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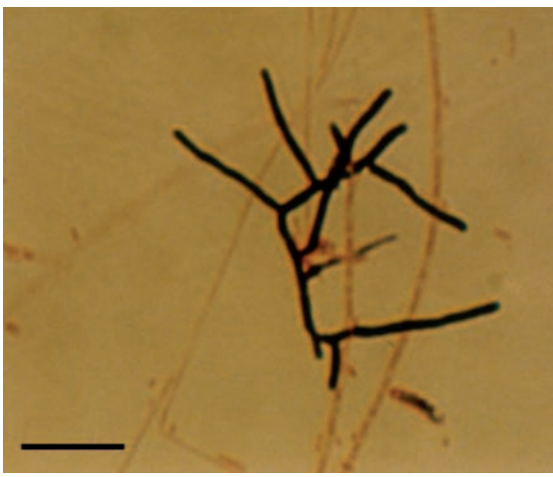
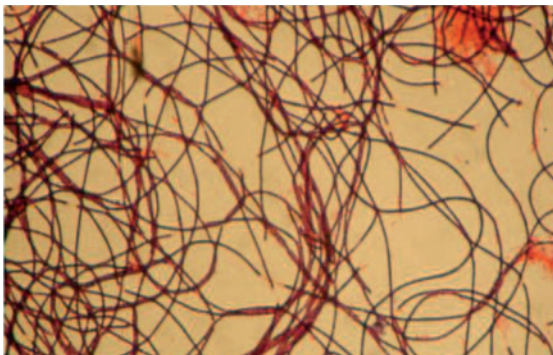
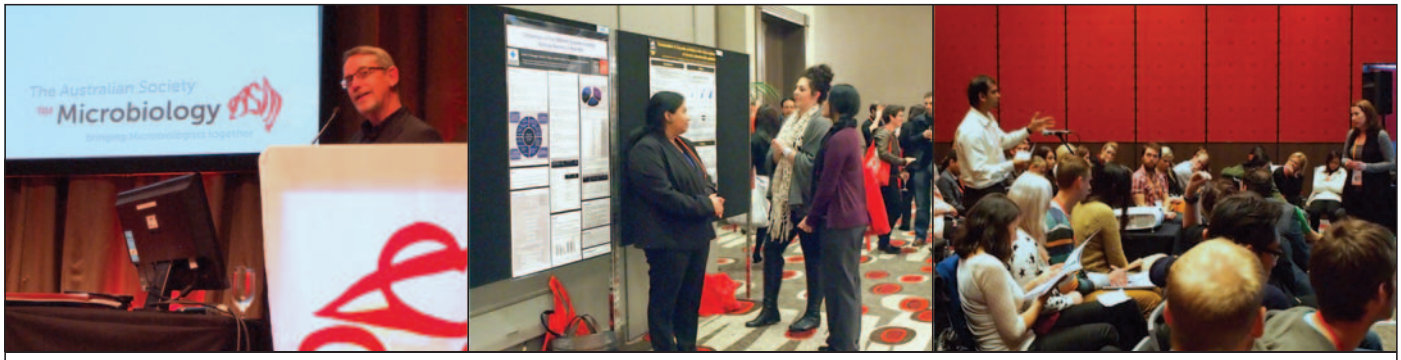
Microbial biodeterioration and biodegradation

A special issue in association with the Russian Microbiological Society

МЕЖРЕГИОНАЛЬНОЕ МИКРОБИОЛОГИЧЕСКОЕ ОБЩЕСТВО







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Dena Lyras
President of ASM

This is my first Vertical Transmission posting as the new President of our Society, after having served as the Vice-President Scientific Affairs since 2014. I would like to thank the Executive Committee and the ASM Council for being confident in my abilities to serve in this important role. I thank Roy Robins-Browne for the wonderful leadership he provided over the last two years, and I am grateful that he will continue to support and guide me and the ASM over the coming year.

Several changes in people holding key ASM roles have recently occurred. Jonathan Iredell has stepped away from the Executive Committee. Kate Seib has replaced me as Vice-President Scientific Affairs and Rebecca LeBard has taken over from Jack Wang as Vice-President Communications. Anthony Baker is serving as Vice-President Elect Corporate Affairs. I would like to say a very big thank you to Jonathan Iredell and Jack Wang, and acknowledge the impact of their involvement in ASM – both helped to move the Society forward successfully during a time of difficult change.

I also want to acknowledge and thank Stephen Graves for his major contribution to ASM and Council. Stephen is stepping down from his role as Chair of the Clinical Microbiology Standing Committee, a position he has held for many years. He will be succeeded by Jonathan Iredell who, as a former President of the Society and clinician, will ensure that Clinical Microbiology is well looked after.

Our recent Annual Scientific Meeting in Brisbane was a great success. We have changed the style and format of our annual meetings to increase speaking opportunities, particularly for our early and mid-career members, and time for networking. We invited 72 national speakers for symposia at this meeting, and provided 72 speaking opportunities for those who submitted abstracts. The invited plenary speakers were excellent and inspirational. The various specialised workshops that were scheduled throughout the meeting were diverse and well attended. In particular, our student and early career day was a tremendous success and showcased our Nancy Millis Student Award speakers, and our poster sessions were a social and scientific success.

The Local Organising and Scientific Program Committees, chaired by Kate Seib and Nick West, respectively, deserve our grateful thanks and warm congratulations on a valuable, enjoyable and stimulating conference that showcased the wonderful world of Australian microbiology. Another excellent Annual Scientific Meeting is planned for Adelaide next year from 30 June to 3 July 2019 – please add the dates to your diary.

The focus and activities of the ASM have changed in recent years, in parallel with the rapid changes in science and microbiology that have occurred – we have embraced these changing times. We have set up or aligned ourselves with several specialist meetings, have provided more financial support for our members to attend our annual meeting, and have increased the number and range of our awards. We are committed to inclusion and promoting diversity, and our 2018 meeting embraced these ideals. I will keep you updated on the positive changes we are making, and welcome your input and ideas on how we can provide what our members need from the Society.

Finally, please visit our website: www.theasm.org.au to see a list and photos of our award winners for 2018, as well as information regarding upcoming meetings, awards, and, for those who may be interested, our financial statements and minutes of recent meetings. You may also like to follow, and contribute to ASM on Twitter, @AUSSOCMIC, or on Facebook to make sure you keep up with the latest news, trends and developments in microbiology in Australia and around the world.

Elizaveta Bonch-Osmolovskaya

President of the Russian Microbiological Society

The Russian Microbiological Society has had a long and complicated history, reflecting the recent history of our country. It was established in 1957, in the Soviet Union, as the *All-Union Microbiological Society* and in the mid-80s comprised more than 5000 members. Several distinguished Russian microbiologists were its Presidents: the last one was Professor Elena Kondrat'eva, famous for her investigations of bacterial photosynthesis. However, the Society did not survive all the changes of the 90s, and, in spite of an attempt to revive it in 1992, did not exist until 2003. At that time, it was organised again as *Russian (Interregional) Microbiological Society* by a group of microbiologists, Professor Valery Gal'chenko being its President from 2004 to 2016. During these years the situation in the Russian scientific community changed significantly in comparison with the 90s: governmental funding grew, young and well qualified people came to microbiology, eager not only to isolate and characterise new microorganisms, but to apply genomic and metagenomic analyses to microbial metabolism and ecology. Though incorporated into the world of science, they needed also an internal platform for communications and navigation in our rapidly developing and transforming scientific field. That made us initiate radical changes in the structure and activities of our Society.

Since 2016, Professor Elizaveta Bonch-Osmolovskaya is the President of the Russian Microbiological Society, and Professor Irina Ivshina is the Vice-President. The Secretary of the Society is Dr Anna Perevalova. The Board includes well-known microbiologists from different parts of Russia, with the complementary fields of expertise: Valery Gal'chenko, Alexander Netrusov, Marina Donova, Olga Karnachuk, Olga Il'inskaya, Nikolai Pimenov, Nikolai Ravin, Olga Turkovsjaya, Darima Barkhutva, Natal'ya Kolotilova. The Society consists of 17 regional branches, and the board is in constant connection with the Chairs of the branches. Current activities of the Society can be followed via its internet site (http://microbiosociety.ru/). The main version of the website is in Russian, but it contains links to the homepages of international organisations and events, as well as the references to the latest news, both in the world and Russian microbiology. A special page is devoted to the history of Russian microbiology where Dr Natal'ya Kolotilova, publishes the information about the prominent microbiologists in the Russian and Soviet era. The Society also has its webpages in most popular social networks, the easiest way to distribute valuable information among younger generations.

The Russian Microbiological Society is a member of the Federation of European Microbiological Societies (FEMS). Professor Alexander Netrusov is our delegate in FEMS, providing constant contact and exchange of information with FEMS authorities. FEMS financially contributes to the meetings we organise, both national and international. An important advantage of FEMS membership is its support to young scientists, who, due to FEMS fellowships, get an opportunity of short-term (2–3 months) visits to a European microbiology laboratory. In general, membership in FEMS gives us the feeling of belonging to an international family of microbiologists, a family where you always find necessary support and understanding.

The main activity field of the Society is the organisation of regular national meetings. Since the revival of the Society in 2003 it has organised conferences for young microbiologists that take place every fall at the *Winogradsky Institute of Microbiology*. Students and post-doctoral fellows from different parts of Russia, as well as from the former Soviet republics present their research findings and attend the lectures of leading microbiologists. The Young Scientists' conferences have become more and more popular, attracting researchers from the neighboring fields such as biotechnology, agricultural and medical sciences, and bioinformatics.

Another field of activity of the Society is the organisation of international conferences: examples include the successful International Conference Microbial Diversity (ICOMID) conferences held in 2005, 2008 and 2016 (http://eng.iegm.ru/conf/ICOMID2016.html). World experts in the fields of microbial diversity and culture collections participate in these conferences. One of the goals of these conferences is to develop international collaboration in order to maintain microbial diversity using it as a source of novel organisms and enzymes. A large-scale international project, BRIO of EC FP 7, involving the scientific groups from Russia, Belgium, Italy and Switzerland serves as an example of such fruitful collaboration. Under this project the Pan-European Rhizosphere Resources Network PERN (http://www.PERN-BRIO.eu) was developed. The Society invites international experts to deliver lectures in modern fields of microbiology and biotechnology.

The main achievement of the new Board of our Society since its election in 2016 is the organisation of the 1st Russian

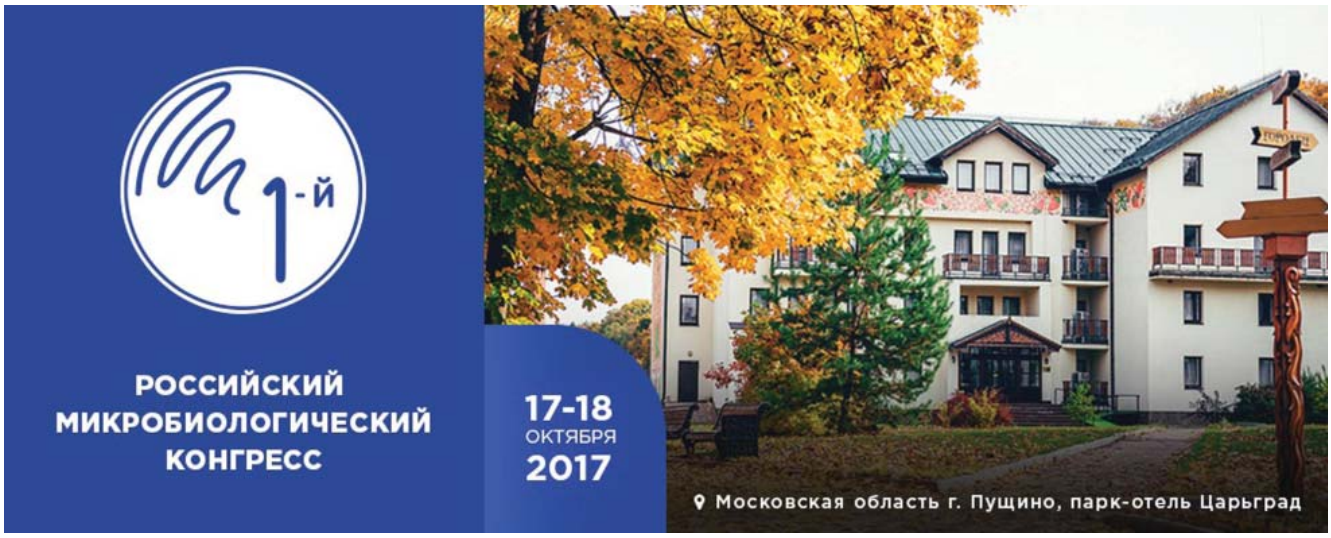

Figure 1. The logo and the announcement of the 1st Russian Microbiological Congress.

Microbiological Congress (Figure 1). Russia is a very big country, and many Russian microbiologists, though familiar to each other from articles and reviews, never met in in person. The young generation of microbiologists, though attending international meetings, never participated in a big Russian scientific event. The 1st Russian Microbiological Congress (http://congress2017.ibpm.ru/) took place on 17–18 October, 2017, in 'Tsargrad' resort near Moscow, a picturesque place on the bank of the Oka River. To organise that event, our Society cooperated with two institutions of the Russian Academy of Sciences: Institute of Biochemistry and Physiology of Microorganisms and Federal Research Center of Biotechnology. More than 300 microbiologists, including 100 young scientists, from 40 universities, institutes and companies from all over Russia participated in the Congress. We obtained generous financial support from the Russian Foundation of Basic Research, FEMS and several companies. Still, in order to keep registration fees affordable for scientists from Russian regions, we decided to keep the event to only two days. That made the schedule very tough and the selection of oral speakers, especially for section presentations, rather dramatic. Nevertheless, we succeeded in making an excellent plenary Program at a truly international level. Twelve plenary lectures were presented in three sessions: 'Ecology and Diversity of Microorganisms', 'Metabolism and Genomics of Microorganisms' and 'Microbial Technologies'. The microbial diversity remained the focus of attention of Russian microbiologists, and the lectures of Svetlana Dedysh, Dmitry Sorokin, and Olga Karnachuk contained the new data of the highest level. The two other sessions were also on a decent level showing that Russian scientists are not newcomers in 'omics' technologies and successfully combine new approaches with bright ideas characteristic for Russian school. On the second day, short oral talks of participants were presented in three parallel sections devoted to the same topics as the plenary sessions. More than 150 posters were presented in the afternoon on the Poster session. The participants, both experienced and young, took active part in the discussions, enjoyed meeting old and new friends, and started new collaborations.

In spite of the lack of time, we used the opportunity given by the Congress, to organise the meeting of our Society members. Among the decisions of the meeting was to make such congresses a regular event. The next one is scheduled for 2020, and then, not to overlap with FEMS Congresses, every even year.

Microbial biodeterioration and biodegradation



İpek Kurtböke, Irina Ivshina and Linda L Blackall

Microorganisms including bacteria and fungi can use a wide variety of organic compounds as their carbon and energy sources and exploit numerous options as electron acceptors facilitating their ability to live in diverse environments. Such microbial biodegradative activities can result in the bioremediation of polluted sites or cause biodeterioration. Biodegradation and biodeterioration are closely related processes, and they often involve the same organisms, processes and materials. Biodegradation can contribute towards recycling of materials in the biosphere and prevent accumulation of undesirable wastes. They can also be used in bio-clean up processes (e.g. oil spills) as well as in bio-transformations such as photosynthetic sulfur bacteria oxidising H_2S, allowing the growth of H_2S-sensitive species. Microbially mediated metallurgy can facilitate recovery of precious metals or desulfurisation of oil. The man-made compound perchloroethylene (PCE) is a major ground water pollutant and it can be fully biodegraded by naturally occurring bacteria like *Dehalococcoides mccartyi*, which generate energy from hydrogen oxidation then reductive dehalogenation in anaerobic respiration. A current long term detrimental impact from plastic waste could be ameliorated if efficacious plastic biodegrading microbes were available. All these microbial characteristics can cleverly be exploited in environmentally friendly processes for the benefit of mankind.

