kingdom.

Recent research conducted by the authors suggests that, on many occasions, the single-species concept may be at fault when applied to microbiology. The primary reasoning for this is that within the natural world microorganisms frequently function not independently but cooperatively as a part of multispecies consortia. Consortia are associations of multiple microbial species that are able to function in a synergistic manner. The functioning of the community is depen-

dent on the contributions of each of the component strains within the consortium. This concept is beginning to have an impact in the medical industry, where there is now a growing realization that many more diseases than previously anticipated are induced by microorganisms. In the determination of the causes of chronic disease, microbial vectors are rarely considered, and there is today a growing recognition that many chronic diseases have their origins in microbial infections of the human body (Ewald 2000). There has not yet been any recognition that the types of consortial infestations seen on the *RMS Titanic* could parallel similar types of infestations in humans. In other words, looking at the pathogen as being but the tip of the iceberg, then its arrival at a site of infection is actually the result of a consortial activity involving many different species of microbes. We readily see only the rusticles that are obvious but not the jelly-like rusticles which are diffused throughout the site being infested; this has been a particular challenge in porous media saturated with groundwater (Cullimore 2000a).

From the present experiential knowledge of the authors, it would appear that there are a number of forms within which these consortia can function. The primary differentiation would be species dependency upon the consortium. Those aggregations in which the member species are essential for, and dependent upon, the functioning of the consortium could be termed vital consortia. In contrast, consortia whose members are transiently interdependent upon one another but can exist individually could be termed transient consortia. In many cases, it would be expected that not all of the component strains in a vital consortium could be cultured independently. However, in the transient consortia, there would be a higher probability that the strains could sustain an independent state. These observations contradict the well-established concept that a living "organism" must have arisen from a single cell in which all the characteristics applicable to all the tissues and structures generated by that organism are retained. In a vital consortium, it would be expected that all the strains essential to the formation of that type of consortium would probably relocate and form as a single community. For transient consortia, it is probable that the various strains involved in these formations may have arrived independently and formed the structure as a result of environmental factors at the site.

All consortia collectively generate structures that become discernible and definable as describable entities. Examples are given here of various obvious and covert living structures that all function in a consortial manner. In each example, it was recognized that the incumbent microbial species might function collectively in an integrated manner within their common habitat. This interdependence means that most strains cannot be easily isolated and cultured as individual species under laboratory conditions. In these studies, it has become evident that there is a need to recognize the nature of often complex and large microbial consortial structures as distinct forms. Consortia should be granted recognition as a separate growth form worthy of recognition. This recognition would be as a separate and definable group within the systems of biological classification. However, with the consortial growths, such species-based concepts cannot func-

tion adequately to formulate the origins of the consortium. In addition, there is the need to separately culture and describe the microbial strains recovered from a consortium. Once this has been achieved, then the reassemblage of the strains in the appropriate environmental setting should trigger the reestablishment of the consortia in both form and structure. This objective may be more achievable for a transient form of consortium than for a vital form.

Each example of a consortium discussed here has been subjected to microbiological investigation at both the structural and species composition levels. Individual species and groups of microorganisms have been isolated and, in some cases, the consortial structures have been reestablished. In each case, the generation of these structures involves the deliberate admission of a range of species into a favorable environment where they will jointly cause the formation of the typical form of growth. Examples of consortia include (a) rusticles recovered from the stem of *RMS Titanic*, (b) clog and tubercle formations infesting groundwater around water wells, and (c) effects of rusticle formation on the maritime industry.

In the evaluation of the environmental impacts that can be associated with the sinking of the *RMS Titanic*, possibly the key factor would be the nature and form of the microbial consortial activities occurring at the site. The primary impact would be the dramatic arrival of the shattered hull, incorporating approximately 31,800 metric tons (t) of iron, on a relatively flat section of the ocean floor. Sequential impacts that could affect the generation of microbial activity include the following:

1. Electrolytic activities associated with the final discharges of any electrical storage systems as a short-term effect and interaction between materials having different electrical potentials as a longer-term effect.
2. Dispersion of foods, beverages, and other organic materials released by the physical disruption of containers. These organics would principally originate from the refrigerated storage rooms in the lower decks and be approximately equivalent to 14 t carbohydrate, 4.5 t protein, and 4 t fat from that source alone.
3. Biodeterioration of the readily degradable organic materials used in the construction of the ship, including cellulosic material such as curtains, sheets, napkins, and paper.
4. Biodeterioration of the more recalcitrant organic materials such as the softwood structures.
5. Corrosion of the metallic structures, particularly those dominated by iron.
6. Structural collapse of the hull.

Each of these events would have a distinct impact on the level of activity of the biota colonizing at the site. For the purposes of this review, biota is considered to include forms of microbial life as well as the more traditionally recognized plants and animals. In the generation of a food web at the site, the microbial component may be expected to perform a key role through the sequential impacts of the effects just listed. In 1996, the most obvious elements of biol-

ogical activity were the rattail fish, crabs, starfish, worms, and sea cucumbers. However, the largest biological component was the rusticle encrustation growing in various manners on the steel structures and gradually weakening the integrity of the ship. Scientific examination of these rusticles revealed that they were indeed generated by consortia of microbial species, feeding partly on the materials being released from the ship but also on the copious amounts of biocolloids and small creatures raining down from higher in the water column in the form of sea snow.

II. Consortial Structures Within Rusticles

From the brief descriptions given here, the case may be made that some living structures such as the rusticles are composed of a multiplicity of species rather than a single species. There would appear to be two levels of organizational structure involved in these consortia. One level would involve interdependence for the common survival of the consortium. In this instance, no one species would be able to physiologically or structurally maintain the structure without the activities of the other component species. The other level would involve the juxtapositioning of the species in relation to each other and also to their relative positioning in the physical structure within which the consortium lives. In many cases, these physical structures would include mechanisms to allow the entry of water, oxygen, and nutrients and passageways for the elimination of potential suppressive products of consortial activity (e.g., carbon dioxide, acidic metabolic products). Mechanisms would also be present to provide some protection to the consortial occupants from any physical disruption or predation. In the rusticles, this support is provided through the generation of geothite-dominated concretious structures, which are extremely porous. The presence of water channels, cavities, and extensive ductwork attest to the need of the consortium for water circulating through the bioconcretions. Microscopic examination at low power, at high power, and using scanning electron microscopy all revealed that complex structures are involved. In the more evolved biota, these complex structural differentiations could form a major factor in the development of specialized tissues and organs universal in these species. From the early observations, the types of extracellular structures observed in the rusticles appear to perform a function parallel to tissue differentiation in the more evolved biota. Possibly, it could be proposed that for microorganisms the formation of "tissues" was essentially extracellular; these could be organic polymer dominated, forming what are loosely described as "slimes." The slimes could come to contain inorganic crystallized and amorphous forms of insoluble substances. In the more highly evolved biota (i.e., plants and animals), the structures emerged largely in an intracellular manner. The classical perception has been to view tissue differentiation as forming the basis for the development of plants and animals with fungal structures being a major precursor route (e.g., differentiation of fruiting caps such as the mushroom *Agaricus campestris*). It is proposed here that the rusticles and parallel consortia represent an early stage in tissue differentiation primarily

driven by extracellular process controlled by microbial consortia. Conversely, in the biota, the cells of the single species control tissue differentiation.