On the flip side, biodeterioration can result in undesirable changes in the properties of materials and equipment due to microbially mediated degradation. Products from animals and plants are largely biodegradable while other products from other sources can be resistant to microbial biodeterioration. Glass, metals, paints, plastics, rubber, pharmaceuticals, cosmetics, and jet fuel systems can be subjected to microbial degradation and bacterial slimes can be a detrimental issue in paper mills. Biodeterioration can be costly to both manufacturer and the customers. Moreover, use of biocides can result in environmental pollution and even if the biodegradative microorganisms are eliminated their hydrolytic enzymes cannot be inactivated leading to loss of quality and strength in leather and wool products. Microbially mediated fouling can result in economic losses particularly in tropical and sub-tropical conditions. For example, wood, natural fibres, camping and sailing materials that are treated with heavy metal containing preservatives can be toxic and detrimental to human, animal and environmental health. Metal corrosion can be mediated by microbial metabolic products including sulfuric acid produced by sulfur oxidising bacteria or by bioelectrochemical phenomena. Post-harvest biodeterioration can create economic losses especially in the tropics due to higher temperatures and moisture levels in these regions. Stored agricultural materials can encourage growth of highly sporulating bacteria and result in occupational diseases such as the farmer's lung, Bagassosis, mushroom worker's lung, Humidifier Fever, and Bysinossis. Respiratory diseases resembling farmer's lung may also be found in cattle and horses exposed to mouldy hay. Microorganisms can cause an earthy-musty odour of water, and can interfere with settling of sewage in wastewater treatment plants. Although biodeterioration might be undesirable (e.g. degradation of shoe-soles) the same process can be useful for biodegradation (e.g. of discarded tyres, oil pollutants).

In this special issue, *Microbiology Australia* focuses on 'microbial biodeterioration and biodegradation' jointly with colleagues from the Russian Microbiological Society. From Russia, Elizaveta Bonch-Osmolovskaya, Alexander Elcheninov, Ksenia Zayulina and and Ilya Kublanov describe new thermophilic microorganisms with

hydrolytic activities, Marina Donova's article involves novel microbially mediated biotechnological methods for steroid production, and the article by Olga Ilinskaya, Alina Bayazitova and Galina Yakovleva covers biocorrosion of materials and sick building syndrome. The sick building syndrome is a growing concern especially in areas subjected to cyclones and severe weather events resulting in pervasive dissemination of fungi and actinomycetes. Irina Ivshina, Elena Tyumina, Elena Vikhareva report on biodegradation of emergent pollutants, particularly pharmaceuticals. Bioclean processes for environments contaminated with hazardous hydrocarbons and metals are highlighted in papers by Maria Kuyukina, Anastasia Krivoruchko, Irina Ivshina, and by Inna Solyanikova, Natalia Suzina, Ludmila Golovleva. Viktoria Shcherbakova and Olga Troshina cover the biotechnological prospects of permafrost bacteria and archaea. The President of the Russian Microbiological Society, Professor Elizaveta Bonch-Osmolovskaya, also contributes an article on the Society.

Diverse topics from Australian-based scientists include

- microbiologically induced chloramine decay by KC Bal Krishna, Maneesha Ginige, and Arumugam Sathasivan,
- actinomycetes as allergens in farm environments by Candice Brinkmann and İpek Kurtböke,
- microbial thiocyanate biodegradation by Mathew Watts and John Moreau,
- bacteriophage control of foaming in activated sludge tanks by Steve Petrovski and Robert Seviour,
- methods to test microbial corrosion by Scott Wade and Linda Blackall, and
- internal corrosion of pipelines by Laura Machuca Suarez and Anthony Polomka.

Scientists from Singapore (Enrico Marsili, Staffan Kjelleberg and Scott Rice) describe biofilms and microbially influenced corrosion.

In this historic interaction between *Microbiology Australia* and the Russian Microbiological Society we, the guest editors, salute the readers of the articles in this special issue from the four corners of the world 'From Russia and Australia with love' and finish with Louis Pasteur's words:

> *I beseech you to take interest in these sacred domains so expressively called laboratories. Ask that there be more and that they be adorned for these are the temples of the future, wealth and well-being. It is here that humanity will grow, strengthen and improve. Here, humanity will learn to read progress and individual harmony in the works of nature, while humanity's own works are all too often those of barbarism, fanaticism and destruction.*

İpek Kurtböke and Irina Ivshina together with the members of the Russian Microbiological Society and the Russian Academy of Sciences at the IVth International Conference MICROBIAL DIVERSITY (ICOMID) Conference held in Moscow 23–25 November 2016.

Biodegradation of emerging pollutants: focus on pharmaceuticals



Irina Ivshina[A,B,D], *Elena Tyumina*[B] *and Elena Vikhareva*[C]

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A priority environmental problem is pollution and disturbance of natural environments by emerging pollutants – substances of various origins and structures and with known and/or potential ecotoxic effects. One of the most dangerous groups of emerging pollutants is pharmaceutical substances due to their highly stable chemical structure and pronounced biological activity. They are found in soil, bottom sediments, surface, sewage, groundwater and drinking water. Uncontrolled release of pharmaceuticals in open ecosystems is potentially dangerous, entailing environmental consequences. Their negative impacts on living organisms are evident. This has driven the search for effective ways to neutralise persistent pollutants. In Russia, pharmaceutical pollution of the environment has commenced recently and is still presented as research with a local focus. In particular, the dynamics and metabolic mechanisms of pharma pollutants by *Rhodococcus* actinobacteria, outstanding among other microorganisms for their capacity to degrade a great diversity of degradable pollutants, are most intensively investigated. These studies are implemented at the junction of organic chemistry, molecular biology, biotechnology, and pharmacology. They include a set of interrelated fundamental tasks, such as developing drug detection methods in the cultivation media of microorganisms, elucidating the relationships between the systematic affiliation of microorganisms and their ability to degrade chemically different drug substances, as well as studying the degree of biodegradability and toxic effects of new compounds on the degrading microorganisms, and also the features of their decomposition and co-metabolism. Solving these tasks is important to enable understanding of the environmental fate of pharmaceuticals and to create prerequisites for innovative technical solutions in the advanced treatment of pharmaceutical wastewater. It is also essential for the development of environmentally safe approaches to hazardous pharmaceutical waste management.

Emerging pollutants are a new global environmental concern. The term emerging pollutants refers to natural or synthetic substances found in ecosystems. Their ecotoxic impacts on the environment and humans are already known, whereas the occurrence and environmental fate are uncontrolled or unregulated[1,2]. Emerging contaminants are not necessarily new substances. Such ecotoxicants comprise compounds long present in the environment, but now detected due to improved analytical methods. In 2016, the Norman network listed more than 1,000 most frequently reported emerging pollutants[3]. This list includes pharmaceuticals, personal

care products, pesticides, industrial and household chemicals, metals, surfactants, industrial additives and solvents.

Of particular concern are the release and accumulation of highly persistent and bioactive pharmaceutical pollutants in the environment. According to aus der Beek *et al.*[4], about 700 pharmaceuticals were found in the aquatic ecosystems of 71 countries. Pharmaceutical substances and their metabolites have been detected in soil, sediments, surface, sewage, ground and even drinking water[4–8]. Trace amounts of drugs found in bottled water is an unprecedented case[9]. Stumm-Zollinger and Fair[10], and Hignite and Azarmoff[11] first reported on pharmaceuticals in wastewater. The toxicity and biodegradability of these compounds was first discussed in the 1980s by Richardson and Bowron[12].

Since the late 1990s, pharmaceutical pollutants present in natural ecosystems have been seen as an emerging environmental problem[13]. The limited knowledge about their negative impacts on animals and humans remains the weak link. Despite the relatively low concentrations (ng/L to µg/L) of pharmaceutical pollutants in nature, their constant replenishment can lead to high permanent concentrations and stimulate negative effects on humans and the environment. The best-known case is the decline in vulture populations (*Gyps bengalensis*, *G. indicus*, *and G. tenuirostris*) in the Indian subcontinent. Toxic exposure was caused by veterinary-used diclofenac in South Asia. Birds fed on cattle carcasses medicated with the anti-inflammatory drug diclofenac died of intoxication and kidney failure[14]. Pharmaceutical pollutants can move across food chains. British scientists have found diclofenac in otter wool, indicating diclofenac contamination of aquatic ecosystems, fish and fauna, the habitat and food for these animals[15]. Several studies reported on feminisation of male fish caused by a synthetic hormone 17-α-ethinyl-estradiol in their habitat[16,17].

For most drugs detected environmentally, potential acute and chronic effects on ecosystem components have not yet been studied. Though drug influence on animals and humans is intensively studied, and reports describing impacts of widely used antipyretics and analgesics on plants have appeared[18,19], they are not sufficiently studied in ecologically relevant microorganisms. Upon contact with these xenobiotics, microorganisms detoxify them, and as principal biosphere constituents, they are sensitive to changes in the habitat. The relevant studies have recently commenced.

Pharma pollutants in ecosystems of Russia

According to aus der Beek *et al.*[4], the prevalence of antibiotics and analgesics in the environment, including non-steroidal anti-inflammatory drugs, is a typical situation for the eastern European countries and Russia.

Investigations on pharmaceuticals and their metabolites in wastewater and surface waters in Russia are few and concentrated mainly in the Central and North-Western regions. Table 1 summarises data on pharmaceutical occurrences and concentrations detected in aqueous samples. The average concentrations of pharmaceuticals found in surface water, untreated wastewater, and treated wastewater were 136, 360 and 181ng/L, respectively.

Pharmaceuticals-related studies of aquatic ecosystems and bottom sediments in the Northwest region detected a number of over-the-counter medications, including the psychostimulant caffeine, anti-inflammatories ketoprofen and diclofenac, and antispasmodic drotaverine hydrochloride[20].

Recent studies under the project 'Implementation of the Baltic Sea Action Plan in Russia' (BASE) detected 20 pharmaceuticals in the wastewater of St. Petersburg, though the initial targets were only diclofenac and ethinylestradiol as the most cosmopolitan pharma pollutants[6]. The effluent contained diclofenac ranging from 355 ng/L in summer to 550 ng/L in winter. The researchers calculated the predicted environmental concentration (PEC_{river}) in the Neva River to be *circa* 5 ng/L. At the same time, a significantly increased diclofenac concentration in the wastewater effluent was found compared to that in the influent. This phenomenon is apparently explained by the release of conjugated diclofenac metabolites during secondary wastewater treatment. The ethinylestradiol concentration was 0.4 ng/L.

Sampling performed at water intakes and reservoirs (Moscow region) revealed 105 pharmaceuticals and their residues[21]. Diclofenac (0.025–0.35 ng/L), caffeine (26 ng/L) and tetracycline (0.662 ng/L) were most frequently detected. Considering the potential risks of pharmaceutical pollutants for living organisms, the authors used the PASS program (Prediction of Activity Spectra for Substances) tailored to simulate the drug toxicity. According to the structural formula of an organic compound the PASS program estimates its probable biological activity[21]. They predicted the possible toxic effects (embryotoxicity, carcinogenicity, mutagenicity, etc.) on living organisms and developed an ecotoxicological map of some Moscow aquatic ecosystems.

Because of the limited knowledge on the topic of this review, the priority research in Russia is still focused on environmental detection and identification of pharmaceuticals, their effective analyses in wastewater, and clinical trials of low drug concentrations against

Table 1. Pharmaceuticals detected in the environment (in ng/L) in Russia.

Therapeutic group	Pharmaceuticals	Source	Concentration	Reference
Antibiotic	Amoxicillin	Wastewater (influent)	525	6
	Ampicillin	Wastewater (influent)	32	6
		Sediments	0.005[A]	21
	Azithromycin	Wastewater (influent)	332	6
	Ciprofloxacin	Surface water (lake)	271	20
		Wastewater (influent)	871	6
	Clarithromycin	Wastewater (influent)	230	6
	Norfloxacin	Wastewater (influent)	502	6
	Tetracycline	Wastewater (influent)	124	6
		Surface water (storage lake)	6.62	21
	Triclosan	Sediments	500–23 600[A]	20
	Trimethoprim	Wastewater (influent)	457	6
	Erythromycin	Wastewater (influent)	216	6
Analgesic	Codeine	Wastewater (influent)	191	6
Antihistamine	Ranitidine	Wastewater (influent)	252	6
Lipid-lowering	Bezafibrate	Wastewater (influent)	48	6
Anticancer	12-Methyl-tridecanoic acid	Sediments	38	21
Antiepileptic	Carbamazepine	Wastewater (influent)	76	6
Psychoactive	Caffeine	Surface water (river)	3.8–446	20
		Surface water (river)	26	21
		Sediments	27[A]	21
NSAIDs	Ketoprofen	Surface water (river)	260	20
		Wastewater (influent)	756	6
	Diclofenac	Surface water (river)	270	20
		Wastewater (effluent)	355–550	6
		Wastewater (effluent)	0.025–0.35	21
		Surface water (storage lake)	0.025	21
Antihypertensive	Enalapril	Wastewater (influent)	611	6
	Enalaprilat	Wastewater (influent)	461	6
Antiplasmodic	Drotaverine	Surface water (lake)	36.1–41.1	20
		Wastewater (influent)	452	6
Hormone	Ethinylestradiol	Wastewater (effluent)	0.4	6

[A]Concentrations are indicated in ng/kg.

humans and other living organisms, including environmentally relevant microorganisms. The latter are capable of pharmaceutical pollutant detoxification in natural ecosystems.

Biodegradation of pharma pollutants

The role of microorganisms role in the environmental degradation of xenobiotics is pivotal. Of those involved in water and soil ecosystem self-cleaning processes, *Rhodococcus* actinobacteria exhibit nonspecific enzymatic actions. They are first to attack compounds novel for microbial cells. In recent years, rhodococci are often considered as promising biodegraders and biotransformers of various xenobiotics. *Rhodococcus*' ecological versatility and exceptional polyfunctionality, high catalytic activity in extreme environments, and biodegradation of organic compounds from many known classes clearly indicate the suitability of rhodococci for degradation of pharmaceutical pollutants[22,23].

Biocatalysis activity studies revealed *Rhodococcus*' ability to decompose chemically diverse pharmaceuticals. Gauthier *et al*.[24] used *R. rhodochrous* ATCC 13808 to biodegrade heterocyclic nitrogen-containing pharmaceuticals, such as sulfamethisole (43.4 mg/L), sulfamethoxazole (32 mg/L), and carbamazepine (9.5 mg/L). These compounds were biodegraded only in the presence of glucose. In co-metabolic conditions, biodegradations of sulfamethisole, sulfamethoxazole and carbamazepine were 14, 20, and 15%, respectively. Each drug biodegradation process did not exceed 36 days and metabolites formed were unstable.

Yoshimoto *et al*.[25] studied rhodococcal interactions with steroid compounds (specifically17β-estradiol). *R. zopfii* Y 50158 degraded this pharmaceutical in the presence of glucose within 24 hours.

R. erythropolis, *R. equi* and *R. rhodochrous* were examined as potential 17α-ethinylestradiol (1.4 mg/L) biodegraders, with *R. erythropolis* being the most active. 17α-ethinylestradiol depletion as the sole carbon and energy source was 10% after 75 hours, and 47% in the presence of glucose within 13 hours[26]. According Larcher and Yargeau[27], *R. rhodochrous* ATCC 13808 completely utilised 17α-ethinylestradiol (5 mg/L) after 48 hours. Complete testosterone (1 mg/ml) decomposition was achieved with resting cells of *R. equi* ATCC 1488728 within 2 days[28]. Biological testosterone degradation proceeded in two stages: formation of 9α,17β-dihydroxyandrost-4-en-3-one and androst-4-ene-3,17-dione further converted into 9α-hydroxyandrost-4-ene-3,17-dione and 9α-hydroxy-1,4-adrostadien-3,17-dione, respectively. The final products contained 3-hydroxy-9,10-secoandrosta-1,3,5(10)-triene-9,17-dione (3-HSA) further degraded to CO_2 and H_2O[28]. There are also studies published on *Rhodococcus* biodegradation of ester-based drugs. Possible clofibric acid biodegradation to clofibrate using *R. rhodochrous* was shown[29].

The authors of this review employed resources of the Regional Specialized Collection of Alkanotrophic Microorganisms (acronym IEGM, WDCM 768, http://www.iegmcol.ru)[30] to investigate biodegradation of pharmaceutical pollutants typically found in ecosystems and widely used in Russia, such as non-steroidal anti-inflammatory drugs (phenylacetic acid derivatives diclofenac and ibuprofen), spasmolytic (an isoquinoline derivative drotaverine or No-Spa)[31,32], analgesic (a phenol group-containing *n*-acetaminophen or paracetamol)[33]. To elucidate biodegradation mechanisms, the genomes of environmentally significant *R. erythropolis* IEGM 267 and *R. ruber* IEGM 231, which actively biodegrade a variety of complex organic compounds, were sequenced[34]. Comparative bioinformatic analysis applied to sequencing data allowed analysis of functional genes, which control the pharma pollutant biodegradation. Kinetics and decomposition patterns of pharma pollutants were studied depending on physiological state of biodegraders (growing, washed, immobilised, and dormant bacterial cells) and their culture conditions (mineral composition, aeration rate and acidity, temperature, initial ecotoxicant concentrations, and selection of effective co-substrates). Regulation mechanisms (induction, inhibition) of *Rhodococcus* catalytic activity towards pharmaceutical pollutants were elucidated. Less hazardous metabolites were identified. The main biotransformation pathways of the parent ecotoxicants were determined. Features of rhodococcal interaction with pharmaceutical pollutants were studied[32]. The pharmaceutical biodegradation process was most significantly enhanced with immobilised rhodococci[31]. The wooden waste available in the Perm region was used as carrier material. To enhance the adsorbent surface affinity to bacterial cells, the carrier was treated with selected hydrophobisers[35]. Stable polyfunctional biocatalytic systems based on immobilised *Rhodococcus* cells were developed. They increased the rate of the biodegradation processes of pharmaceutical pollutants and their metabolites. Additionally, they are characterised by high functional stability over 3–8-month storage and are reusable.

The research results indicate that *Rhodococcus* actinobacteria may act as important bio-oxidants of chemically diverse pharma pollutants typically detected in the environment. The experimental data on mechanisms and tentative detoxification and bioconversion pathways of pharma pollutants together with biochemically and catalytically characterised biodegrader strains may offer new environmentally safe methods for hazardous pharmaceutical waste management. Another practical application is to use actinobacteria-based biodegraders as a suitable model to study the novel pathways

of drug metabolism that allows prediction of metabolites expected from degradation of closely related pharmaceutical pollutants.

Conclusion

There is a rapid growth in pharmaceuticals and novel pharmaceutical agents on the market. This, combined with their exponentially increasing annual consumption, only partial removal of pharmaceuticals and their metabolites in wastewater treatment, and a lack of highly effective disposal methods for hazardous pharmaceutical wastes contribute to the dramatically growing pharmaceutical pollution of the biosphere and an imbalance in natural ecosystems. Society only recently realised the real scope of the imminent danger and the urgent need to work out ways to deal with the pharmaceutical pollution challenge. Environmental disamenities stimulate novel technical solutions on how to mitigate the environmental burden and to assess environmental risks due to possible pharmaceutical pollutant exposure. They force us to seek detoxification and removal methods of these anthropogenic toxicants from aquatic and terrestrial ecosystems to reduce and even exclude the problem completely in future.

In Russia, such studies are still at the stage of intensive accumulation of factual data on both the expansion and analysis of pharmaceutical pollution of the environment (principally water bodies) and drug conversion by microbes. Attempts are being made to propose a set of measures reducing environmental risks associated with drug pollution. However, it will take years of research to evolve fundamentally new, experimentally based solutions that require large investment, and more deliberate strategies to prevent drug release into the environment.

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New thermophilic prokaryotes with hydrolytic activities




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Thermophilic microorganisms are capable of growing on polymeric substrates and have been intensively studied for their enzymes, thermostable hydrolases (glycosidases, proteinases, lipases), which have important applications in many fields of bioindustry: production of detergents, food processing, paper and textile industry, biofuel formation

from organic wastes, etc.[1]. The advantages of thermostable enzymes application are in their higher stability not only against temperature, but also against high or low pH, presence of detergents, etc. High temperature increases solubility of substrates[2], thus making them more available, and significantly decreases the contamination risks. Many highly stable hydrolases, produced by thermophilic bacteria and archaea have been discovered[3–6]; however, due to continuous industrial demand and our knowledge that natural environments are a significant reservoir of genetic and hence functional diversity[7], new thermophilic organisms producing hydrolytic enzymes are still of high interest. Here we present our achievements in isolation of novel thermophilic bacteria and archaea with various hydrolytic activities.

The representative of a new bacterial phylum *Ignavibacteriae* – *Melioribacter roseus* was isolated from the microbial biofilm developing at the outlet of an abandoned oil-exploration well in Western Siberia[8]. The well is 2750 m deep, reaching organic-rich Jurassic deposits, the so called Bazhenov Platform. In the water of the well diverse organotrophic thermophiles were found to be present which presumably degrade buried organic matter[9]. When recovered, those capable of growth by aerobic respiration formed a biofilm, degrading dissolved organic matter present in the water. *Melioribacter roseus* was found both in the well water and in biofilms. It is, to our knowledge, the first thermophilic facultatively anaerobic bacterium capable of degrading cellulose and its derivatives. The genome of *M. roseus* contains numerous genes of various glycosidases, glycosyl transferases, carbohydrate esterases and polysaccharide lyases that correlate with its ability to utilise diverse polysaccharides[10]. Some of these genes were heterologously expressed in *Escherichia coli* and characterised[11,12]. Due to the presence of numerous terminal oxidases, *M. roseus* can grow by aerobic or anaerobic respiration with nitrate, ferric iron, or arsenate as electron acceptors[8,13]. It is capable of utilising acetate and performing complete mineralisation of complex organic substrates in anaerobic conditions (for example, by iron respiration). The capacity to use various substrates including insoluble or poorly soluble polysaccharides and a number of electron acceptors including insoluble ones makes *M. roseus* an excellent candidate for utilisation in microbial fuel cells where the energy of organic wastes is converted to electricity.

Planctomycetes is a large and environmentally relevant group of bacteria found in almost all types of ecosystems. It is known that planctomycetes (with uncultivated anammox bacteria as an exception) perform aerobic degradation of organic matter including complex natural compounds such as polysaccharides and proteins, and have complex cell organisation, multistage life cycle and large genomes[14]. Sequences of 16S rRNA genes belonging to planctomycetes were often found in DNA samples from thermal environments, but no thermophilic planctomycetes were described until now. We isolated novel planctomycetes from the hot springs of Kuril Islands and Lake Baikal area, shallow water submarine vents of Italy, and a deep subsurface gold mine in South Africa[15–17]. Three new isolates, *Thermogutta terrifontis, Termogutta hypogea* and *Thermostilla marina*, multiply by budding and belong to class *Planctomycetia*, while the fourth, *Tepidisphaera mucosa*, multiplies by binary fusion and forms a new order *Tepidisphaerales* in class *Phycisphaerae*. Thus, at the moment thermophilic species are known for both classes of cultivated planctomycetes. All isolates are moderate thermophiles (growing optimally at 50–60°C) and facultative anaerobes (capable of growth by fermentation (all), nitrate or sulfur reduction (all excluding *T. mucosa*)). As mesophilic planctomycetes, thermophilic members of this group are able to grow on numerous polysaccharides, including most resistant ones (such as xanthan gum, xylan, etc). The genome of *T. terrifontis* R1 was found to contain genes encoding diverse hydrolases and lyases (our unpublished data). A thermostable esterase from *T. terrifontis* was heterologously expressed in *Escherichia coli* and characterised[3].

Most cultivated representatives of organotrophic hyperthermophilic and thermophilic archaea of two well-studied phyla, *Crenarchaeota* and *Euryarchaeota*, can grow with peptides and proteinaceous substrates[18], but much less of them are able to degrade polysaccharides. 16S rRNA genes analysis in *in situ* enrichments set in Kamchatka hot springs[19], showed that archaea efficiently compete with bacteria for polymeric substrates even at up to 70°C, temperatures considered favourable for bacteria. Similar archaeal 16S rRNA gene sequences were found in numerous Kamchatka hot springs[20] and helped to isolate a themophilic archaeon *Fervidicoccus fontis*, the first representative of a novel order *Fervidicoccales* in phylum *Crenarchaeota*[21,22]. Among terrestrial hyperthermophiles, the ability to grow on polymeric substrates is a specific feature of the representatives of the archaeal genera *Desulfurococcus* and *Thermogladius*, which belong to phylum *Crenarchaeota*. Though preferring peptides, some of these archaea can also utilise polysaccharides: different strains of *Desulfurococcus amylolyticus* grow on starch or cellulose[23–26]. The ability to grow on cellulose was also shown for *Thermogladius calderae*[27]. However, it should be noted that the genome analysis of the cellulotrophic *D. amylolyticus* strain (formerly known as *D. fermentans*) and *T. calderae* (formerly known as *T. cellulolyticus*) did not reveal any exo- or endoglucanases genes of known families[28,29].

Alfa- and beta-keratins are highly resistant proteins of animal fur or bird feathers, respectively. The ability to grow on keratins was found in some thermophilic bacteria[30]. We found that hyperthermophilic archaea of genera *Desulfurococcus* and *Thermogladius* were able to grow on keratins, completely degrading them at 85°C[31]. Zymography of *Desulfurococcus* spp. grown on keratin, showed the presence of the endopeptidases, attached to the cell surface.

A hyperthermophilic representative of the phylum *Euryarchaeota* – *Thermococcus sibiricus* was isolated from a high-temperature oil reservoir in Western Siberia[32,33]. While initially *Thermococcus sibiricus* was described as a peptolytic microorganism, the additional experimental tests showed its ability to grow on numerous polysaccharides. Its genome analysis showed the presence of various glycosidase genes, some of which were located in the gene island, presumably horizontally transferred from a hyperthermophilic bacteria of phylum *Thermotogae*[34]. It was assumed that this organism was buried with the deposits of Jurassic ocean and continued living in a deep subsurface geothermally heated environment slowly degrading energy-rich polymeric substrates of deposited organic residues.

An outstanding representative of the same genus, *Thermococcus* sp. strain 2319x1, was isolated from the hot vent in intertidal zone of Kunashir Island (Southern Kurils). This organism was able to grow efficiently on numerous substrates, including polysaccharides, like amorphous cellulose, carboxymethyl cellulose, xylan, xyloglucan, lichenan, alginate and amorphous chitin. Its genome analysis showed the presence of a unique multidomain glycosidase[5], consisting of three glycoside hydrolase (GH) domains and two carbohydrate-binding modules (CBM) with the domain order GH5-12-12-CBM2-2. The full-length gene, as well as its truncated versions, was heterologously expressed in *E. coli*. The analysis of activity of the complete multidomain glycosidase (MDG) and its truncated versions has shown a vast number of substrates differing in polymerisation degree, type of bond and type of monomers.

Representatives of genus *Thermococcus* are, perhaps, the most easily cultivated hyperthermophilic archaea: the number of species in this genus at present exceeds 30. Most *Thermococcus* species were isolated as peptolytic organisms: they grow on hydrolysed proteins, fermenting peptides with elemental sulfur as the electron acceptor, stimulating the growth by avoiding the inhibitory effect of molecular hydrogen formed in the course of fermentation. The habitats of *T. sibiricus* and *Thermococcus* sp. 2319x1 are characterised by the presence of organic substrates of marine origin, ancient in the first case and modern in the second: dead masses of marine alga. That explains the outstanding hydrolytic capacities of these organisms.

Thermostable enzymes from new thermophilic bacteria and archaea, some of which were represented above, could be used in various fields of biotechnology, such as food and biofuel industry (glycosidases), or poultry wastes recovery (keratinases).

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Microbiotechnologies for steroid production

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Structural modification of steroids by microorganisms, known since the 1950s, is nowadays a base for industrial production of many steroid hormones and their high-value precursors. Phytosterols, renewable biomaterials of plant origin, are recognised now as most attractive, low-cost and available raw materials for the pharmaceutical industry.

Microbial technologies have been developed for production of value-added androstane steroids – androstenedione, androstadienedione, 9α-hydroxy androstenedione in a single biotech stage from phytosterol to yield over 30% (w/w) of the high-purity crystalline products. The bio-processes are based on the activity of selected mycobacterial strains (*Mycobacterium neoaurum* VKM Ac-2015D, 1816D, *Mycobacterium* sp. VKM Ac-1817D) capable of performing cascade reactions of the selective sterol side chain degradation and steroid core oxidation, which are the parts of sterol catabolic pathway.

New generation microbial technologies are based on the application of the engineered strains in combination with new approaches for the enhancement of hydrophobic steroid bioconversions. Heterologous expression of eukaryotic steroidogenesis genes in saprophyte mycobacteria allowed single-step biotechnological production of the valued steroid hormones such as testosterone, boldenone and progesterone from phytosterols. Novel manufacture schemes based on the one-pot two stage microbial conversions, or combined chemical-microbiological syntheses allow more key steroid intermediates to be produced from phytosterol. A range of the biotechnologies for production of hydroxylated steroids has been developed using selected filamentous fungi capable of regio- and stereospecific hydroxylation of androstane and pregnane steroids.

The results indicate great potential of actinobacteria and filamentous fungi for steroid production. Selection of the suitable strains, metabolic engineering of steroid catabolic pathways in combination with chemical derivatisation, fungal oxyfunctionalisation of steroids and effective down-stream procedures enable creation of improved bioprocesses and effective schemes to produce a large number of high-value steroids.

Steroids (*Greek, stereos = solids*) represent a specific class of terpenoid lipids that contain in their structure gonane core of four fused cycloalkane rings (A–D) (Figure 1). The steroid superfamily includes various structures such as sterols (e.g. cholesterol, sitosterol, ergosterol), bile acids, corticoids, cardiac aglycones, vitamin D, insect molting hormones, etc. These compounds fulfil essential vital functions in living organisms of the animal and plant kingdoms. They control various aspects of cell proliferation and differentiation, play a role as sex hormones in the reproduction of vertebrates, provide electrolyte and glucose homeostasis in higher organisms, regulate signal transduction pathways by the binding to the respective intracellular receptors and some of them serve also as signaling molecules in cell-cell interactions. Bile acids are extremely important for the vertebrate digestion: the so-called neurosteroids function as allosteric modulators of neurotransmitter receptors, etc.[1–5]. In eukaryotes, the hormones, bile acids and other essential steroids are produced from cholesterol, an important component of the cell membranes, playing a role in membrane fluidity, cell differentiation and proliferation.

High biological activities provide great importance to steroid preparations for medicine: more than 300 steroid therapeuticals are clinically approved[6,7]. Along with antibiotics, steroids represent the best-selling category, being a significant sector of the global pharmaceutical market and this trend is forecast to continue in the future[6,7].

Nowadays, phytosterols (**I**) that are the mixtures of plant sterols such as sitosterol, stigmasterol, campesterol and others, are recognised as most cheap and available raw materials for steroid industry. Phytosterols are structurally close to cholesterol. They are produced in huge amounts from soya, pine, or wastes from cellulose production plants. Because all steroid hormones share several common precursors, production schemes for different kinds of steroid hormones (sex, adrenocortical, anabolic etc.) include phytosterol bioconversion to C_{19}- steroids such as androstenedione (AD, II), androstadienedione (ADD, **III**), 9α-hydroxyandrostenedione (9-OH-AD, **IV**), testosterone **(XIII)** (Figure 1) and others.

Since their discovery in the early 1950s, microbial biotechnologies play a significant role in the steroid pharmaceutical industry thus replacing multistage, complicated and environmentally risky organic syntheses. Growing demand for steroid pharmaceuticals stimulates development of new cost-effective and ecologically