It is common to view the biota as being composed of a range of single-species organisms that operate independently, with few exceptions, and reproduce using some mechanism which causes like-structured organisms to be generated. Microorganisms do not follow this mandate; instead, a number of species function in a consortial manner to create a common habitat structure within which all the species cohabit. It has become apparent that structures incorporating microbial communities include that range of species essential to the basic functioning of the consortium. It clearly becomes impractical to isolate each of the species, particularly where a high level of interdependency has evolved.

It is therefore proposed that definable extracellular structures that have been formed out of, nurtured by, and matured as a result of the activities of an incumbent consortia of microbial species should be recognized and classified as a distinctly separate group within the living world. Where there are essentially a complex of different species forming populations that integrate into community structures, some form of "communication" must exist to allow mutualism. This exchange may be based partly upon the availability of space, electron acceptors, the redox potential, and, perhaps, upon some more direct form of cellular signal that may be chemical or physical in nature. This coordination of microbial behavior into complex patterns is known as "quorum sensing" (Straus 1997). The environmental impacts associated with the sinking of the *RMS Titanic* therefore relate to the stimulation of a myriad of consortial microbial activities that subsequently affect the rest of the local biota.

To this end, it is further proposed that a system of classification needs to be introduced in which the defining first word would be *consortium*; this would mark the living "organism" being described as consisting of a microbial consortium. Construction of the consortium is based on a minimum number of member species arriving at a suitable niche, which would then allow the consortium to generate a structure possessing the typical features expected for that form of growth (i.e., replication of a definable living growth as a result of a commonality of circumstances). These consortia would initiate the biodegradation of the structures and the relocation of any recalcitrant materials. The basic parameters that could be used to identify consortia at the community rather than at the individual or molecular level were defined by Cullimore (2000b). This approach involved culturing the sample within selective culture conditions including juxtaposition of diffusing selective nutrient and oxidization fronts to generate locational environmental patterns and reactions resulting from metabolic functions. The reactions and activities observed are formed into a reaction pattern sequence (RPS) that is typical for particular consortia. In generating RPS data, it now becomes possible to recognize the major bacterial types that may be contributing members of the consortium.

In the determination of environmental impacts, it has been a common practice to attempt to describe microbial activity primarily at the species and genome level because this is deemed to be intrinsically more accurate. However, such

events do not recognize the importance of consortial activity as an essential component of the environmental process. For the rusticles on the *RMS Titanic*, the use of this level of precision would have proved expensive and inappropriate to the determination of the microbial components active within the rusticles. One significant outcome of the research on the *RMS Titanic* has been the recognition of the major importance of microbial consortia as the prime generators of the rusticles.

III. Rusticle Growth Rates

Since the discovery of the *RMS Titanic* in 1985, there have been a number of opportunities to examine this deep-sea wreck. It is, in fact, the world's first deep-ocean archeological site. The *Titanic* rests under approximately 4000 m of 1 °C water, with pressures in excess of 41,000 KPa. One of the first features that stood out in the images of the ship was the mass of rustlike growths (Pellegrino 2000), which occurred as a growing mass of iron-rich bioconcretions on the steel surfaces of this once-elegant ship. These bioconcretions were first noted growing on the outside and within the ship's structures. The scale of these growths led to the adoption of the term "rusticle" as a derivation of two words, "rust" and "icicle." The term "rust" was elected because the growths had a predominantly rusty color and the texture resembled flakes of rust growing on steel (Cullimore 1999). The most dramatic, but not necessarily the largest, growths hung over the sides of the hull, somewhat paralleling the structure of icicles.

In character and form, these growths resemble the speleothems that have been observed in natural limestone caves. These rare speleothems, originally considered to be secondary mineral growths, appear to be of subaqueous origin and show many similarities to the deep oceanic growths observed on the *RMS Titanic*. In their simplest form, both the rusticles and the speleothems could be described as elongate structures incorporating organic filaments coated by, or included in, a shell of iron oxide or calcite. In the Lechuguilla Cave in New Mexico, the speleothem growths are described as hanging down in a manner that closely resembles the rusticles at the *RMS Titanic* (Davis et al. 1990). These cave growths are irregular, consisting of iron oxide stalactites and calcite-encrusted columns; these are all of ancient origin (more than 100,000 yr ago) and are now dry and inactive. Microscopic examination reveals that the speleothems are primarily iron oxide deposits covering organic filaments. These encrustations were deposited via oxidative reactions that may have been initiated by bacteria. Other unusual cave features related to rusticles in form, but not based on iron oxides, include "pool fingers." These stalactiform subaqueous growths are calcite-encrusted organic strings, interconnected by curved bridge structures. In Wind Cave, South Dakota, hollow subaqueous calcite speleothems known as "helictite bushes" that grow in an upward branching pattern, have been identified. These growths again closely resemble rusticles and pool fingers; they include fossil bacterial traces but may be more closely related to the submarine

"white smokers" than to either rusticles or the pool fingers (Davis 1989; Davis et al. 1990; LaRock and Cunningham 1995).

The bioconcretious rusticles vary in color, texture, size, and form. The variations of color, particularly the brilliant orange-brown color of the rusticles, are due to the highly oxidized ferric iron content. Closer examination of the rusticles by Pellegrino and Cullimore (1997), Wells and Mann (1997), and Mann (1997) revealed that the rusticles are complex structures involving water channels, reservoirs, complex iron platelike structures, threadlike spans, porous matrices, and ducts connecting to the outside. Within the rusticle structure appear to be a number of different microbial strains occupying specific sites. These strains were identified, using the Biological Activity Reaction Tests (BART™, Droycon Bioconcepts Inc., Canada), to include sulfate-reducing bacteria (SRB), iron-related bacteria (IRB), heterotrophic aerobic bacteria (HAB), denitrifying bacteria (DN), and archaeobacteria, together with a range of fungi. Six different forms of rusticles were noted; however, all bore the common characteristics of diverse and site-focused bacterial consortia (Fig. 1).

The supporting structures appeared to be dominated by a meshlike, heavily mineralized matrix in which goethite was dominant. The presence of goethite in rusticles was confirmed by Garzke et al. (1997). In addition, an iron oxide sulfate complex known as green rust $[Fe^{2+}_{3.6} Fe^{3+}_{0.9}(O^-, OH^-, SO_4^-)_9]$ was found (Garzke et al. 1997). A large hanging rusticle recovered from the ship in 1996 was analyzed by electron diffraction x-ray, which revealed that iron was the dominant atom within the range of atoms tested. The relationship was (dominant atom first) $Fe > Na > S > Cl > Ca > Mg > Si > P > Mn$. There was a considerable variation in the elemental composition for the various samples analyzed, reflecting the heterogeneous nature of the structures within the rusticles. Where goethites dominate the structure, the iron (Fe) concentrations are very high whereas other components within the rusticle (e.g., the water channels and porous regions) have lower iron levels. Rusticles also vary widely in size and form.

Video imagery revealed rusticle sizes ranging from tiny tubercles or encrustations to massive, braided, or ropelike bioconcretions exceeding 3–4 m in length. The rusticle form also varied from flat, platelike growths to convoluted and intricate growth patterns. One major environmental impact of the sinking has therefore been the creation of a growing biomass dominated by rusticles that are gradually mobilizing the iron from the ship's steel into various oxidized forms which are moving away from the ship's structures to create associated biomass. Such an environmental impact has serious potential implications for the maritime industry.