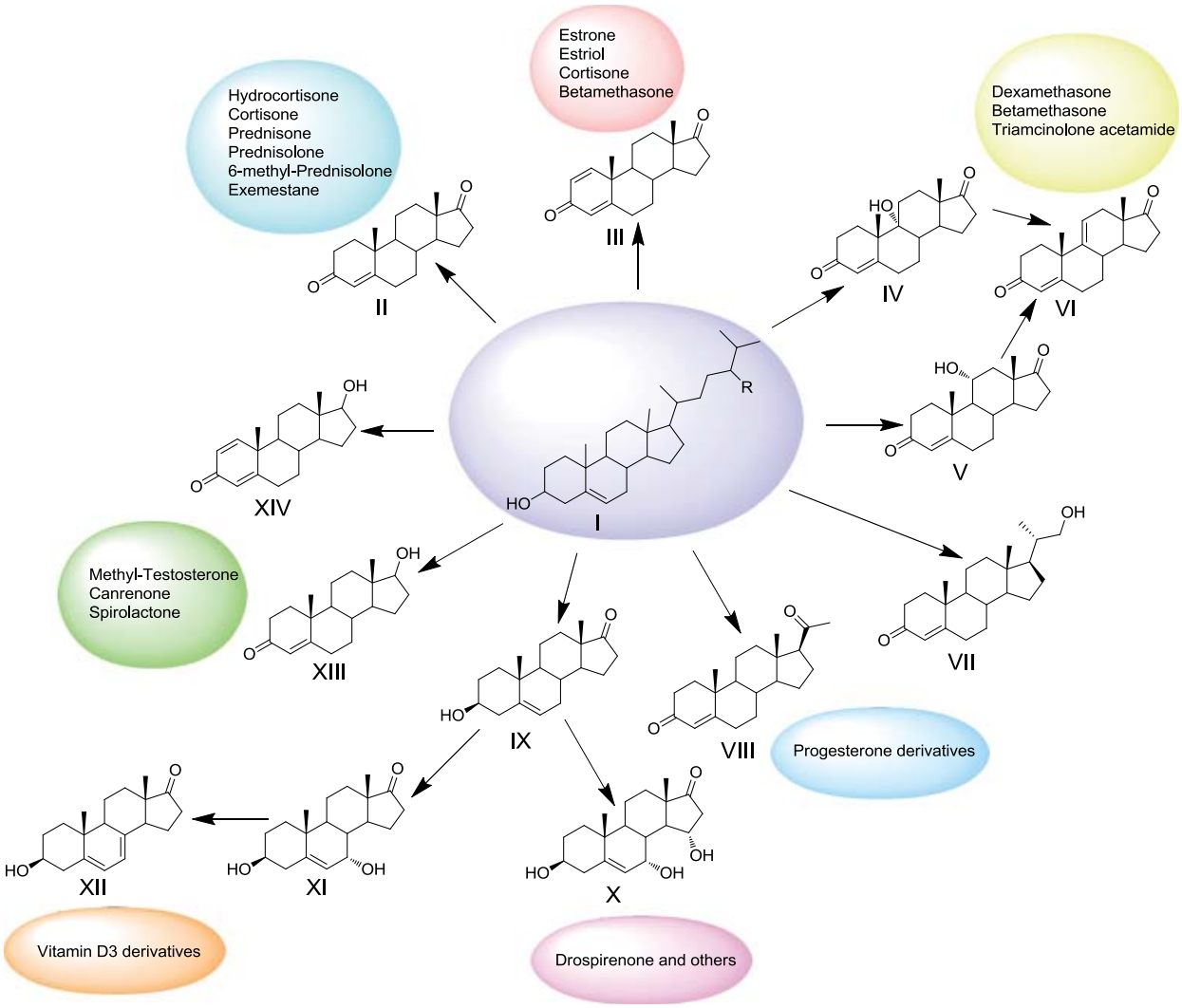

Figure 1. Valuable steroids produced from phytosterol (**I**) using microbiotechnologies, producer strains and active pharmaceutical ingredients (APIs) on their base: **II**, androst-4-ene-3,17-dione (AD) produced by *Mycobacterium neoaurum* VKM Ac-1815D; **III**, androsta-1,4-diene-3,17-dione (ADD), by *Mycobacterium neoaurum* VKM Ac-1816D; **IV**, 9α-hydroxy-androst-4-ene-3,17-dione (9-OH-AD), by *Mycobacterium* sp. VKM Ac-1817D; **V**, 11α-hydroxy-androst-4-ene-3,17-dione, by one-pot two-stage bioconversion with *M. neoaurum* VKM Ac-1815D and *Aspergillus ochraceus* VKM F-830; **VI**, androst-4,9(11)-diene-3,17-dione, by chemoenzymatic synthesis (via **IV** or **V**); **VII**, 20-hydroxymethylpregn-4-ene-3-one (20-HMP, BA), by *M. neoaurum* VKM Ac-1815D and relative strains; **VIII**, progesterone, by engineered *Mycobacterium smegmatis* mc2 155 strain; **IX**, 3β-hydroxyandrost-5-en-17-one (dehydroepiandrosterone; prasterone, DHEA), by *M. neoaurum* VKM Ac-1815D using chemical protection/deprotection of 3β-hydroxyl function; **X**, 3β,7α,15α-trihydroxyandrost-5-ene-17-one, by *Fusarium graminearum* and relative fungal strains; **XII**, 3β-hydroxy-5,7-diene-17-one, by bioconversion with 7β-hydroxylating fungal strain followed by chemical modification; **XIII**, androst-4-ene-3-one-17β-ol (testosterone), by engineered *M. neoaurum* strain; **XIV**, androst-1,4-diene-3-one-17β-ol, boldenone, *by recombinant M. neoaurum strain*.

friendly biotechnologies. Microbial steroid bioconversion may provide in one biotech stage single steroid modifications, or cascade reactions that are a part of catabolic pathways intrinsic to microorganisms, with cofactor regeneration and with mild conditions. It expands the toolbox of organic synthesis thus enabling production of both well-established and new steroid derivatives of potential biological and pharmacological activity that are otherwise inaccessible[8].

There are some data showing that steroids originated hundreds millions years ago[9], and microorganisms evolved, exposed to a variety of steroid substrates thus resulting in numerous metabolites and enzymatic activities. Natural steroid substrates serving as carbon sources have originated from plants (e.g. sitosterol, stigmasterol), or animal steroids excreted into the environment such as cholesterol, estrogens, androgens, or bile acids. Steroid microbial degradation plays a significant ecological role, being a key process for biomass decomposition, as well as removal/detoxification of steroid pollutants.

Most steroid transforming bacteria were isolated from soil, but recent metagenomic studies showed global distribution of microbial steroid degraders with the prevalence of *Actinobacteria* and *Proteobacteria* also in eukaryote hosts, aquatic environments and other habitats[10].

Most effective phytosterol degraders have been found from mycolic acid rich actinobacteria of *Corynebacterineae* suborder such as

representatives of *Mycobacterium, Rhodococcus* and *Gordonia*. It was assumed that the mycolic acid rich cell wall of these Actinobacteria may contribute to the effective transportation of lipophilic substances such as steroids.

Actinobacteria are known to catabolise phytosterol via the 9(10)-secosteroid pathway[11]. Along with degradation of the aliphatic side chain, different modifications of the steroid core occur during sterol bioconversion, such as 3β-hydroxy-5-ene to 3-keto-4-ene moiety transformation, Δ1-dehydrogenation, 9α-hydroxylation. Metabolic engineering allows overproduction of the valued steroids by exploiting the cascade reactions that are the part of the degradative pathway[12].

We have developed microbial technologies based on phytosterol biotransformation by whole-cell actinobacteria, and especially, by the selected strains of *Mycobacterium neoaurum* VKM Ac-1815D, 1816D and *Mycobacterium* sp. VKM Ac-1817D, which provide effective production of AD **(II),** ADD (**III**) and 9-OH-AD (**IV**), respectively. These androstane steroids are the key intermediates in the synthesis of various steroid drugs. The microbial technologies developed enable full phytosterol conversion at the loadings over 12 g/L to yield 60–72% (of theor.) for 96–140 h fermentations. Effective down-stream procedures provide high purity (over 96%) crystalline products and the overall yield of the target steroids is over 30% (w/w).

Based on the results of the full genome sequencing and genome-wide transcriptomic profiling the specific genes and gene clusters, which are essential for steroid modifications have been revealed[13,14]. The data were used for the generation of engineered strains with improved biocatalytic capabilities for production of AD (**II**), 20-hydroxymethyl pregn-4-ene-3-one (**VII**) and other value-added steroids (Figure 1). The recombinant strains capable of single-step converting of phytosterol to testosterone (**XIII**), 1-dehydrotestosterone (**XIV**), progesterone **(VIII)** have been generated using heterologous expression of eukaryotic steroidogenic systems in mycobacterial hosts[15,16]. Effective production of dehydroepiandrosterone (DHEA, **IX**) from phytosterol **(I)** has been provided by the combination of the chemical protection-deprotection of the oxygen functionality at C3 with selective side chain degradation of the 3-substituted sterols using *M. neoaurum* VKM Ac-1815D (Figure 1).

Unlike diverse bacteria, most fungi are not capable of full degradation of the steroid skeleton, but detoxify steroids as fungitoxic molecules by oxyfunctionalisation of the steroid core, or other reactions. These features are very important for biotechnological application since they provide effective production of clinically important hydroxylated steroids. One-pot two-stage microbial conversion of phytosterol using *M. neoaurum* VKM Ac-1815D and *Aspergillus ochraceus* VKM F-830 enable effective production of 11α-hydroxy-AD (**V**), which is a key precursor in the syntheses of halogenated corticoids[17]. Regio- and stereospecific hydroxylations of DHEA at positions 7α, 7β as well as 7α,15α-dihydroxylation using selected fungal strains enabled effective production of the valued 3β-ol-5-ene derivatives[18] (**X, XII**), thus allowing further effective schemes to drospirenone and its derivatives (Figure 1). Combination of chemical derivatisation with steroid hydroxylation by selective fungal strains allows production of the $\Delta^{5,7}$-steroids[19] (**XII**), which can be used in the syntheses of vitamin D3 derivatives. Effective procedures for the isolation and purification have been developed which provide high (>96%) purity of the final crystalline products. The number of valued products that can be produced from phytosterol using our biotechnologies and active pharmaceutical ingredients (APIs) that can be synthesised from these steroidal products are presented in Figure 1.

Thus, selection of the suitable strains, metabolic engineering of the mycobacterial phytosterol catabolic pathway in combination with chemical derivatisation, fungal oxyfunctionalisation of steroids and effective down-stream procedures enable creation of improved bioprocesses and effective production schemes for obtaining of a vast number of the high-value steroids.

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Biography

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Biocorrosion of materials and sick building syndrome



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The problem of biological damage of mineral building materials and structures based on them is multifaceted and covers all types of industry. The most destructive biocorrosion impacts are on building materials in cities with a large water area. Various types of microorganisms, including pathogens, and especially the filamentous fungi of the genera *Aspergillus*, *Penicillium*, *Trichoderma*, etc., occupy the surfaces of mineral building materials, cause their destruction, disturb the ecological balance of cities and endanger the human health. The term 'sick building

syndrome' (SBS) is used to describe a situation when the residents of a building experience acute health- or comfort-related effects that seem to be linked directly to the time spent in the building wherein no specific illness or cause can be identified. Biological contaminants, in particular micromycetes, can present one of the possible causes of SBS. Here, we assessed the biodeterioration level of structural materials on the basis of fine-grained concrete widely used in construction practice and architecture. First, we determined the strength characteristics of the material that has been biologically damaged; second, we identified the damaging micromycetes and analysed their metabolic activity related both to the induction of biocorrosion and to the impacts of fungi on human health. Applying a new integrated approach, which combines methods of molecular microbiology and genetic toxicology with standard methods for determining the strength of building structures, we confirmed the relation between biodestructive and pathogenic properties of micromycete isolates.

At present, the world's losses from biodeterioration are estimated at billions of dollars and are more than 7% of the total cost of industrial products. Physical, chemical and biological factors of mortar and concrete corrosion are in close interrelation. Because concrete is a capillary-porous body, microorganisms, settling on the surface, then spread into the interior causing corrosive processes by the secreted metabolites. Biocorrosion of construction materials and structures has now become an important environmental problem, as according to statistics, the urban residents spend about 95% of their time indoors. Since the prospects for the overwhelming majority of the population are in city housing, the relevance of studying the biocorrosion of building materials and its consequences for the environment and human health is constantly increasing. One of the consequences is sick building syndrome (SBS), which is considered to be a multifactorial health problem, being a medical, psychological and social phenomenon[1–3] at the same time. The spaces that contain sufficient levels of chemicals, allergens and microorganisms to make those who live or work in the space sick are known as 'sick buildings'. These are mainly incommodious old buildings contaminated by microorganisms, especially filamentous fungi inducing biocorrosion of construction materials. At least 600 species of fungi are in contact with humans, and less than 50 are frequently identified and described in epidemiologic studies of indoor environments[2]. In this study we found the connection between destructive and health-damaging properties of filamentous micromycetes.

According to the Federal State Statistics Service (2016), the population of the Republic of Tatarstan is 3 868 730 people, of which the urban population is 76.41%. Biological damage to building structures is a common problem, especially in cities. Filamentous fungi dramatically worsen the characteristics of materials on which they grow causing partial or complete biodegradation. We have isolated micromycetes from the inner walls of some old buildings in Kazan city and found that fungal communities represented the class *Eurocytomycetes* and family *Trichocomaceae*. Pan-fungal primers specific for the conserved sequences of 18S and 28S rRNA genes common to all fungi have been used. The predominant genera (97%) were filamentous fungi *Penicillium* and *Aspergillus*: *A. niger* and *A. fumigatus* were the most common species. In some cases, micromycetes of the genera *Alternaria* and *Trichoderma* were found. Simultaneously, our studies of fungal infection in patients who visited the Laboratory of fungal diseases at the Kazan Research Institute of Epidemiology and Microbiology revealed the prevalence of aspergillosis. This is partly due to the contamination of many buildings with micromycetes of the genus *Aspergillus*, which can cause mycogenic allergies, atopic dermatitis and mycotoxicoses[4], the probability of which is significantly increased in environments with high numbers of these organisms, which contribute to SBS[1].

Three main processes cause biodeterioration: mechanical, assimilative (because building materials are a source of nutrition and energy for microorganisms) and dissimilative (the interaction of building materials with aggressive metabolites of microorganisms)[5,6]. Since the ability of fungi to produce secondary metabolites like single-, double- and tribasic organic acids and enzymes plays an important role in the process of dissimilative biodeterioration[7,8] as well as in pathogenesis[2,9,10], we focused our experimental work on measurements of secreted proteases and lipases of *Aspergillus* strains isolated from old buildings and from patients diagnosed with onychomycosis and otomycosis.

Pathogenic fungi secrete hydrolytic enzymes that enable them to breach and invade host tissues. The most highly recognised extracellular hydrolytic enzymes include proteases and lipolytic enzymes. Proteases from fungi induce inflammatory responses by altering the permeability of the epithelial barrier and by inducing the proinflammatory cytokines through protease-activated receptors[10]. Lipolytic enzymes have been shown to influence growth, morphology, adherence, and dissemination of fungal cells across the host[9]. We found that isolates from buildings showed a higher level of lipolytic activity compared to clinical strains (Figure 1*a*). The level of proteases was low in both groups, with slight prevalence in the activity of clinical isolates. Growing on keratin-containing substrate, *Aspergillus* strains produced a coat around the hair, but did not destroy the hair itself (Figure 1*b*). All the isolates studied are able to synthesise oxalic acid. The malic acid was found in culture fluids of 93% micromycetes, acetic acid was synthesised by 67%, citric acid by 60%, lactic acid by 33% and acetic acid by 13% of isolates. Acids promote the wash-out of calcium from concrete thus increasing the biodeterioration. On the other hand,

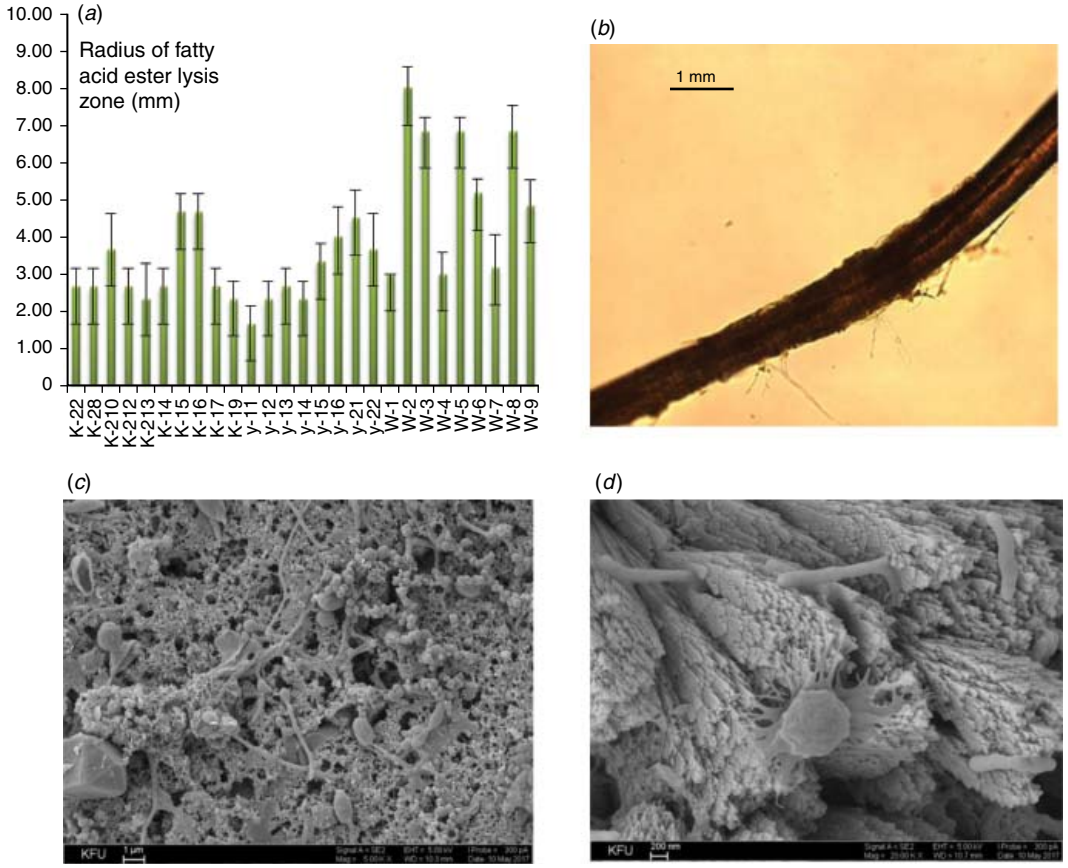

Figure 1. Lypolytic (*a*) and keratinase (*b*, light microscopy) activities of *Aspergillus* strains isolated from old buildings (W), from patients with diagnosed onychomycosis (K) and otomycosis (Y) measured by hydrolysis of linoleic acid methyl ester added to Sabouraud agar. Germination of *A. niger* W-2 spores in microcracks of concrete by low (*c*) and high (*d*) resolution scanning microscope LSM 780, Carl Zeiss, Germany.