The hull of the *RMS Titanic*, torn into three main sections, lies on the ocean floor in an oxidative environment where animal life can also be observed. In the deeper regions of the hull there is a strong probability that more reductive conditions may exist that would not only totally change the nature of the dominant microbial consortia but also influence the physical and chemical nature of the local environment. Gerhules and Alford (1990) observed such a phenome-
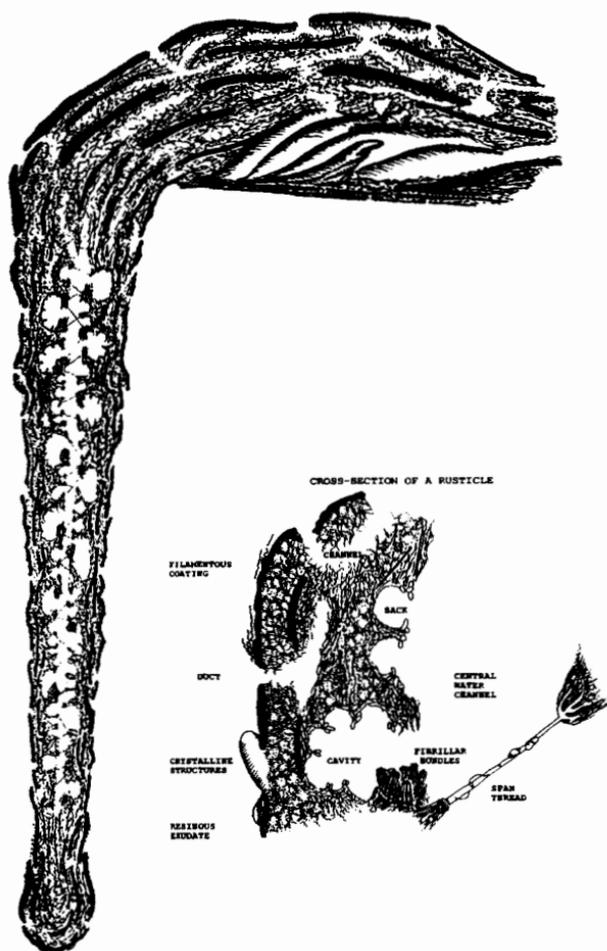
Fig. 1. Illustration of the typical components in a dissected hanging rusticle. The left side of the diagram is a vertical cross section through a hanging rusticle showing a central water channel with saclike extensions into the porous cortex of the rusticle. The outside of the rusticle is coated with iron-rich plates. To the lower right is an illustration of the structures found associated with the central water channel; these include the spans of threadlike materials forming a major structural support (upper left), the cavities and sac within which water may collect, the very porous concretious cortex, and the iron-rich plates toward the outside rusticle. Passageways (ducts) connect the central water column with the outside environment through these plates. Scales: left-hand diagram, 1 mm is equivalent to 20 mm; inset diagram, lower right, 1 mm is equivalent to 0.1 mm. (Figure originally published on page 102 of Ghosts of the Titanic by Charles Pellegrino 2000, published by HarperCollins Publishers Inc., New York.)

non when attempting the rehabilitation of a severely biofouled well in Ontario, Canada. Here, there was success in removing biological plugging that extended more than 15 m from the oxidative zone in the water well to very reductive zones well back in the formation material. In chemically analyzing the various water samples during posttreatment monitoring, it was noted that the various metallic elements appeared to have clustered along the gradient from oxidative to reductive. This gradient showed a grouping as follows (from oxidative to reductive with squared brackets indicating the grouping): [iron, copper], [zinc, manganese, titanium, chromium], [lead, barium, vanadium, cobalt], and [molybdenum, nickel, molybdenum], indicating, as a part of the bioaccumulation of some metallic cations, their location in the reduction–oxidation gradient. This location is dictated by the cationic species with some, such as iron, being at the very oxidative edge of the biomass whereas others such as nickel and molybdenum are at the reductive edge of the biomass. The consortial plugging occurring around this water well is therefore behaving like a biologically driven chromatograph.

Because the rusticles recovered from the *RMS Titanic* were growing in an oxidative environment, it is therefore not surprising that the dominant metal was iron, constituting 20%– 36% of the dry weight of the material. In the environmental industry, the absence of a particular metal does not mean that it is not present in that environment but simply that it was not detected in the sample. This finding does not exclude the possibility that all the cation in question has been bioaccumulated upstream from the position at which the sample was obtained. When undertaking environmental management of a porous medium that is biofouled, there remains always the possibility that hazardous chemicals could be bioaccumulated within the matrices of the biomass that could be released when there is a destabilization in that biomass. These accumulations could be viewed as the "icebergs" just over the horizon, as disasters that could one day happen without warning.

IV. Rusticle Relevance to Maritime Industries

The investigations to date of the rusticles have revealed that they are capable, under suitable conditions, of extracting iron from steel at significant rates. This biological extraction has the potential to seriously compromise the physical structure of a ship. The time frame for such compromise would appear to be based on the visual evidence. This evidence has been gathered from various sunken vessels (e.g., *Bismarck, Yorktown, Derbyshire*, the Japanese submarine I-52, and the *RMS Titanic)*, located at various sites around the world. There remains the potential for the covert growth of rusticles within ships during the normal operational life span. Covert growth means that the rusticles could thrive at sites within the body of the vessel, particularly those that are not commonly inspected, and where conditions are conducive for growth.

In a ship's structure, areas that are most vulnerable include welded areas and zones which have severe stress concentrations (Mansour et al. 1997). It is esti-

mated that two bulk tankers are lost every month, with 45% of these losses caused by heavy weather and structural damage. This category is further described as "strained crack in hull," concluding that structural failure is the major cause for the rising number of bulk carrier losses, as can be seen from the December 12, 1999, sinking of the *Erika* during a storm off the French coast. The 25-year-old tanker *Erika* broke in two, spilling about 13,600 t of fuel oil, polluting 400 km of beaches, and killing or maiming 300,000 seabirds. The classing agency for the tanker, Registro Italiano Navale Group (Internship classification & Management System Cert. Society), reported the initial findings into the cause of the accident as pointing to a small structural failure in hull structure. This structural failure led to further cracking and finally to the collapse of the hull (Hauley 2000). These structural failures can be a result of corrosion and fatigue cracking, which, in conjunction with biological attachment from within the tanker itself, can result in the loss of a ship's integrity (Ma et al. 1997). Such catastrophic failures of shipping have considerable long-term environmental pollution implications through the release of both degradable organics and the more recalcitrant inorganic materials, including toxic metals and radionuclides.

A range of factors would be important in considering the potential for these rusticles to grow rapidly enough to compromise the normal lifespan and seaworthiness of a ship (Cullimore and Johnston 2000b). These factors could include, but are not necessarily limited to, suitability of steel surfaces on which rusticles can form and function, a high level of humidity or a water-saturated environment, oxidative conditions, salt concentration in the water greater than 1.4%, temperature gradient, turbulence, nutrients, electrically charged surfaces, and neglect. A typical example of a condition where these rusticles could infest and compromise the integrity of the ship is between hulls and in compartments where a confined environment could provide conditions conducive to growth.

The most likely sites for a rusticle infestation to occur require a number of variables to be achieved, including surfaces or areas where the steel is poorly protected with paint, embrittled by stress, electrically charged in any way, involved in rhythmic movement of water over the site, positioned on a temperature gradient, or where available water contains sufficient nutrients to support growth. Where a site is not subjected to regular inspections, for example, on a monthly basis, or rusticle growth is not suppressed through the use of biocides or physical removal, the growths can then begin to extract iron from the steel and weaken the afflicted steel structures. It is a common practice to presume that the appearance of rusty encrustations are merely the result of physio-chemical activity and an inevitable part of the normal deterioration that may be expected. Traditionally, the appearance of rust within an enclosed chamber has not been viewed as a living mass that is "eating" away at the steel, but rather the rust is seen as an inevitable chemical event for which solutions may be ineffective over the long term.

In the water well industry, it is now acknowledged that most of the plugging and clogging events that occur down a well are actually biologically derived.

Comparable studies have revealed that the same groups of bacteria are involved in these events both down in water wells and deep down at the site of the *RMS Titanic*. Similar rusticle structures are observed at both sites. The question therefore becomes whether steel-fabricated ships floating on the surface, or the *RMS Titanic*, a splintered steel structure lying on the ocean's floor, are subject to the same bacterial challenges as water wells, which involve steel structures set into the groundwater. The arrival of nonindigenous organisms, such as the zebra mussel (*Dreissena polymorpha*) plaguing North American water systems, appears to have arisen from covert passengers in or on seagoing vessels. By analogy there may be "microbial" passengers traveling in the bilge areas and other damp dark places within ships that can cause problems to the ships themselves. In water wells, these nuisance bacteria can so substantially reduce water flow into a well that it has to be abandoned. The developing practice in the water well industry to prevent these infestations has evolved into the Sustainable Water Well Initiative in Canada. The objective is to extend the operating life span of wells by routine testing and suitable preventative maintenance or radical treatment procedures, depending on the state of the bacterial infestation. In the Canadian prairies, 200,000 operating wells have been found to have, on average, a life span of 15 yr. The capitalization of these wells has been estimated to be minimally CAN$1 billion, and the annual replacement and/or rehabilitation costs are CAN$67 million.

In the maritime industry, a similar scale of impact on the life span of ships also causes heavy replacement costs, pollution, or unscheduled repairs. These costs could be partially curtailed if the true level of these microbial impacts was found to be significant and controllable. In the shipping industry, there is also an average life span for ships, but sudden catastrophic sinkings continue to occur on a regular basis. By improving detection and control procedures to include microbial events that can weaken the structure of the ship, it should be possible to lengthen the life span of ships in much the same manner as water wells are becoming more sustainable.

V. Rusticle Biomass on the *RMS Titanic*

The examination of rusticle growth rates on the *RMS Titanic* has only been possible through annual or biennial expeditions to the wreck site. Through the use of video and high-resolution imagery, growth rates and patterns can be closely analyzed. In 1996, the first detailed examination of rusticles from the *RMS Titanic* was begun. This examination has permitted us to quantify rates and modes of rusticle growth on the outside of the bow section of the ship; however, the outside of the stern and the interior of the wreck cannot be clearly quantified because these areas remain largely unexplored. One aspect of this examination was to estimate the percentile coverage of the various parts of the bow section, together with the estimated thickness of the rusticles at these various sites. From the 1998 Expedition, comparative assessments through video imagery showed clear evidence that the rusticles continue to grow, and there is

evidence that the biomass is approximately 30% greater than the mass observed in 1996.

Video surveys of the bow section of the *RMS Titanic* in August 1996 and 1998 allowed a quantitative estimate of the total volume of rusticles. This estimate is based on the area of the steel covered by rusticles at various points on the ship. Calculations reveal that the bow section of the ship had a total mass of 590 ± 30 t of rusticles in 1996, increasing to 800 ± 40 t in 1998. The iron content in the rusticles had presumably been extracted from the ship's steel structures and was now accumulated within the rusticles. The iron content has risen from 160 ± 10 t of iron in 1996 to 220 ± 12 t in 1998. Although a significant amount of iron is present within this mass of rusticles, there remains a concern as to the rate at which iron is being released from the rusticles into the oceanic environment. Iron concentrations vary in rusticles along with their size, weight, and density of the ducts on the rusticle surface. These estimates are based on examination of rusticle specimens recovered from both the 1996 and 1998 Titanic expeditions.

VI. Focused Accumulation Sites for Iron Within Rusticles

One concern arising from the examination of the *RMS Titanic* was the manner in which the iron is accumulating in the rusticles. It is known that the rusticles have a very large surface area and a highly porous concretious structure; however, little was known of the sites where the iron accumulates. Accumulation occurs as various forms of ferric oxide and hydroxide, dominated by goethites. To examine the sites of iron accumulation in the rusticles, a Veterinary Grade Radiograph machine MinXray 903 Type B-85, MinXray Inc., 3611 Commercial Ave., Northbrook, Illinois 60062-1822, operated at 100 kV, 1/20 sec, using Kodak high-speed film was used. Five rusticles, collected from the 1996 and 1998 expeditions, were investigated using radiographic examination (Cullimore and Johnston 2000a). It was found that the iron within the rusticle structure was not evenly distributed throughout (Fig. 2) but was concentrated into two major regions. The first region where iron was dispersed resembles "cloudlike" structures. The second region had very dense channeling of the iron into localized regions that spread weblike throughout the rusticle. The first impression was that the regions channeled with a high iron content bear resemblance to a primitive blood system.

The radiographic images confirmed the complex nature of the rusticle channels and the fact that the rusticles had entrapped small artifacts, coal fragments, and glass shards that billowed over the bow immediately after its collision with the ocean floor. Microbiological examination revealed that the microbial consortia within the rusticle were not evenly dispersed but remained concentrated within localized regions of the rusticle. No correlation could be established between the sites of iron concentration and the various bacterial consortia present in the rusticle.

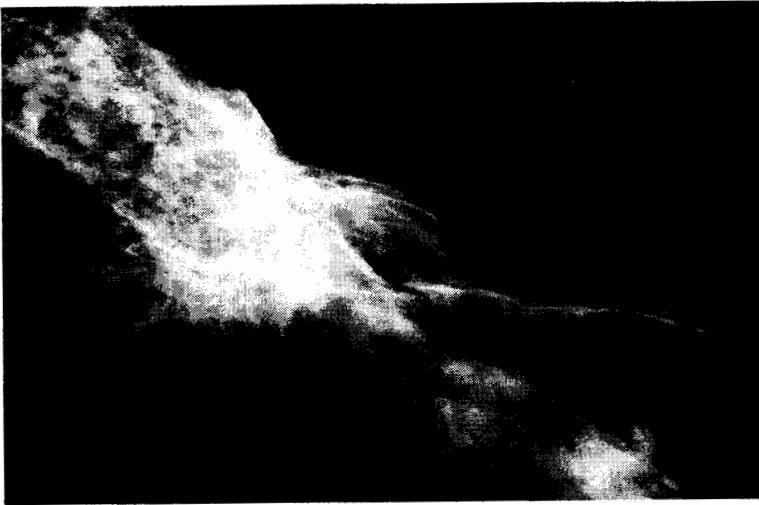It has been determined, based on extraction results, that a cycle has been

Fig. 2. Radiographic image of a rusticle recovered from the 1998 Titanic Expedition. This radiographic image represents one "shoulder" of a rusticle recovered from the bow section of the ship. The image reveals the density of the iron (the lighter the image, the greater the density of iron). The close-up image reveals there are complex structures within the rusticle body.

established in which iron is being biologically extracted from the steel of the ship into the rusticle structures. The iron is then exported into the oceanic environment as "red dust" (RD) and "yellow colloids" (YBC). The rate of extraction is increasing beyond the predicted 1996 rate of 0.09 t of iron being mined by the rusticles/d. This rate of dispersion of the iron through the release of RD and YBC into the greater oceanic environment is a critical factor in assessing the true nature of the environmental impact of the sinking of *RMS Titanic*. Thus, there is clearly a potential for some of the iron to enter into the surface biosphere as a result of events such as the consumption of fish and other animals from the oceanic environment. The iron in both the RD and YBC is likely to be consumed by the oceanic biota and, through that means, to enter the food chain. It is therefore possible that some of the iron from the *RMS Titanic* could actually find its way back into the blood of humankind and become a minor player in blood hemoglobin, primarily after entry into and passage through the phytoplankton biomass.