Table 1. Alteration of strength characteristics of concrete during 28 days of exposure in pure Czapek Dox medium and medium inoculated by spores of *Aspergillus* W-2.

Brand of concrete[A]	Characteristics of concrete strengths, MPa[B]					
	Resistance to compression, R_c			Resistance to bending, R_b		
	Initial	Incubated in pure medium	Incubated in inoculated medium	Initial	Incubated in pure medium	Incubated in inoculated medium
M400	6.60	6.55	6.50	32.30	32.25	32.15
M500	7.10	7.05	7.00	42.80	42.75	42.65
M600	9.50	9.45	9.40	60.80	60.70	60.70
M800	10.50	10.49	10.40	74.80	74.80	74.7
M1000	12.20	12.17	12.15	95.20	95.16	95.00

[A]Brand of concrete reflects its average characteristics depending on the amount of cement used in a mix.
[B]Compression and bending strengths are measured by breaking cylindrical concrete specimens in special testing machine and are calculated as failure load divided by cross-sectional area (reported in megapascals, MPa).

pathogenic fungi acidify the environment as a strategy to damage host tissues, and acidification of the host tissues promotes the expression and activity of fungal proteases[11].

To date, there are more than 200 different methods to test biostability of building materials. Along with international standards, there are national standards for particular countries (STD 141C/6271/2-86 for USA; BS 1133 for UK, DIN 53739-84 for Germany, NF X 41-514-81 for France; JIS Z 2911-87 for Japan, GOST 9.048-89 for Russia). According to our data, the most aggressive strain was *A. niger* W-2 isolated from an old hospital building in Kazan city. This isolate possessed the highest levels of lipolytic activity and acid

production. After 28 days of contact with *A. niger* W-2 in the growth medium, the values of concrete sample stability were determined as described earlier[12]. As seen from Table 1, the resistance coefficients to bending and compression (R_b and R_c) after contact with the growing strain decreased by no more than 1%, indicating that there are no significant changes in the concrete specimens. However, these minimal changes determined by the standard test develop over time and gradually contribute to the destruction of concrete. Penetrating into the thickness and germinating in cracks, *A. niger* W-2 destroys the concrete (Figure 1*c, d*). Moreover, colonisation of the building material by toxigenic fungi raises the question of a subsequent exposure of residents to aerosolised mycotoxins. The greatest part of the aerosolised toxic load is found in particles whose size corresponds to spores or mycelium fragments. However, some toxins were also found on particles smaller than spores that are easily inhaled and can deeply penetrate into the respiratory tract[13].

A list of major strains in hospital environments (Centre F. Baclesse, Normandy, France) compiled according to their frequency, concentration level, and/or capacity to produce mycotoxins *in vitro*, is as follows: *A. fumigatus, A. melleus, A. niger, A. versicolor, Cladosporium herbarum, Purpureocillium lilacinum*, and *Penicillium brevicompactum*[14]. No mutagenic activity was found in bioaerosols. The results of genotoxicity testing by the reversion of auxotrophic *Salmonella typhymurium* stains to prototrophy under exposure to the culture fluid of *A. niger* W-2 (Ames assay) were also negative. Nevertheless, some *Aspergillus* strains assigned to section Nigri are well known as ochratoxin and/or fumonisin producers and showed toxigenic potential *in vitro*[15]. It could be concluded that active biodegraders from the genus *Aspergillus* contribute to the onset of the SBS.

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Hydrocarbon- and metal-polluted soil bioremediation: progress and challenges



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The problem of soil contamination with petroleum hydrocarbons and heavy metals is becoming particularly acute for large oil-producing countries, like the Russian Federation. Both hydrocarbon and metal contaminants impact negatively the soil biota and human health, thus requiring efficient methods for their detoxification and elimination. Bioremediation of soil co-contaminated with hydrocarbon and metal pollutants is complicated by the fact that, although the two components must be treated differently, they mutually affect the overall removal efficiency. Heavy metals are reported to inhibit biodegradation of hydrocarbons by interfering with microbial enzymes directly involved in biodegradation or through the interaction with enzymes involved in general metabolism. Here we discuss recent progress and challenges in bioremediation of soils co-contaminated with hydrocarbons and heavy metals, focusing on selecting metal-resistant biodegrading strains and biosurfactant amendments.

Environmental impacts of hydrocarbon and metal soil co-contamination

Contamination of soil environments with petroleum hydrocarbons (in the form of crude oil, fuels, organic solvents and other petroleum products) and heavy metals is becoming prevalent globally. Moreover, many contaminated industrial and municipal sites around the world are co-contaminated with organic and metal pollutants[1–3]. In fact, the largest emission sources for heavy metals are energy-related activities associated with oil extraction and refinery, as well as fuel combustion for heat and transport[4–6]. Trace metals most frequently found at oil-contaminated sites include arsenic, barium, cadmium, chromium, lead, mercury, nickel, vanadium, and zinc. For example, Russian heavy oils are enriched with metals, especially V and Ni, and to a lesser extent with Cd, Pb and Zn, which can contaminate soil along with hydrocarbons during accidental oil-spills or petroleum gas burning. Geological reserves of vanadium in heavy oils of the largest petroliferous basins of the Russian Federation are estimated at 1.3 million tonnes, of which 0.2 million tonnes are extracted along with oil[7]. Both hydrocarbon and metal contaminants impact negatively the soil biota and human health. Oil constituents (e.g. low molecular weight aliphatics, light aromatics, polycyclic aromatic hydrocarbons, and phenols) are highly toxic and carcinogenic. Also heavy metals present in oil can be accumulated by plants, thus leading to toxic reactions along the food chain. Moreover, trace metals occur in crude oil partly as organo-metallic compounds (e.g. geoporphyrins of V, Ni, Cu and Zn) and form stable complexes with asphaltenes, thus making their removal a difficult task[8,9]. Even more difficult is the assessment of environmental risks caused by simultaneous oil and heavy metal pollution. Software modelling is considered a powerful tool for integrating various elements in quantitative risk assessment, such as site characterisation, contaminant fate and transport, exposure assessment and risk calculation[10]. We previously developed a model that can be used in the site-specific risk assessment to

evaluate potential human health and environmental risks from terrestrial oil spills. The software developed allows estimation of hydrocarbon and metal impacts through various exposure pathways from different media. It is important that the model was validated and tested in pilot scale projects on management and bioremediation of crude oil contaminated site under cold climate conditions in the Perm region of Russia[11].

Metal toxicity for hydrocarbon-degrading microorganisms

Bioremediation of soil co-contaminated with hydrocarbon and metal pollutants is complicated by the fact that, although the two components must be treated differently, they mutually affect the overall removal efficiency. Biodegradation is considered to be an environmentally friendly and cost-saving process for removing organic contaminants, particularly hydrocarbons. In contrast, the non-biodegradable metal component must be removed, detoxified or stabilised within the site using microbial biosorption and phytoremediation. Heavy metals are reported to inhibit biodegradation of hydrocarbons by interfering with microbial enzymes directly involved in biodegradation or through the interaction with enzymes involved in general metabolism[12]. It should be noted that metal toxicity is related to the concentration of bioavailable metals rather than to the total metal concentration as the latter may include soil-adsorbed, precipitated or complexed metal species[1]. Metal speciation and the resulting bioavailability are greatly affected by soil properties, such as pH and ion exchange capacity, clay type, mineral and organic matter content. Soil contamination with oil could further impact metal bioavailability through the complexing with heavy petroleum fractions, especially asphaltenes[9]. Therefore, the extent of the combined metal and hydrocarbon stress on soil microbiota cannot be estimated simply as a function of co-contaminant concentrations, it should be determined for each specific case of soil contamination, considering the physiology and ecology of hydrocarbon-degrading microorganisms.

Most literature data suggest that inhibition of biodegradation increases progressively with the increasing concentration of bioavailable metal. However, in several studies, low metal concentrations stimulated biodegradation and after reaching the threshold concentration, the metal toxicity began to increase with increasing their concentration. Such stimulatory effect of low metal concentrations could be due to reducing the competition between metal-resistant degraders and metal-sensitive non-degraders in soil populations, thus stressing the importance of ecological impacts of toxic metals[1]. Alternatively, high metal concentrations in co-contaminated soil create a selective pressure for

Table 1. Heavy metal resistance and emulsifying activity of actinobacterial strains from the Regional Specialised Collection of Alkanotrophic Microorganisms, Perm, Russia.

Genus, species (number of strains)	Minimal inhibitory concentration (mM)								Emulsion index E_{24} (%)
	Cd^{2+}	Cu^{2+}	Ni^{2+}	Pb^{2+}	Zn^{2+}	CrO_4^{2-}	MoO_4^{2-}	VO_3^-	VO_4^{3-}	
Dietzia maris (5)	0.1 ± 0.02	6.0 ± 2.0	6.0 ± 2.0	8.0 ± 2.5	6.0 ± 3.4	20.0 ± 4.0	10.0 ± 2.0	>250	90 ± 20	26.2 ± 5.1
Gordonia rubripertincta (5)	0.8 ± 0.4	14.0 ± 7.4	12.0 ± 4.0	7.0 ± 2.5	4.5 ± 2.9	10.0 ± 2.0	14.5 ± 7.1	>150	80 ± 24	41.0 ± 3.9
Gordonia terrae (6)	0.4 ± 0.3	7.5 ± 2.5	4.3 ± 1.6	4.7 ± 2.9	1.7 ± 0.9	3.9 ± 3.0	3.8 ± 1.3	>200	58 ± 31	25.7 ± 9.1
Rhodococcus erythropolis (10)	0.3 ± 0.1	13.0 ± 9.0	6.3 ± 2.6	14.0 ± 6.2	4.6 ± 2.9	90 ± 83	8.0 ± 4.6	>200	48 ± 7.5	44.7 ± 3.3
Rhodococcus fascians (5)	0.6 ± 0.3	11.5 ± 7.4	10.0 ± 5.5	10.0 ± 5.5	5.6 ± 7.4	9.1 ± 6.5	13.0 ± 8.6	>250	40 ± 12	36.4 ± 10.2
'*Rhodococcus longus*' (8)	0.1 ± 0.03	6.0 ± 2.5	6.9 ± 2.4	6.3 ± 2.2	2.9 ± 1.8	15.0 ± 5.0	7.3 ± 3.6	>200	62 ± 21	42.5 ± 3.7
Rhodococcus opacus (7)	0.2 ± 0.1	4.3 ± 1.6	5.0 ± 0.6	7.9 ± 5.3	3.8 ± 1.5	14.3 ± 5.0	4.6 ± 2.7	>250	34 ± 15	45.7 ± 4.5
Rhodococcus rhodochrous (3)	0.1 ± 0.01	5.8 ± 1.9	10.0 ± 5.0	10.0 ± 2.0	4.2 ± 1.2	20.0 ± 4.0	8.3 ± 2.4	≥250	67 ± 24	41.2 ± 8.1
Rhodococcus ruber (12)	0.2 ± 0.1	10.4 ± 3.2	7.0 ± 3.3	12.1 ± 6.9	3.2 ± 1.3	12.9 ± 6.3	7.1 ± 6.4	≥250	83 ± 24	38.9 ± 7.3

metal-resistant oil-degrading microorganisms. We previously isolated and characterised a large number of vanadium-tolerant bacterial strains from soils contaminated with crude oil and refinery wastes[13]. The vast majority of isolated strains were resistant to high concentrations of vanadium salts and appeared to be capable of biosorption of the metal from the medium. Moreover, a non-specific resistance of selected actinobacterial cultures to heavy metals correlated positively with their growth on hydrocarbons and biosurfactant production (estimated as emulsifying activity) (Table 1)[14]. Thus, microorganisms exposed to metal- and hydrocarbon-polluted environments developed several mechanisms to tolerate a cumulative stress caused by metal ions (using efflux, complexation, or reduction) and toxic hydrocarbons (using modifications of the cell envelope and degradation[15]). Our recent findings suggest the involvement of proton- and sodium-dependent efflux pumps in the organic solvent tolerance of *Rhodococcus* actinobacteria[16], so these efflux systems could serve as cell adaptation mechanisms to both hydrocarbons and heavy metals.

Bioremediation approaches to hydrocarbon- and metal-polluted soils

Recent advances in bioremediation of co-contaminated environments have focused on the use of metal-resistant hydrocarbon-degrading bacteria (indigenous or bioaugmentation cultures) and different treatment amendments to mitigate metal toxicity. Of the latter, clay minerals, pH modifiers and chelating agents are widely used to reduce heavy metal mobility and bioavailability in soil[1]. Biosurfactants, microbially produced surface-active compounds, show promise for enhancing soil bioremediation due to their possible dual action (Figure 1): (i) micelle solubilisation of hydrocarbons; and (ii) metal detoxification via complexation[1,17,18]. The possibility of *in situ* biosurfactant production by oil-degrading microorganisms, indigenous or introduced during bioaugmentation, can be advantageous for soil bioremediation[19]. However, despite numerous laboratory demonstrations of positive biosurfactant effects on hydrocarbon biodegradation, there are still many gaps in our understanding of mechanisms of their action in soil co-contaminated with hydrocarbons and heavy metals.

Bioremediation strategies currently applied to soils affected by different types of pollutants include natural attenuation, biostimulation and bioaugmentation, phytoremediation and vermicomposting[20,21]. These strategies can be also used in combinations, thus increasing the efficiency of complementary approaches. For example, bioaugmentation-assisted phytoremediation resulted in the highest removal of petroleum hydrocarbons from soil co-contaminated with diesel and heavy metals (Cu, Pb and Zn) followed by bioaugmentation, phytoremediation and natural attenuation applied separately[20]. While the synergistic effect of plants and rhizosphere microorganisms has been demonstrated in many bioremediation experiments, more research is required to confirm such effects for the simultaneous treatment of organic and metal contaminations.

To date, only a few field trials have been performed on bioremediation of environments co-contaminated with organics and heavy metals. In the Northeastern Bulgaria oil deposit, waters contaminated with crude oil and toxic heavy metals (Zn, Cd, Cu, Pb, Mn, and Fe) were treated using the constructed wetlands[22]. Both oil and heavy metal concentrations decreased significantly due to hydrocarbon biodegradation and metal reduction/biosorption by indigenous microorganisms. Similarly, in our experiments, the oilfield wastewater treatment in a fluidised-bed bioreactor using *Rhodococcus* cultures co-immobilised on sawdust resulted in the efficient removal of hydrocarbons (68%) and heavy metals (75–96%)[23]. Using pilot bioreactors, the dual-bioaugmentation strategy was evaluated for soil co-contaminated with cadmium and 2,4-dichlorophenoxyacetic acid (2,4-D)[24]. A dual-bioaugmentation procedure involved the addition of cadmium-resistant *Pseudomonas* strain H1 in reactors inoculated with a cadmium-sensitive 2,4-D degrader

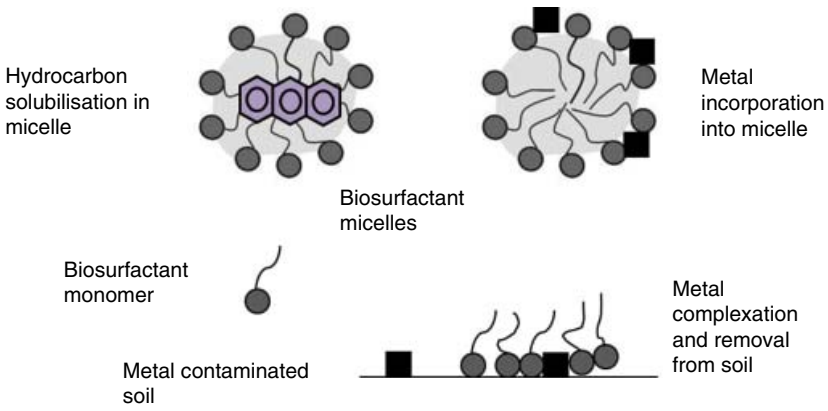

Figure 1. Mechanisms of hydrocarbon and metal removal from soil by biosurfactants (modified from Mulligan[17]).

Ralstonia eutropha JMP134, resulting in the enhanced biodegradation of the organic fraction. Overall, bioaugmentation with metal-resistant and oil-degrading bacterial cultures appears to be a viable approach in the remediation of co-contaminated soil and water.