VII. Water Wells and Rusticles

A parallel can be drawn between the rusticles at the *RMS Titanic* and growths that occur within the various forms of wells that interface with groundwaters (Cullimore 1993; Cullimore and Johnston 2000a). The origin of the iron accumulating in these growths was, for the rusticles growing in the wells, mostly

coming from the groundwater and surrounding geology. On the ship, the iron appears to be coming from the steel structure of the ship itself.

Downhole video inspection of water wells that are becoming biologically plugged reveals growths and concretions similar to those seen in the aforementioned caves and at the *RMS Titanic*. Injection and extraction wells at bioremediation sites reveal startlingly similar concretious growths. For example, a bioremediation site in Holland utilizes horizontal extraction wells as a part of the bioremediation process, extracting chlorinated hydrocarbons such as trichloroethylene and polycholoroethylene. The bioconcretious growths in the 10.16-cm well screen appeared within the first 2 yr of operation, causing a 50% loss in hydraulic production. These growths appear so morphologically similar to those observed at *RMS Titanic* that they may be called "sibling" species.

VIII. Laboratory Evaluation of Corrosive Processes

The first stage in the investigation has been to develop a methodology to determine whether microbial growths in the form of rusticles can cause iron extraction from a targeted steel plate. To achieve this outcome, a Biological Activity Reaction Test (BART™, Droycon Bioconcepts Inc., Canada; Cullimore and Alford 1990) was modified (Fig. 3). The BART™ test uses a 15-mL water sample
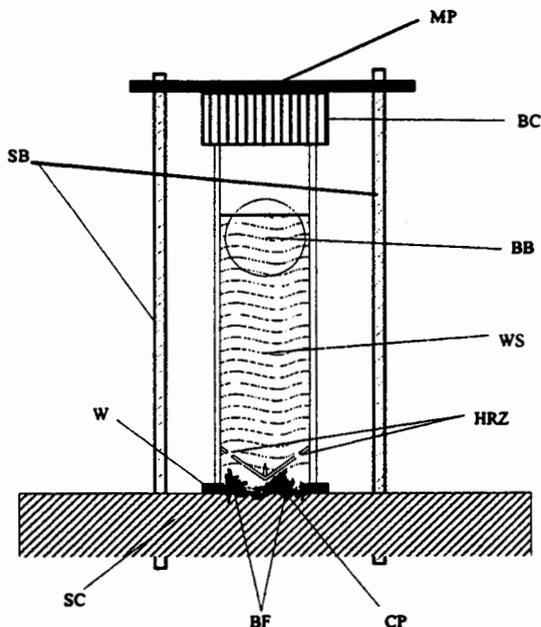

Fig. 3. The biological activity reaction test (BART™, Droycon Bioconcepts Inc., Canada) has been modified to determine corrosion of the surfaces of steel coupons.

that creates a reductive/oxidative (redox) front with a rising nutrient gradient selective to the bacteria being investigated (e.g., sulfate-reducing bacteria). To contact the mild steel coupon, six 3-mm holes were drilled through the basal cone of the test vial, and the vial itself was placed under pressure onto a 26-mm rubber washer to prevent leakage.

The BART test vial is modified to encourage the growth of bacteria within the water sample (WS) (see Fig. 3) on the steel coupon (SC). To achieve this, the BART test is placed on a circular rubber washer (W) and pressed down using a main plate (MP), which is screwed tightly on by two screw bolts (SB). The water sample is added before this procedure by unscrewing the BART cap (BC) and adding 15 mL of the water sample, at which time the BART ball (BB) floats up to create a redox gradient. Holes (HRZ) are cut in the base of the test vial to allow the bacteria to grow directly on the steel and form biofilms (BF). Corrosive pitting (CP) can be observed at the contact sites of the steel coupon (CP).

To conduct the test, a 1% (w/v) suspension of rusticles recovered from the site of the *RMS Titanic* was made using a 4% sterile solution of sea salts. The 15-mL suspension was aseptically injected through the cap into the test vial to set up a redox gradient selective for a specific group of bacteria. The tests were conducted at room temperature (21 °C) for 60 d. Each steel coupon was examined for biological corrosion or extraction by reflectance microscopy (×10 and ×40). It was observed that biological growths appear to be attached to the steel surfaces on which the test vial had been positioned. Examination of the growths by both photomicrography and radiography revealed a range of structures that resembled rusticles in color, texture, and form. The observed rusticle-like structures grew from the surface of the mild steel to an average height of 2 mm, occupying 60% of the volume (after 60 d), having a wet volume of approximately 0.37 mL, a dry weight of 0.09 g with an iron content of 22%; this resulted in approximately 0.2 g of iron being biologically extracted from the steel in the 60-d period. Removal of the rusticle structures attached to the steel and subsequent cleaning of the steel was achieved using a chemical immersion technique. The steel coupon was immersed in a 1.0% solution of hydrochloric acid for 60 min, followed by a sterile distilled water rinse. The steel was again microscopically examined for evidence of biocorrosion and/or extraction. Pitting was observed covering 20% of the steel's surface, with the pits ranging in diameter from 0.1 to 0.8 mm with a depth of 0.4–1.0 mm.

This experiment revealed that, under reductive conditions, the biocorrosive processes could be initiated within 60 d at room temperature. These processes generated biological growths that resembled rusticles on a microscale. The next stage of this study will be to apply this methodology to coupons of steel from the *RMS Titanic*. This experiment will provide somewhat controlled conditions to observe the forms of corrosion or extraction that occur on this particular steel. Field experience has revealed that corrosion or extraction tends to be dominated by lateral flaking and dissolution of the steel directly under maturing rusticle growth.

IX. Environmental Cost of Covert Rusticle Growth

There is a clear chain of evidence that suggests microbial growths such as rusticles, in the form of slimes, tubercles, nodules, and encrustations, can extract elements such as iron from the steel into these structures. This type of functioning bioconcretion is evident at the *Titanic* site. These growths are contributing to the deterioration of the *RMS Titanic* through biodegradation; it is a natural extension to consider the implications of these biofunctions at other sites where conditions may be conducive for such growth. It becomes clear that the rusticles have become dominant in an environment where there is high salt concentration (i.e., highly conductive), available organic material such as the "sea snow" found at the wreck site, and steel surfaces.