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Biotechnological perspectives of microorganisms isolated from the Polar Regions

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Polar permanently frozen grounds cover more than 20% of the earth's surface, and about 60% of the Russian territories are permafrost. In the permafrost environments, the combination of low temperature and poor availability of liquid water make these habitats extremely inhospitable for life. To date, both culture-dependent and culture-independent methods have shown that permafrost is a habitat for microorganisms of all three domains: *Bacteria*, *Archaea* and *Eukarya*. An overview of applying psychrophilic and psychrotolerant bacteria and archaea isolated from Arctic and Antarctic permafrost ecosystems in biotechnological processes of wastewater treatment, production of cold-adapted enzymes, etc. is discussed here. The study of existing collections of microorganisms isolated from permanently cold habitats, improved methods of sampling and enrichment will increase the potential biotechnological applications of permafrost bacteria and archaea producing unique biomolecules.

Even though microorganisms were found in the permafrost more than 100 years ago, intensive studies of microbial communities in the Arctic and Antarctic began at the end of the last century. Cold-adapted microorganisms do not only survive at low temperatures, but can show significant growth and metabolic activity (respiration and biosynthesis) at temperatures down to −20°C, and even at −39°C[1,2]. The number of publications describing new prokaryotic taxa isolated from the polar ecosystems has increased by eight times between 1990 and 2015 (Figure 1).

Cold-adapted microorganisms face diverse challenges inherent to living in cold environments: low enzyme activity and low rates of biochemical reactions, altered transport systems, decreased membrane fluidity, and cold denaturation of proteins[3]. To get over these challenges, they have developed remarkable adaptations. Psychrophiles produce higher amounts of unsaturated and methyl-branched fatty acids and shorter acyl-chain fatty acids to increase the membrane fluidity[4]. They also produce cold-shock proteins to aid in different cellular processes as well as antifreeze proteins that inhibit ice crystal growth. Furthermore, all components of their cells must be suitably adapted to cold temperatures. The key adaptation to cold is the synthesis of cold-adapted enzymes that show an improved flexibility of enzyme conformation and maintain high specific activities at low temperatures.

The vast majority of current industrial processes are performed under harsh conditions, including extremely high and low temperatures, acidic or basic pH values, and elevated salinity. Standard enzymes have specific requirements for maximal function and perform optimally in narrow ranges of physical and chemical conditions. Cold-adapted enzymes have a combination of specific adaptations in their structural features that give them more flexible structures than mesophilic and thermophilic enzymes. This trait allows for high catalytic activity at low temperatures[5] and

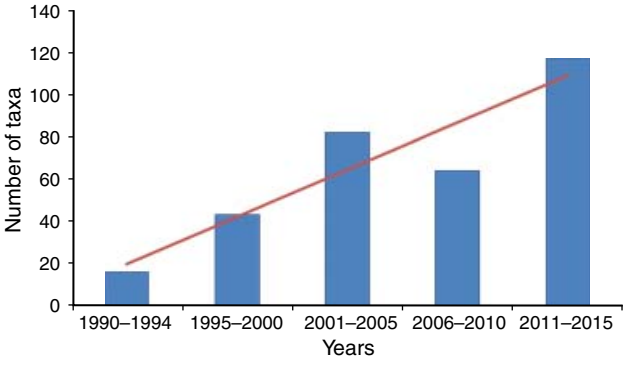

Fig 1. Distribution of the number of new prokaryotic taxa from the Polar regions validated from 1990 to 2015.

thermolability. In recent years, scientific and industrial efforts to discover and develop novel cold-adapted enzymes have increased substantially. The intrinsic characteristics of cold-adapted enzymes, such as high activity at low temperatures and thermolability, are extremely valuable for biotechnological applications in a wide variety of industries from molecular biology to food and beverage preparation[6]. Consequently, psychrophilic enzymes are replacing mesophilic enzymes in many different industrial processes. Due to the limitations of cultivating psychrophiles for production of cold-adapted enzymes, the current strategy is to clone and express genes encoding the desired product in mesophilic hosts prior to the operation in a bioreactor[7].

Biotechnological applications of psychrophilic and psychrotolerant microorganisms include cold-water detergents, food additives and flavour modifying agents, biosensors, and environmental bioremediation. Cold-active lipases catalyse the hydrolysis of fats and remove fatty acid stains from tissues at low temperatures[8–10]. Recently, high-performance lipase from *Pseudomonas stutzeri* PS59 has been identified and proposed for use. This lipase has its optimal activity at 20°C, pH 8.5, and its activity is not inhibited by various surfactants and oxidants[11]. Despite a number of successes achieved in recent years in the study of cold-active enzymes for the production of detergents, there is a clear need for even more active enzymes from psychroactive microorganisms. Enzymes that can work optimally at low temperatures (15–25°C), but retain high activity over a wide range of temperatures (from 5–60°C), and in the presence of surfactants and at alkaline pH will have future applications in detergent compositions. Enzymes, which possess such universal properties, have already been obtained in the form of recombinant proteins[12]. Proteases are enzymes that catalyse the hydrolysis of peptide bonds in proteins breaking them into shorter fragments. They also play an important role in the washing industry for removal of protein stains. Recent studies have focused on the discovery of new cold-active proteases in psychrophilic microorganisms derived from the Arctic and Antarctic ecosystems[13].

Psychrophilic and psychroactive microorganisms synthesise various molecules to protect the cell from freezing or to minimise the harmful effects of ice crystal formation[3]. Antifreeze proteins (AFPs) are ice-binding proteins that have the ability to change the crystalline structure of ice and suppress ice growth in two directions. For the first time, the presence of a protein leading to thermal hysteresis (TH) in bacteria was demonstrated by Duman and Olsen[14], and the *Moraxella* sp. strain became the first bacterial producer of AFPs[15]. At the beginning of studies of the antifreeze phenomenon, the highest values of TH were found in insects (3–6°C) and fish (0.7–1.5°C). AFPs isolated from plants and microorganisms had a significantly smaller TH: from 0.2 to 0.5°C for plants, less than 0.1°C for bacteria. Later, an AFP with high activity was detected in the Antarctic bacterium *Marinomonas primoryensis*[16], which is able to reduce the freezing point by 2°C. Up until now, the antifreeze activity has been detected in a small number of bacteria, mainly in Antarctic isolates. This fact directly indicates a role for AFPs in bacterial adaptation to cold. To date, antifreeze proteins have been detected in actinobacteria of the genera *Micrococcus* and *Rhodococcus*, γ-proteobacteria of the genera *Colwellia, Marinomonas* and *Pseudomonas* and bacteria belonging to the genera *Flavobacterium* and *Chryseobacterium*[17]. Screening of anaerobic prokaryotes in the All-Russian collection of microorganisms (VKM) showed AFP of a new type in the spore-forming bacterium *Clostridium tagluense* strain 121^T isolated from the Canadian permafrost[18]. Metagenomic analysis of several permafrost Arctic samples of various origins and ages has been carried out[19] and the screening for AFP will be our next step.

Psychrotolerant microorganisms are of great value for bioremediation of hydrocarbon-contaminated soil in Polar Regions, because they can maintain activity under extreme environmental conditions. Several strains of psychrotolerant bacteria have been isolated from the oil-contaminated soil in Antarctica and studied in detail[20,21]. Low-temperature anaerobic digestion (AD) has been successfully applied to treat a vast range of wastewater types, such as phenolic, chlorinated aliphatic, brewery, pharmaceutical and glucose-based wastewaters. The evidence of comparable treatment efficiencies to mesophilic setups has also been recorded, as well as the successful treatment of complex wastewater. Despite successful applications, there is a lack of fundamental knowledge regarding the mechanisms underpinning AD. The future full-scale implementation of AD, and particularly the development of promising new applications, such as low-temperature AD, is severely impaired by this knowledge gap. Methanogenic populations have been the focus of many low-temperature AD studies due to their crucial role in biogas formation and biofilm integrity[22]. As our preliminary tests have shown, permafrost methanogens (Table 1) can be used for the process start-up at moderate and low temperatures. Other possible applications of psychroactive isolates from VKM are also presented in the Table 1.

Nature provides a vast source of biocatalysts. However, the probability of finding the right enzymatic activity for a particular application relies on the available technical capabilities to efficiently assess this large microbial diversity. This capability is mainly mediated by technologies, such as metagenomic screenings, genome mining, and direct enzymatic exploration[23,24]. Metagenomic screenings and genome mining require that the search for a novel enzyme is based on genetic sequence homology to already described enzymes. But the discovery of new enzymes in this way does not always give accurate information, especially for less studied organisms like psychrophiles.

An alternative method is presented in the direct exploration of cold-adapted enzymes and AFPs based on functional screenings of

Table 1. Psychrophilic and psychroactive prokaryotes from Arctic and Antarctic permafrost in VKM.

Species	Source of isolation	Growth temperature (optimum)	Application
Bacteria			
Clostridium algoriphilum 14D1^T VKM B-2271^T	Cryopeg in permafrost, Arctic	0–20°C (5–6°C)^A	Low temperature waste treatment
'*Clostridium frigoriphilum*' 14F^T VKM B-2368^T	Cryopeg in permafrost, Arctic	0–15°C (6°C)^A	Low temperature waste treatment
Clostridium tagluense 121 VKM B-2271^T	Permafrost, Arctic	0–28°C (15°C)^A	Low temperature waste treatment, AFP producing
'*Clostridium deceptionensis*' G4 VKM B-2784^T	Volcano ground, Antarctica	0–15°C (6–7°C)^A	Low temperature waste treatment
Celerinatantimonas yamalensis C7^T VKM B-2511^T	Cryopeg in permafrost, Arctic	0–34°C (18–22°C)^A	Nitrogen-fixing at near zero temperature, cold-adapted enzymes
Sphaerochaeta associata GLS2^T VKM B-2742^T	Permafrost, Arctic	5–37°C (30–34°C)	Low temperature waste treatment
'*Psychrobacter murincola*' 1pS VKM B-2269	Cryopeg in permafrost, Arctic	2–37°C (18–20°C)^A	Cold-adapted enzyme
'*Psychrobacter murincola*' 2pS^T VKM B-2270^T	Cryopeg in permafrost, Arctic	2–37°C (16–18°C)^A	Cold-adapted enzyme
Psychrobacter arcticus VKM B-2377^T	Permafrost, Arctic	0–28°C (22°C)^A	Cold-adapted lipase and esterase
Psychrobacter cryohalolentis VKM B-2378^T	Cryopeg in permafrost, Arctic	0–30°C (22°C)^A	Cold-adapted lipases
'*Desulfovibrio algoritolerans*' K3S^T VKM B-2877^T	Cryopeg in permafrost, Arctic	0–36°C (26°C)^A	Low temperature precipitation of heavy metals
Desulfovibrio arcticus B15^T VKM B-2367^T	Cryopeg in permafrost, Arctic	0–28°C (24°C)^A	Low temperature precipitation of heavy metals
Archaea			
'*Methanosarcina gilichinskiia*' JL01^T VKM B-2370^T	Permafrost, Arctic	10–37°C (25–28°C)^A	Low temperature biogas production
Methanobacterium arcticum M2^T VKM B-2371^T	Permafrost, Arctic	10–42°C (37°C)^A	Low temperature biogas production
Methanobacterium veterum MK4^T VKM B-2440^T	Permafrost, Arctic	5–37°C (30°C)^A	Low temperature biogas production

^{A}Capable of subzero growth.

enzymatic activities in large collections of microorganisms, such as VKM. Studies of microbial communities in cold ecosystems indicate that psychroactive prokaryotes represent a large variety of novel and understudied microorganisms. Research of existing collections of microorganisms isolated from permanently cold habitats, the development of improved methods of sampling and enrichment will increase the potential biotechnological application of permafrost bacteria and archaea producing unique biomolecules.

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The role of non-spore-forming actinobacteria in cleaning up sites contaminated by persistent pollutants and the ability of these microorganisms to survive under unfavourable conditions



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Years of research has shown that actinobacteria, including *Rhodococcus*, *Gordonia*, *Arthrobacter*, *Microbacteria*, play an important role in cleaning up sites contaminated by persistent organic pollutants. Under special conditions, actinobacteria of different genera are able to form specific forms, cyst-like resting cells (CLC), which maintain the viability during long-term storage (for at least 5–6 years, *our unpublished results*). These cells quickly germinate when conditions become favourable for growth. As a result, actinobacteria can be used as a basis for creating highly efficient biological preparations for cleaning up the soil with high levels of toxic contaminants such as (chloro) phenols, (chloro)biphenyls, polycyclic hydrocarbons, oil[1].

Among the most important environmental concerns today is contamination with toxic and persistent pollutants. Oceans, soils, groundwater, air, living organisms are increasingly polluted due to human activities[2]. The most serious problem is created by the presence of halogenated aromatic compounds, polycyclic aromatic hydrocarbons and various oil components[3–5]. A great majority of these compounds are by-products derived from manufacturing processes and found in waste of by-product-coking and petrochemical industries[6,7]. In some industrial regions, technogenic load on the environment is caused by emissions and waste dumping from large enterprises of the chemical industry, potassium industry, and power plants. One of the largest environmental polluters in Chapaevsk (Samara Region, Russia) is the Middle Volga chemical plant[8]. A number of large enterprises for the processing of potassium-magnesium and sodium salts are located in Perm Krai, Russia[9]. Vast quantities of halides in wastewater and the brine sludge are formed as a result of the activities of such enterprises. These components, released by potassium salt mining and processing plants, pollute the environment with compounds of sodium, potassium, magnesium, and other elements, which leads to soil salinisation. Hence, wastes generated during the production process of fluorine- and bromine-containing organic and inorganic compounds are major contributors to the pollution of soils and water bodies[5]. These compounds are characterised by low water solubility and can be strongly adsorbed by biological tissues[2]. A strong technogenic impact on the environment is often aggravated by ecological factors that are unfavourable for microbial destruction of pollutants, such as low temperature, high contents of mineral substances, and high or low pH of the substrate. Nevertheless, the unique soil microflora developing in such areas can both survive and decompose different classes of xenobiotics under extreme environmental conditions[10,11].

Actinobacteria are able to degrade a great variety of persistent compounds, for instance, phenol, chlorophenols, (chloro)benzoates, chlorinated biphenyls, oil. Biopreparations for soil and water detoxification contain bacteria of one or few strains. Generally, these biopreparations include representatives of actinobacteria. A combination of microorganisms with different viability and metabolic strategy (e.g. *Rhodococci* and *Pseudomonads*) increases the efficacy of biopreparations.

To date, there is limited research in microbiology on survival of the species of non-spore-forming microorganisms in nature under conditions of carbon, nitrogen and phosphorus deficiency, species-specific growth factors necessary for growth and development, and under the influence of aggressive physical factors of the environment that may be temporary or seasonal (fluctuations in temperature and humidity, pH of the medium, the presence of aggressive chemical compounds-contaminants of anthropogenic origin, etc.). Cyst-like resting cells (CLC) of non-spore-forming actinobacteria, representatives of the genera: *Rhodococcus*[12], *Gordonia*, *Arthrobacter*[13] and *Microbacterium*[14] were obtained in experimental laboratory conditions. These actinobacteria are widely distributed in various natural habitats (including extreme ones) and, apparently, have the ability to rapidly and effectively adapt to structural rearrangements remaining in a viable state.

It is shown that the studied non-spore-forming actinobacteria, under unfavourable growth conditions, for example, depletion of the growth substrate, are capable of entering into a resting state (Figure 1). Model strains of bacteria of two genera: *Arthrobacter* and *Microbacterium*, have the ability to form, under experimental conditions, small (volume $\leq 0.1\ \mu m^3$) and ultra-small (from 0.01 to $0.02\ \mu m^3$) viable cyst-like resting cells (US CLC). Experimentally obtained CLC are characterised by a high density of the cytoplasm, a compacted nucleoid and a dense cell wall with an external additional cover in the form of a fibrillar capsule (single CLCs of *M. foliorum* BN52), or represented by conglomerates of such ultra-small CLCs (*A. agilis* Lush13).