In examining existing engineered steel structures present within the oceanic environment, it is evident that current management strategies, over the lifetime of such structures, would be ineffective and restrictive to the determination of observable occurrences of rusticle-like growths. There are two main points at which these growths may become key to the ongoing life of a structure. The first critical point is that these growths may commonly be generated at covert sites, remaining unobserved, yet still remain able to create catastrophic failure of the structure. Loss of buoyancy, leaking, and total loss of structural integrity could follow, resulting in the sinking of the vessel and rendering forensic proof for this type of failure difficult to determine. The second critical point relates to abandoned steel-fabricated structures, such as pipelines, oil rigs, drums of ocean-dumped hazardous waste, reactor casings of lost atomic submarines, and plutonium triggers aboard these submarines. Rusticle colonies now have the potential to seriously compromise the structural integrity and containment of these artifacts. To a lesser degree, rain- and salt-exposed crevices in the supporting structures of bridges may be vulnerable to rusticle activity.

Historically, there has been very little understanding or examination of the impact of microbial activity on metallic structures. The extent and significance of these biologically induced events must rely heavily on the limited knowledge that has been gained with respect to biocorrosion and on the understanding of the function and aggressivity of microbial communities (consortia) to establish and proliferate in extreme environments. The covert microbial growth and degradation of steel structures has the potential to cause economically significant environmental problems.

Potential maritime examples range widely, but perhaps one that has a long-term and serious concern is the disposal of steel drums containing toxic or nuclear wastes into the marine environment over the past 50 yr. The chain of events established by this practice involves the loading of an industrial steel drum with radioactive material and allowing it to free fall to the ocean floor. At the time this practice was established, popular opinion was that the ocean floor was a sterile environment divorced from the terrestrial environment. In the event of a free fall of the loaded drum, the first concern is generated by the collision between the drum and the ocean floor, as well as high-pressure implosion of air

pockets contained within the drum itself. These impacts may cause seam failures or the embrittlement of the mild steel of the drum. These sites then become focal points for initial attachment and growth of microorganisms. The form and extent of the growth would depend on the nutrients in the environment (positive impact) and the level of radiation being generated by the wastes (negative impact).

In the case of a negative impact caused by radiation, two microbial mechanisms can counter this influence on their growth. The first of these mechanisms is that some microorganisms, such as the bacteria belonging to the genus *Deinococcus*, have a very high resistance to radiation (e.g., 1–2 Mrad) due to unique cell-wall-surface structures and DNA-repair systems. Under suitable environmental conditions, these bacteria could therefore grow over the surface of the steel containment drum, causing a loss of integrity. An alternative mechanism occurs where the indigenous microbial consortia are protected from radiation by the nature of the concretious growths that these microbes form. The microbially induced concretious growths contain a high metal content, retarding the impact of the radioactive contents of the drum. Initial growth may occur through the metal-rich biocolloids, such as red dust from the growth of rusticles, which attach or come to rest on the outer surfaces of the steel structure. Consequent growth may then generate conditions that cause corrosion of the steel structure, followed by perforation and leakage of the contained radioactive waste into the environment. The biological embarrassment of steel containment structures such as these rums is likely to occur over an extended period of time, yet the potential for large-scale environmental contamination continues to be significant and largely unpredictable.

The potential for corrosion by rusticle-like concretions on drums filled with toxic material is probable and problematic. This concept is founded on the dual premise that (1) there is no restricting radiation field to suppress microbial activity, and (2) the contained toxic material may well be selectively toxic to humankind and the biota but not toxic to many species in the microbial community. For example, cyanide is clearly perceived to be a powerful toxic compound; however, many microorganisms are resistant to cyanide and some can even degrade it as part of the process of natural self-purification commonly seen when toxic spills occur. The sequences involved in the event of the degradation of toxic containment vessels would follow a pattern of impact, embrittlement, localized corrosion, and, finally, perforation. Perforation would be followed by localized leakage, with a probable accumulation of the toxic material within the concretious growths. Bioaccumulation events would occur at the viable cells present and active within the concretious growth. Finally, biodegradation or dispersion, depending on the nature of the toxic material, would occur. Growths such as rusticles would therefore both accelerate, through corrosion and perforation of the steel structure, and reduce, through bioaccumulation with or without biodegradation, the release of toxic materials from the compromised structure.

Underwater pipelines offer a number of problems relating to the potential biological compromise that could involve the activity of rusticles. The contents

of the pipeline, whether it is abandoned or in use, may be critical to the nature of the growth. For example, an underwater gas pipeline may suffer from corrosive physical pitting or perforation, leaking methane into the surrounding environment where it would be utilized by the methane-consuming (methanotrophic) bacteria (MCB). These MCB may be incorporated into the concretious consortium-forming rusticles. Consequently, early microleaks of gas or oil may serve as the primary organic feedstock for the rusticles, which then become much more aggressive and, in so doing, structurally weaken the pipe, causing sudden and dramatic pipeline failures. Abandoned pipelines would carry only static perched material that may form a smaller feedstock for microbial growth, but the lack of forced flow and pressures may allow the microorganisms to gain entry into the pipeline itself and initiate reductive corrosive processes from directly within the pipeline. Concurrently, the pipeline could also be challenged with oxidative corrosive processes being generated by rusticle-like structures. The form of these attacks on the integrity of the steel pipeline would be very much affected by the nature of the variable electrical charges along the pipeline because anodically charged sites tend to be the focal point of the microbial activity. Many pipelines are protected, during their active life span, from these types of events through a process of cathodically charging the outer surfaces, which reduces microbial attachment and growth. Once the pipeline is abandoned, corrosion prevention practices are halted, and the gradual process of microbially induced corrosion and deterioration continues unimpeded.

In offshore drilling operations such as those for oil and gas, the steel structures being used for such operations are known to be subjected to corrosive processes generally thought to have originated from infestations of sulfate-reducing bacteria (SRB). The presence of this type of activity is clearly signaled by hydrogen sulfide-driven electrolytic corrosion, the presence of copious black-sulfide-rich slimes, and, often, the "rotten egg" odor of the gas. This group of microorganisms forms a major component of the consortia of rusticles and can be found cloistered within the bioconcretions formed by this process. Rusticle growths on the outside of a drilling rig would clearly be recognized, treated accordingly, and removed during routine maintenance procedures. The same thing may not happen when rusticles grow within the ballast tanks of these rigs. Where this process is occurring, there is a risk that the growth may compromise the structural integrity of these ballast tanks in a manner that could lead to sudden and catastrophic failures.

Abandoned steel rig structures, particularly when immersed in seawater beneath the light penetration zone, are more likely to become infested with bioconcretious structures resembling rusticles. Residual hydrocarbons would form a major part of the feedstock supporting the growth, and the primary focal regions for growth are likely to be oxidative over steel that is, in some manner, embrittled to facilitate microbial attachment and growth. The rate at which these rusticle-like structures would begin to form and compromise the steel cannot be estimated because the experiential base is largely limited to a few sunken ships, with the RMS *Titanic* being the one most vigorously investigated. Ships sunk in

shallower depths, such as the *RMS Britannic*, show very different forms of biological encrustations with a much greater diversity of life forms. The relative significance of the relationship between these different species and the rate of corrosive challenges to the steels on the vessels remains to be determined.

The potential environmental costs of biological activities on submersed steel structures are a combination of positive and negative impacts. Positive impacts relate to the recycling of the iron from the structures back into the natural oceanic cycles. This impact relates, in part, to the *RMS Titanic* investigation, forming perhaps a fitting conclusion to the investigation of the ship herself. Of particular interest is the fact that iron has now become recognized as a key limiting factor in the rate of oceanic carbon dioxide fixation through the phytoplankton. The iron released from these submerged steel structures may therefore play a significant role in increasing the ability of the oceans to fixate carbon dioxide and therefore, in some small way, thus reduce the potential impact of increased carbon dioxide on global warming. From there, the iron would gradually move into the global biosphere so perhaps, some time in the distant future, there will be at least one or two iron atoms from the *RMS Titanic* pulsing through every human body. The same may be said to occur in the fullness of time for the many other steel ships now resting on the ocean's floor and gradually deteriorating. This result may be viewed as a positive long-term environmental effect.

Some negative environmental impacts are more diverse and difficult to predict. These impacts may relate more to the releases of toxic, potentially carcinogenic, or recalcitrant chemicals of concern that were previously confined by steel but are now being released, at least in part, as a result of biological activity. These effects become very difficult to predict, challenging to contain, and may impact in many different manners over various time scales. In the past, when a ship sank at sea, there was little concern for its impact on the environment. Today, a ship sinking means simply that the contents of that ship have now become subject to a different pattern of release that is not in the domain of humankind. Impacts, therefore, become speculative because the knowledge has yet to be gained, and in that regard the *RMS Titanic* is one of the signposts pointing the way to understanding these effects.

There is a continuing need for dedicated research and development to address the capacity of rusticles and other microbial communities to cause sudden losses in steel strength and integrity. These losses must be recognized and effectively managed throughout the life span of the steel structure, but also after usage. This postusage monitoring will, in effect, require a more complete understanding of biologically induced corrosion events and how these activities are impacting the environment.

Three different replicable consortially driven forms of growth are proposed to form the foundation of a new scheme for the recognition of living entities formed and dynamically driven by a multiplicity of microbial species that may commonly contain bacteria and fungi. Each of these entities would have a generic name that would relate to a commonality of form and consortial composition, whereas the species name would reflect the habitat where the entity was most

commonly observed growing. The following represent suggestions within the mandate just discussed. Rusticles are defined as the "type" consortium.

The other two consortially formed living entities discussed here relate to the black plug layering in golf courses and the plugging of water wells. In these events, the consortia possess two common characteristics. First, the growth is formed by the glycocalyx to produce a coherent body of "slime." Second, there is an occlusion or loss in permeability (plugging) in the infested porous medium. For the black plug layer, this can be a dramatic event, with permeability falling several orders of magnitude over a matter of weeks. This change is caused by a rapid shift in the voids from being essentially saturated with water to a state of being totally occupied by the "slime" (glycocalyx) generated by the consortium. For the plugging, the loss in hydraulic conductivity can be a very gradual happening as the biofilms forming the plug (glycocalyx) only slowly fill the void space. There are thus two major consortial groups proposed under this heading: (1) the black plug layering consortium and (2) the plug-forming bacteria that commonly accumulate iron along with other metals within their growths. As both these forms of growth occur within porous media, their form and structure become more difficult to determine because direct observation is not possible except under laboratory conditions.

In these consortia, there is a form of social "intelligence" that is one step beyond "quorum sensing," for here the various incumbent species through cooperation (assigning everything to its proper proportion) generate a concord within which all incumbents can mutually benefit. Although this might not be true of the bulk of plants and animals, it may be reasoned that most microorganisms forming a part of the larger environment do form into consortia, which may vary from tenuous to essential and commonly are within an extracellular matrix of some type. In understanding each of these component species, their role within the consortia needs to be understood as an integrated part of the social "intelligence" that is inherent in the consortium. We clearly have come to accept the defining role of species in the classification of living organisms. Perhaps, when examining the microbial kingdom, classification should now be based on the living entities generated by consortial synthesis and maturation rather than on a study of those components that can, through convenience, be readily observed using traditional scientific concepts.

Perhaps the most challenging and fascinating aspect of the presence of these often covert consortia is their role in the evolution of life forms on this planet. Present concepts cloister species as unique entities capable of independent existence. Given the extreme environments prevailing during the early development of the biosphere on planet Earth, it would not be unexpected to find that the simple life forms evolving at that time would either have found specialized environments such as high salinity, extreme heat, or radical pH, or developed some mechanisms for a compensatory synergistic development with other species. Today, the Archeaobacteria remain dominant in the extreme environments. In the surface biosphere, we have yet to determine the full extent and nature of the microbial synergy that would have led to the evolution of complex and

robust consortia such as the ones now on the *RMS Titanic*, in golf course greens, and bioplugging water wells.

It is not unreasonable to conjecture that these consortial forms may be considered as living fossils dating back 3 billion yr to the inception of multicellular life and represent not only definable life forms but the beginnings of tissue differentiation. Of all the consortia so far discussed and recognized, the rusticles display the most differentiation. Through the cooperative activities of a range of Eubacteria, Archaeobacteria, and Fungi, the rusticle shows differentiable structural forms and activities that would be more expected of an animal than of a collective group of protists. The central water channel, reservoirs, passage-ways, and ducts to the outside possessed by the rusticles indicate a rudimentary circulatory system. In the laboratory, the venting of gases (principally CO_2) would imply that the gases could act as a pneumatic mechanism for moving water through the rusticle. The large plates and columns rich in ferric iron perhaps represent a primitive skeletal structure providing physical integrity to the consortial matrix. The very porous nature of the consortium, with a high sorption capacity, would allow the retention of nutrients, metals, and both anions and cations in forms that would augment the ongoing growth of the rusticle. The metal in the hull of the *RMS Titanic* has come to form essentially a culture substrate for the growth of rusticles.

It would therefore be of considerable scientific interest to determine in a more precise manner the roles that microbial consortia may well have played in the evolution of the animal and plant kingdoms in an open-minded analytical manner. Marcus Aurelius (1969b) considered the need to address the dogma that sometimes impedes progress by observing:

> The Pythagoreans bid us in the morning to look to the heavens that we might be reminded of those bodies which continually do the same thing and in the same manner perform their work, and also be reminded of their purity and nudity. For there is no veil over a star.

In our dogmatic application of the classification of living systems using the species concept, we have perhaps pulled a "veil" over the understanding of the dynamics of living entities that do, in the form of consortia, flourish.

X. Conclusion

There is little doubt that the *RMS Titanic* was traveling close to her maximum speed as she entered that fatal field of ice. Time was dictating speed, and arrogance spiced with greed ignored the ice, but there was a collision with the ice and the ship sank quickly with a tragic loss of life. Today the "unsinkable" ship lies broken on the ocean floor, but not at rest, for Nature is now recycling the iron, transforming the ship, and folding it back into the web of life. The time is now to learn the lesson woven by Nature into the ship as the ship yields back her being to become only a distant memory.

The lessons relevant to environmental pollution and toxicology relate to the nature of the microbial events that are now moving the iron and other elements

away from the hull into the local oceanic environment. Perhaps the most significant is that a complex of microbial consortia have, in a very obvious manner, created rusticles throughout the oxidative environments both outside and within the hull. These consortia have collectively generated bioconcretious-like materials within and upon which the consortia operate. A major net outcome of this is the export of iron from the steel via the rusticles into the oceanic environment as red dust and yellow biocolloids. Within these particles are contained the biologically extracted iron and other metallic cations that are primarily bioaccumulated within the complex of polymeric structures which surround the microbial cells forming the consortium. Had the rusticles not been so obvious, then the condition would have been very similar to the biofouling that occurs in and around water wells where it is not possible to view these rusticle-like structures formed by the consortia.