Under favourable conditions, the process of germination of resting cells of non-spore-forming bacteria occurs. As a rule, the process of activation of growth is already discernible at the ultrastructural cellular level during the first 0.5–1 h after cell seeding procedure. Intrapopulation variability of *R. opacus* was shown to be coupled

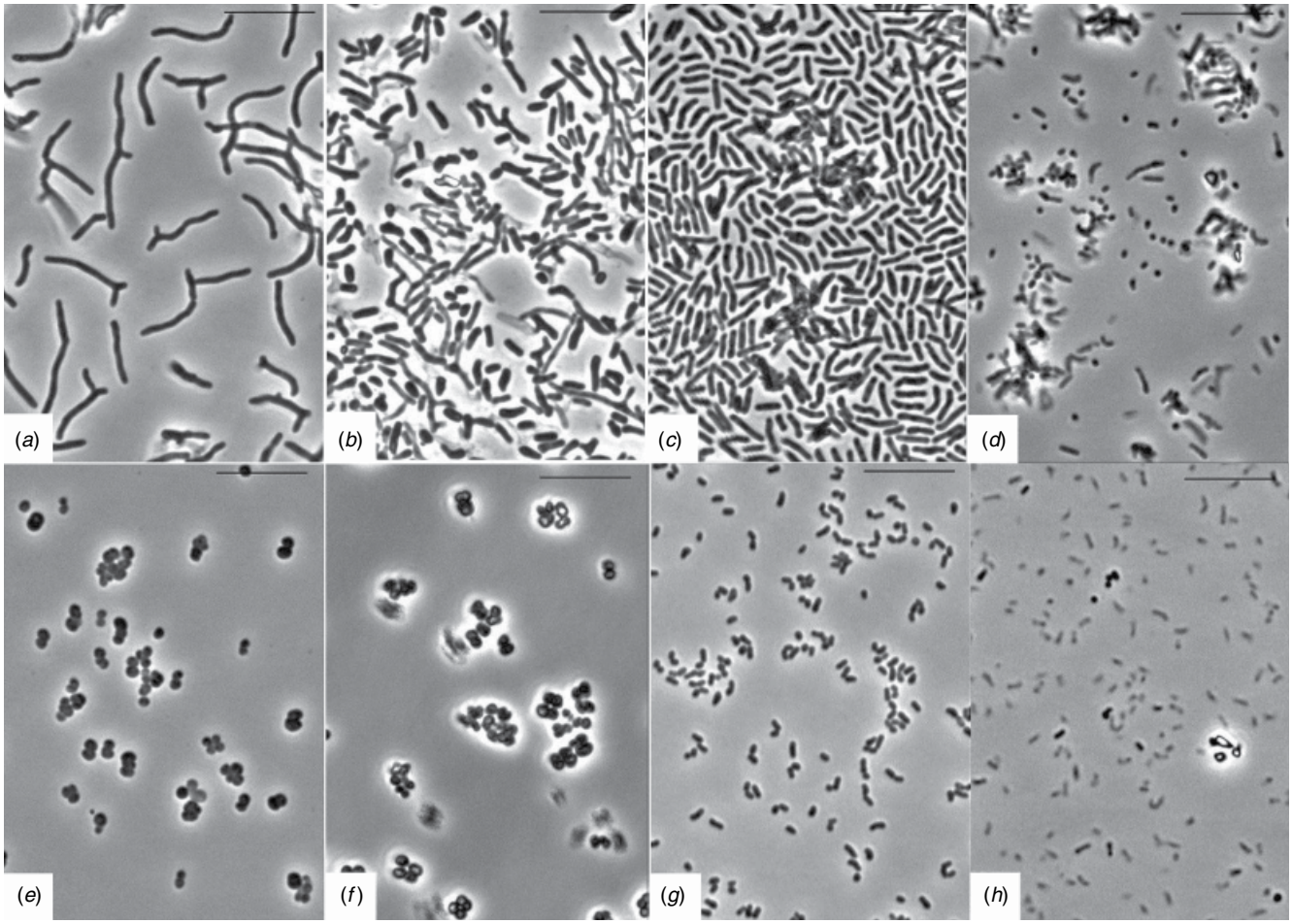

Figure 1. Vegetative (*a*,*c*,*e*,*g*) and cyst-like cell dormant (*b*, *d*, *f*, *h*) forms of *Rhodococcus opacus* 1CP (*a*, *b*), *Gordonia polyisoprenivorans* 135 (*c*, *d*), *Arthrobacter agilis* Lush 13 (*e*, *f*), and *Microbacterium foliorum* BN52 (*g*, *h*). Phase contrast microscopy. Scale bar: 10 µm.

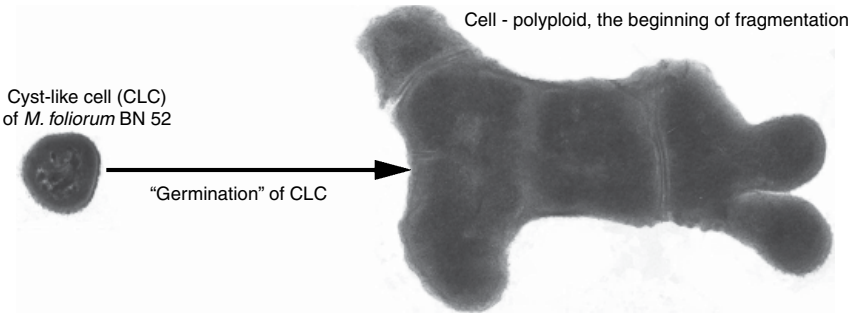

Figure 2. Germination of CLC form of *M. foliorum* BN52. Initial steps result in formation of large polyploid cells undergoing fission at the next stages of germination. In the schematic image, ultra-thin sections of bacteria taken at the same magnification were used (figures in this scheme are taken from Solyanikova *et al*.[14]).

with germination after dormancy stage, and diversity of the emerged colonial variants depended upon the physiological age (storage time) of CLC and additional stress impacts (thermal treatment)[12].

The transition to the vegetative growth of conglomerates of ultra-small CLC *A. agilis* Lush13 was accompanied by disintegration of conglomerates into single US CLCs followed by their active growth and division. A distinct feature of the process of 'germination' of the CLC of this type is simultaneous formation of a dense extracellular and intercellular fibrillar matrix. The transition from a resting state to vegetative growth of the *A. agilis* Lush13 CLC resulted in the formation of microcolonies of vegetative cells enclosed within a dense fibrillar matrix, the 'cocoon'. It can be assumed that in this case, the bacterium realises the protective mechanism of rapid multiplication within the fibrillar matrix, which protects the population of young vegetative cells against sudden fluctuations in external factors and unexpectedly unfavourable conditions. In common laboratory practice *A. agilis* Lush13 cultivated cells do not form a fibrillar matrix[13].

The transition to vegetative growth of the CLC strain *M. foliorum* BN52 was followed at the first stages by the formation of large cellular forms, polyploids, where the cytoplasm and nucleoplasmic volume was ~40 times greater than the volume of vegetative cells under laboratory cultivation conditions (Fig. 2)[14]. In the subsequent stages of the 'germination' of the CLC of strain BN52, the nucleoplasm was split into discrete nucleoids and multiple fragmentation of large cells into ultra-small 'single nucleoid' cell forms (with a volume of 0.01–0.02 μm^3). The process of 'germination' of US CLC was complete when 15 to 20 'daughter' vegetative cells were formed. At the same time, under laboratory conditions of cultivation binary fission is the most common form of reproduction in bacteria; such fission is complete when two 'daughter' cells are formed.

The obtained experimental data are consistent with the literature data on the detection in a variety of natural biotopes of a large number of ultra-small bacteria and bacterial cell forms (70–90% according to various data)[15–17]. It is obvious that small and ultra-small viable cell forms have the advantage of spreading in the environment, since they can penetrate into habitats that are inaccessible to large single-celled organisms[18]. Thus, our studies have shown that the transition of the CLC to active growth differs significantly from conventional binary fission under cultivation conditions on laboratory nutrient media. One can assume that in natural conditions, bacterial cells implement the following mechanisms: (1) the mechanism of 'protection of offspring', realised in the natural environment by forming a protective outer matrix-cocoon around young dividing cells; (2) the mechanism of directed reduction in the size / volume of daughter cells to provide an advantage in colonising hard-to-reach ecological niche; and (3) cell numbers increasing by the division of giant polyploid cells, boosting the growth of the cell population in a short time.

The following properties of CLC are important for survival in nature: (1) the ability to quickly reset active metabolism and cell division under favourable conditions; (2) stress resistance; and (3) increased phenotypic variability, which is expressed after CLC germination as a spectrum of phenotypes, with one of them being probably most adapted to the new environment[12]. Using the strain *R. opacus* 1CP as an example, it was shown that the renewal of growth after resting stage in actinobacteria can be accompanied by an expansion of the range of utilisable substrates[12]. The most likely explanation for this phenomenon is a change in the rate of induction/repression of genes responsible for different metabolic networks of the whole cell and facilitating the reorganisation of their regulation.

Conclusion

Actinobacteria can degrade a wide range of compounds under varied environmental conditions due to high metabolic rates, the presence of highly specific enzymes, and the ability to transfer biodegradation genes. Under adverse conditions these bacteria are able to form specific surviving forms, the so-called cyst-like resting

cells (CLC). Thanks to the formation of the CLC, the actinobacteria retain their viability for many years in starvation, drier, and higher temperature conditions. Under favourable conditions, the CLC can germinate quickly. The mechanism, which is based on the formation of polyploid cells, can be accompanied by a structural and functional reorganisation of the genomes of forming 'daughter' cells, which in turn can lead to the appearance of new properties in forming cells, which can increase the adaptability of cells to new environmental conditions.

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Biographies

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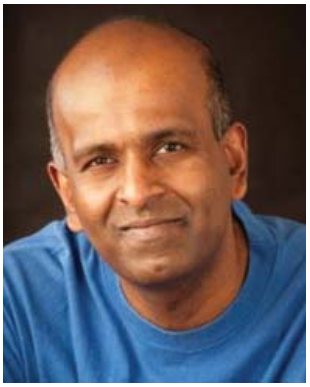
Is nitrite from nitrification the only cause of microbiologically induced chloramine decay?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Nitrite, produced by ammonia oxidizing bacteria (AOB), was traditionally thought to be the only cause of microbiologically mediated decay of chloramine. The development and application of microbial decay factor method and bacterial community studies, for the first time have revealed many other factors such as soluble microbial products (SMPs) and bacteria other than AOB mediating the decay of chloramine.

In all states of Australia, chloramine (NH_2Cl is formed using chlorine and ammonia – $Cl_2+NH_3 \rightarrow NH_2Cl+HCl$) has been used as a secondary disinfectant in drinking water distribution systems to comply with drinking water quality guidelines. About seven million people in Australia drink chloraminated water. Chloramine offers several advantages over chlorine: chloramine continues to remain active in long water-supply systems even in warmer climates; it inhibits the growth of pathogens such as *Naegleria fowleri*[1]; and it produces fewer carcinogenic disinfection by-products[2] in water distribution systems.

Although somewhat resilient towards decay (i.e. to Cl^- and free NH_3), as the chloraminated water travels along the distribution system, the disinfection residual gradually starts to decrease due to self-decay and chemical interactions of chloramine with water constituents (such as organic carbon, bromide, iron) that are prevalent in the distribution system[3]. The continuous release of free NH_3 in smaller concentrations trigger growth of ammonia oxidising bacteria (AOB) in chloraminated distribution systems and AOB oxidise ammonia to nitrite that accelerates the decay of chloramine. With a focus on controlling accelerated decay of chloramine, much of the research was focused traditionally towards AOB communities in chloraminated systems. The AOB *Nitrosomonas oligotropha* and *Nitrosospira* were found ubiquitous in chloraminated systems[4,5]. In addition to AOB, heterotrophic bacteria (measured by the heterotrophic plate count method) were observed dominating specifically after/or closer to the onset of nitrification and in some instances, an increase of abundance has even been observed prior to the onset of nitrification[4,6]. The pH and temperature can impact AOB activity as well as chemical instability of chloramine[3,6]. The utility operators find it difficult to predict at which combination of chlorine, ammonia and temperature, nitrification starts to take place in a distribution system. Hence, they operate their reservoirs with frequent rechlorination to control free NH_3 to the lowest possible level. However, even small operational interruptions lead to nitrification with an accelerated loss of chloramine in distribution systems.

Questioning the traditional belief that nitrite produced by AOB is the only cause of microbial chloramine decay and with the idea that chloramine decay should be measured to control residuals, Sathasivan *et al*.[7] developed a microbial decay factor method to distinguish chemical and microbial mediated components of the decay.

The method used filtration (0.2 µm) or inhibition (100 µg-silver L^{-1}) to eliminate the microbial activity. According to the results of many full-scale water supply systems, chloramine decays rapidly despite nitrite concentrations being insignificant in bulk water. Unware of the overall microbial diversity along a chloraminated system, past research concluded nitrifiers were responsible for such decay. On comprehensively analysing bulk water samples of chloraminated distribution systems, Sathasivan *et al*.[8] for the first-time reported the prevalence of many phases that have varying influence on the decay of chloramine. The first phase identified refers to chemical decay that is often observed in treated water and is at the front end of the system. The second is the mildly nitrified phase recognisable by the presence of a low nitrite concentration (<0.010 mg-N L^{-1}) and a mild decay of chloramine (<0.0101 h^{-1}). This phase is distinctly apparent prior to the onset of nitrification. The third and final phase is called severe nitrification, where an accelerated decay of chloramine (>0.0101 h^{-1}) is noted. The prevalence of these three distinct phases suggests several different mechanisms of decay and there could be different microbes that are responsible and involved in each of these phases. Understanding and quantifying the decay mechanisms in each of these phases could lead to a better control of chloramine decay.

A lab-scale chloraminated distribution system[9] was operated to capture the aforementioned three phases with the aim to facilitate a deeper understanding of the microbial decay mechanisms taking place in chloraminated distribution systems. The lab-scale system successfully simulated all three phases[8] that are encountered in full-scale chloraminated systems. The chloramine residuals in the first two reactors (R-1 and R-2) were high with mild nitrification (nitrite < 0.010 mg-N L^{-1}) and a rapid decrease of the residual was observed in the subsequent reactors (R-3 to R-5) with an onset of severe nitrification in R-3 (Figure 1). The bulk water pH dropped from 8.1 (in R-1) to 7.7 (in R-5) along the reactors.

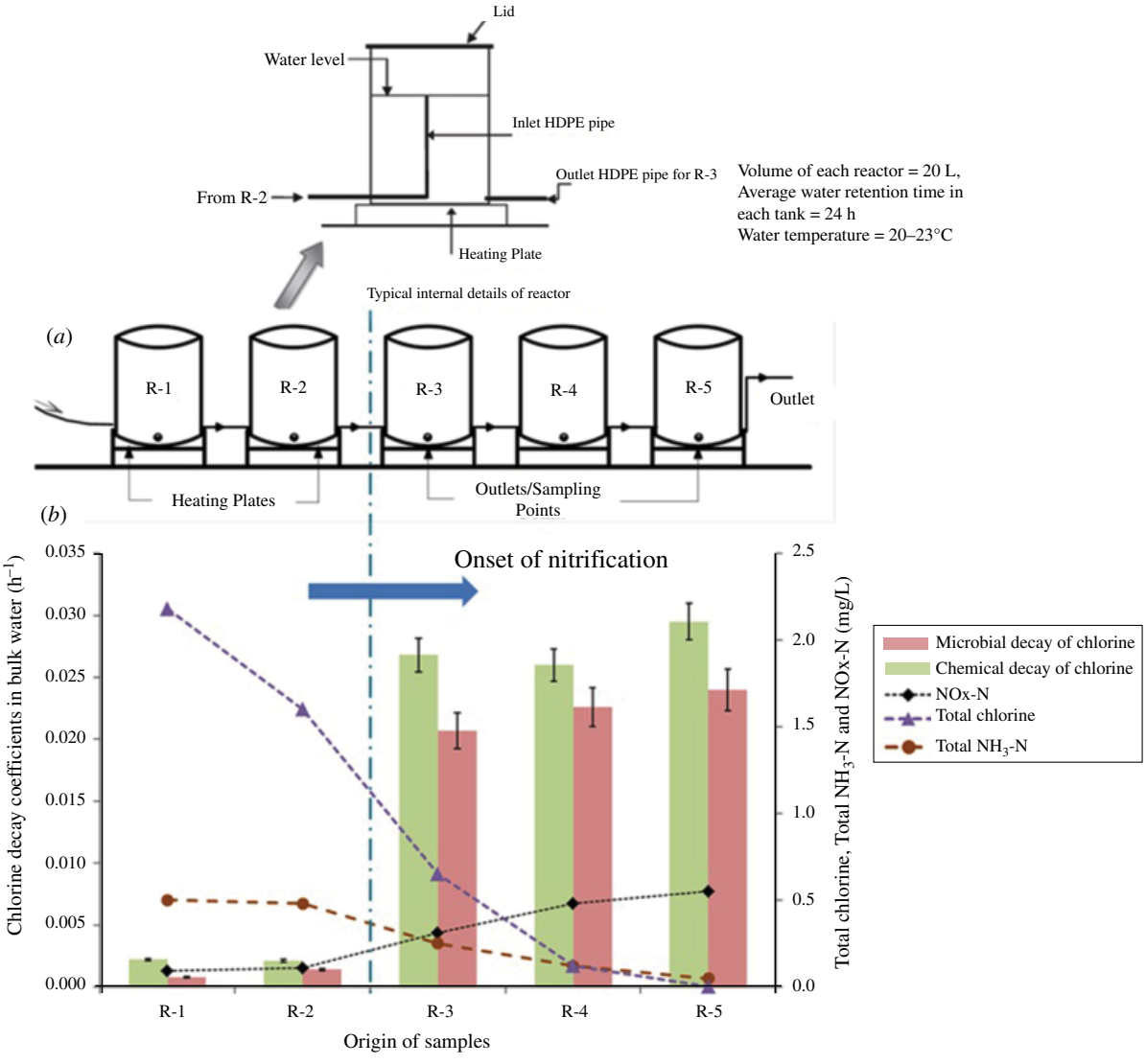

Figure 1. Reactor assays. (*a*) A schematic diagram of the lab-scale reactor system. (*b*) Chemical parameters measured along the reactors (R-1 to R-5) with chloramine decay due to chemical reactions (by microbial decay factor method) and microbial activities in the bulk water.