In bioremediation work, there is still the mindset that every function has to be performed by a single species and not by a consortium. The outcome of this lateral thinking from the diagnosis of acute diseases is that most biologically driven events are thought to be a direct result of the activity of a single species of microbe. As a result, much effort is presently being devoted to the molecular-level determination of very particular species that are considered to be totally responsible for the problem or solution being managed. No consideration is given to the potential for one or more consortia to be effectively driving the problem or the solution. In the next two or three decades, the prime lesson from the environmental impacts of the *RMS Titanic* on the oceanic environment will be that the biological activities observed have been totally driven by microbial consortia. In these coming decades, the role of these sometimes covert consortia impacting in many environments, including the human body, will need to be addressed and recognized if a more mature management of environmental resources and remediation is to be achieved. Today, the frontiers are moving away from the *RMS Titanic* to the plugging of oil wells, the curing of concrete, the nature and diagnosis of chronic diseases, the rehabilitation of plugged water wells, and the extreme adaptability of these microbial consortia to extreme environments both on this planet and elsewhere.

Summary

The *RMS Titanic* sank in 1912 and created a historical event that still ripples through time. Stories were told and lessons learned but the science has only just begun. Today the fading remains of the ship resemble the hanging gardens of Babylon except that it is not plants that drape the walls but complex microbial growths called rusticles. These organisms have been found to be not a species, like plants and animals, but to be structures created by complex communities of bacterial species. Like the discovery of tube worms in the mid-oceanic vents, the nature of these rusticles presents another biological discovery of a fundamental nature. Essentially these microbial consortia on the *RMS Titanic* have generated structures of a mass that would rival whales and elephants while grad-

ually extracting the iron from the steel. Rusticle-like consortia appear to play many roles within the environment, and it is perhaps the *RMS Titanic* that is showing that there is a new way to understand the form, function, and nature of microorganisms. This understanding would develop by considering the bacteria not as individual species functioning independently but as consortia of species functioning in community structures within a common habitat. This concept, if adopted, would change dramatically the manner in which a microbial ecologist and any scientist or engineer would view the occurrence of a slime, encrustation, biocolloid, rust flake, iron pan, salt deposit, and perhaps even some of the diseases that remain unexplained as a disease of unknown cause.

Acknowledgments

References

Alford GA, Cullimore DR, Monea M, Ostryzniuk N (2001) Device for the Blended Cathodic Disruption of Biofouled Sites. U.S. Patent Pending.

Ballard R (1987) The Discovery of the Titanic. Warner Books, New York.

Cullimore DR (1993) Practical Manual of Groundwater Microbiology. CRC Press, Boca Raton, FL.

Cullimore DR (1999) IV Titanic: the connection between rusticles and clogging. In: Alford, GA. Cullimore R (eds) The Application of Heat and Chemicals in the Control of Biofouling Events in Wells. Lewis, Boca Raton, FL.

Cullimore DR (2000a) Microbiology of Well Biofouling. Lewis, Boca Raton, FL.

Cullimore DR (2000b) Consortial bacterial forms. In: Practical Atlas for Bacterial Classification. Lewis, Boca Raton, FL, pg. 131–135.

Cullimore DR, Alford GA (1990) Method and apparatus producing analytic culture. U.S. Patent 4,906,566.

Cullimore DR, Johnston L (2000a) The impact of bioconcretious structures (rusticles) on the *RMS Titanic*: implications to maritime steel structures. In: Proceedings of the Annual Meeting of The Society of Naval Architects and Marine Engineers, Vancouver, British Columbia, October 2000, paper 9.

Cullimore DR, Johnston L (2000b) Biodeterioration of the *RMS Titanic*. Canadian Chemical News, November/December. The Chemical Institute of Canada, Ottawa, Canada.

Davis DG (1989) Helictite bushes, a subaqueous speleothem? Natl Speleol Soc Bull, 51: 120–124.

Davis DG, Palmer MV, Palmer AN (1990) Extraordinary subaqueous speleothems in Lechuguilla Cave, New Mexico. Natl Speleol Soc Bull, 52:70 B86.

Droycon Bioconcepts Incorporated (1999) BART™ Biological activity reaction test user manual. Droycon, Saskatchewan.

Ewald PW (2000) Plaque Time: How Stealth Infections Cause Cancers, Heart Disease, and Other Deadly Ailments. Simon and Schuster, New York.

Garzke WH, Brown DK, Matthias PK, Cullimore R, Wood D, Livingstone D, Leighley HP, Foecke T, Sandiford A (1997) *Titanic*, The Anatomy of a Disaster. Report from the marine forensic panel (SD-7). Proceedings of the 1997 Annual Meeting of the Society of Naval Architects and Marine Engineers, Ottawa, Canada.

Gerhules J, Alford A (1990) Application of physico-chemical treatment techniques to a severely biofouled community well in Ontario, Canada. In: Howsam P (ed) Water Wells Monitoring, Management, Rehabilitation. Spon, London.

The Harvard Classics edited by Charles W. Eliot, LL.D.1 P. F. Collier & Son Corporation, New York, 1969.

Hauley B (2000) Safety charter targets old tankers, flags of convenience. Maritime Wkly Maritime Report Eng News (March).

LaRock EJ, Cunningham KI (1995) Helictite bush formation and aquifer cooling in Wind Cave National Park, South Dakota. Natl Speleol Soc Bull, 57:43–51.

Ma K-T, Orisamolu IR, Bea RG, Huang RT (1997) Towards optimal inspection strategies for fatigue and corrosion damage. In: Proceedings of the 1997 Annual Meeting of the Society of Naval Architects and Marine Engineers, Ottawa, Canada, SD-7.

Mann H (1997) A close-up look at Titanic's rusticles. Voyage 25 47–48 pp.

Mansour AE, Wirsching PH, Lucket MD, Plumpton AM, Lin YH (1997) Structural safety of ships. In: Proceedings of the 1997 Annual Meeting of the Society of Naval Architects and Marine Engineers, Ottawa, Canada, SD-7.

Marcus Aurelius (1969a) The Meditations of Marcus Aurelius (trans. G Long) Book V, observation 30, pg. 230. The Harvard Classics edited by Charles W. Eliot, LL.D. P. F. Collier & Son Corporation, New York, 1969.

Marcus Aurelius (1969b) The Meditations of Marcus Aurelius (trans. G Long) Book XI, observation 27, pg. 293. The Harvard Classics edited by Charles W. Eliot, LL.D. P. F. Collier & Son Corporation, New York, 1969.

Pellegrino C (2000) Ghosts of the Titanic. Harper Morrow, New York.

Pellegrino C, Cullimore DR (1997) A study of the bioarcheology of a physically disrupted sunken vessel. Voyage 25 39–46 pp.

Sly LI (1995) Microorganisms, an essential component of biological diversity. Can Soc Microbiol Newsl 42(2):7–10.

Straus E (1997) Mob action, peer pressure in the bacterial world. Sci News 152:124–125.

Wells W, Mann H (1997) Microbiology and formation of rusticles from the *RMS Titanic*. Resour Environ Biotechnol 1:271–281.