The accelerated decay of chloramine with soluble microbial products (SMPs)

When the microbial decay factor method was applied to infer the chemical decay of the nitrified bulk water in each of the reactors in the lab-scale system (Figure 1*a*), the chemical decay rates derived simply could not be explained by the increase of bulk water nitrite concentrations and the decrease of pH alone[10]. The reason for the observed higher chemical decay rates were unclear and were hypothesised to be a result of novel dissolved organic carbon (DOC) constituent and/or SMPs released by microorganisms. A similar behaviour was also noted with bulk water in a full-scale chloraminated distribution system[11]. Further experiments with DOC free chloraminated water in the lab-scale reactor system revealed that accelerated chemical decay was primarily a result of SMPs[12]. SMPs are largely amines, carbohydrates, nucleic acids, proteins, and, polysaccharides. When bulk water was subjected to protein denaturing conditions (such as heat treatment, pH treatment, microwave irradiation, silver addition), it negatively impacted the chemical decay rate and according to this observation, the SMPs that accelerate chloramine decay were hypothesised to be protein-like in nature[12].

In our studies[10,12], the SMPs accelerating chloramine decay was evident only after the onset of nitrification. The reactors R-1 and R-2 (Figure 1*a*), which are upstream of R-3 (where the onset of nitrification occurs (Figure 1*b*)), also foster bacterial growth and these bacteria may also have released SMPs into the bulk water. However, due to a low abundance of bacteria, the bulk water SMPs concentration in these two reactors may not have reached the minimum specific concentration required to facilitate a noticeable decay of chloramine. Similarly, within the nitrified waters, there were microbes other than nitrifiers present[9] and these microbes may also have contributed towards production of these SMPs[13]. While SMPs may accelerate chloramine decay, only a more comprehensive understanding of SMPs could enable development of engineering solutions to mitigate chloramine decay in chloraminated drinking water distribution systems.

Chloramine decay by microbes other than nitrifiers

Both full and lab-scale studies have clearly demonstrated the prevalence of microbial decay of chloramine before the onset of nitrification in chloraminated distribution systems[8,9,13]. Well-known nitrifiers were rarely detected in both bulk water and biofilm (inlet HDPE pipe shown in Figure 1*a*) prior to the onset of nitrification, and bacterial genera such as *Mycobacterium* and *Pseudomonas* were found dominating in R-1 and R-2 of our lab-scale chloramination system (Figure 2). High abundances of *Mycobacterium* (41–61% in bulk water and 48–59% in biofilm) and *Pseudomonas* (2–10% in bulk water and 3–26% in biofilm) were observed before the onset of nitrification (Figure 2). *Mycobacterium* and *Pseudomonas* are both known to resist chlorine and chloramine and have also been observed in full-scale

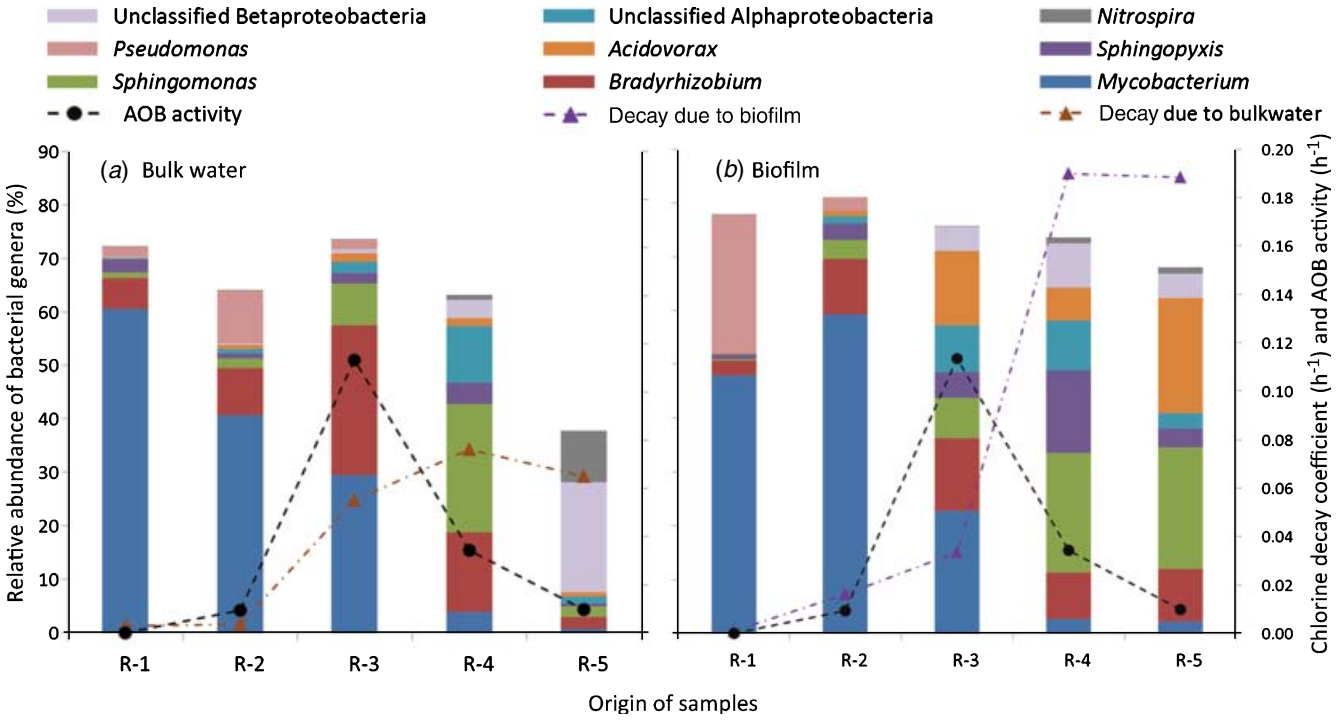

Figure 2. Relative abundances of bacterial genera as determined by metabarcoding of the 16S rRNA genes (detailed in Ginige *et al.*[17]), chlorine decay coefficients and AOB activity. (*a*) Bulk water and (*b*) Biofilm.

distribution systems[14]. While these bacterial genera are known to resist chloramine, the mechanism behind resistance and their role, if at all any, in the initial decay of chloramine remains unknown.

The AOB activity measured as NOx-N (nitrite-N + nitrate-N) production rate (detailed in Bal Krishna *et al.*[9]) peaks in R-3, and then rapidly declines in R-4 and in R-5 (Figure 2). Even with a decrease of AOB activity in R-4 and R-5, an increase of chloramine decay was observed (Figure 2), which could be a result of non-AOB bacterial activities. For instance, when Herath *et al.*[15] operated the lab-scale reactor with chloraminated water containing a high DOC concentration of 10–12 mg L^{-1}, mild nitrification prevailed (nitrite <0.012 mg-N L^{-1}) but chloramine decay rates remained similar to when AOB activity prevailed in the lab-scale system. This observation provides evidence towards the possible involvement of non-AOB in the decay of chloramine.

The abundance of the AOB genus *Nitrosomonas* in reactors R-3 to R-5 ranged between 1.4–2.9% in bulk water and 0.9–1.4% in the biofilm (Figure 2). Accordingly, our lab-scale system was dominated by heterotrophic bacteria, both before and after the onset of nitrification (Figure 2). A decrease of AOB activity coincided with an increased abundance of other bacterial genera such as *Sphingomonas*, and *Acidovorax* (in the biofilm). These bacteria are metabolically diverse and have been demonstrated capable of decomposing halogenated compounds such as chlorinated biphenyls, halogenated diphenyl ethers, haloacetic acids, etc.[16]. The diverse metabolic capacity of these bacteria raises questions on whether they are able to directly or indirectly utilise chloramine.

In summary, accelerated chloramine loss after the onset of nitrification was noted due to SMPs that are protein-like in nature. There was no positive correlation between AOB activity and the chloramine decay rate (both in bulk water and biofilm) in the lab-scale system and this suggests a possible involvement of other bacteria in the decay of chloramine.

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Extrinsic allergic alveolitis-causing actinomycetes in indoor and farm environments

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Suspended airborne particles, of biological origin, can constitute bioaerosols[1,2] and they can be of different origin ranging from farm environments dealing with hay, sugar cane, tobacco, mushroom and cotton to refuse disposal sites to military equipment test chambers. These bioaerosols might carry allergenic and pathogenic agents like viruses, spore forming bacteria and fungi, excreta of insects and mites, moss and fern spores, algal and plant cells; enzymes, antibiotics, endotoxins, mycotoxins and fungal glucans[1]. Although infections from pathogenic viruses, bacteria and fungi may occur in these work environments the commonly reported symptoms relate to allergic rhinitis and asthma, allergic alveolitis (granulomatons pneumonitis) or organic dust toxic syndrome (inhalation fever or toxic pneumonitis)[1,2]. This article will only provide an overview on the non-infectious lung diseases resulting from long-term exposure to the spores of thermoactinomycetes of the order *Bacillales* and thermophilic actinomycetes of the order *Actinomycetales* in indoor and farm environments.

Early reports on the above-mentioned non-infectious diseases resulting from the allergenic and/or immunotoxic properties of airborne biological agents dates to 1877 by the recognition of byssinosis (brown-lung disease) associated with cotton, hemp and flax farming[3,4]. The cause of this disease has later been associated with the exposure to endotoxins from Gram-negative bacteria in 1981[5]. Another similar disease, the *Farmer's Lung Disease* was linked to 'white dust arising from mouldy hay' in 1932[6] and the causative agent was only identified in 1963 as the airborne spores of actinomycetes[7]. Since then such diseases have been frequently reported from many different countries including Finland, Germany, Poland, Switzerland, Sweden, India, Canada, Argentina and Costa Rica due to the storage of substrates prone to infestations by spore forming and Gram-positive bacteria (e.g. farm environments, mills and compost facilities)[8–13]. These diseases can be more prevalent in tropical and sub-tropical environments where thermophilic actinomycetes and fungi thrive due to elevated temperatures and humidity. Farmer's lung is also highly prevalent in regions with high rainfall, such as Doubs, France, during the haymaking season[14].

In this article causative bacterial genera or species associated with the above-listed diseases and belonging to the order *Actinomycetales* (http://www.bacterio.net/actinomycetales.html) will be termed as thermophilic actinomycetes, whereas the ones belonging to the family of *Thermoactinomycetaceae* of the order *Bacillales* (http://www.bacterio.net/thermoactinomycetaceae.html) will be termed as thermoactinomycetes covering the 21 genera currently listed under this family, including *Thermoactinomyces*, *Laceyella*, *Seinonella* and *Thermoflavimicrobium*.

Genera of the thermophilic actinomycetes implicated in hypersensitivity pneumonitis or extrinsic allergic alveolitis (EAA)[15] include *Faenia, Streptomyces*, *Thermomonospora*, *Saccharopolyspora, Saccharomonospora* and *Nocardiopsis.* Thermophilic members of these actinomycete genera are capable of growth at elevated temperatures due to diploconic acid containing spores that are responsible for their survival at elevated temperatures. Whereas, genera belonging to thermoactinomycetes produce highly heat-resistant endospores[2] and such spores can easily be disseminated through bioaerosols[1,2,13,16], *Thermoactinomyces* species can survive in organic materials, such as soil, for up to 100 years as well as at 100°C for 30–40 minutes. Improper storage of large bales of

organic substrates (in airtight buildings) when temperatures reach 50–60°C with a moisture content of >30% can result in the growth and proliferation of endospore forming thermoactinomycetes[13].

Thermoactinomyces species were reported to infest home humidifiers and buildings where humid conditions may be present[16]. *Thermoactinomyces vulgaris* and *Thermoactinomyces intermedius* were isolated from hot water and heating systems of homes and *Thermoactinomyces vulgaris* from the household air[13,16,17]. Thermophilic actinomycetes are also reported to occur in the condensate of refrigerators and air conditioners[17].

The above-mentioned non-infectious lung diseases associated with different occupational environments are termed accordingly: farmer's lung disease, mushroom worker's lung disease and bagassosis (sugarcane mills/farms) and their occurrence may differ in work places but present with similar symptoms that include cough, fever, malaise, weight loss, chills and shortness of breath[9,18,19]. Eye and nose irritation may also be experienced[20]. Susceptible workers that are exposed to the organic dust clouds from disrupted overheated materials, due to storage rooms with inadequate ventilation, may be at risk of acquiring the acute, subacute or chronic form of extrinsic allergic alveolitis[21,22]. The different forms of the disease (acute, subacute or chronic) depend on the exposure concentrations of these bacteria and length of the exposure time[22,23]. Workers may inhale anywhere between 500 000 to 750 000 spores per minute during a concentrated exposure and this may ultimately lead to pulmonary inflammation[15]. Extrinsic allergic alveolitis is a T-lymphocyte dependent granulomatous inflammatory reaction of the alveoli of the lungs[23]. Although a clear dose-response relationship for extrinsic allergic alveolitis and concentration of thermoactinomycetes and thermophilic actinomycetes is yet to be determined symptoms increase in a dose-dependent way[24].

Hypersensitivity pneumonitis has also been described in highly sensitised animals[25]. When animal feed, made up of hay and grain, becomes damp in environments with elevated temperatures (e.g. in a storage unit that may not be completely isolated from rain or other water entry), thermoactinomycete spore concentrations increase thus putting the animals at risk of inhaling antigens of *Thermoactinomyces* species[25]. Cattle have been known to develop hypersensitivity pneumonitis during the winter period when they are confined to their stables. Growth of *Thermoactinomyces* species occurs in their feed and other organic material resulting in inhalation of high concentrations of spores[25,26]. Hypersensitivity pneumonitis may also develop when cattle are moved from a dry stable to lush pastures at the end of summer, where these bacteria may be present[26]. When cattle eat feed containing thermoactinomycetes they excrete viable spores that are spread on fields and within soil that can remain there for many years until suitable germination conditions arise[27].

So far, the limited research that has been done in Australia has mixed results. A report by McNeill[27] around Queensland sugar mills

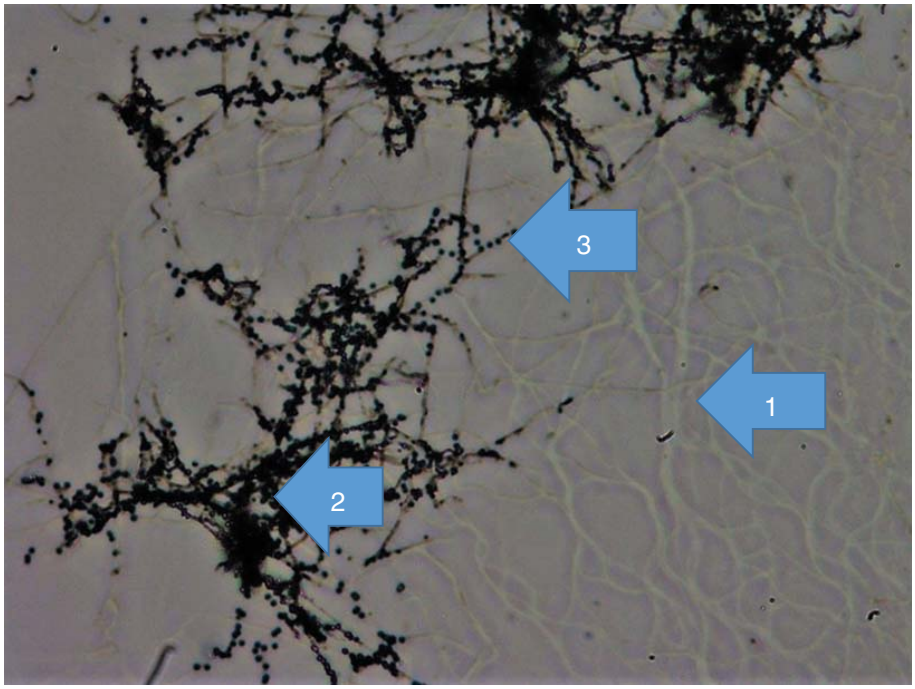

Figure 1. (1) Substrate, (2) aerial and (3) spores produced by the isolate USC-4005 found to be closely related to *Laceyella sacchari*.

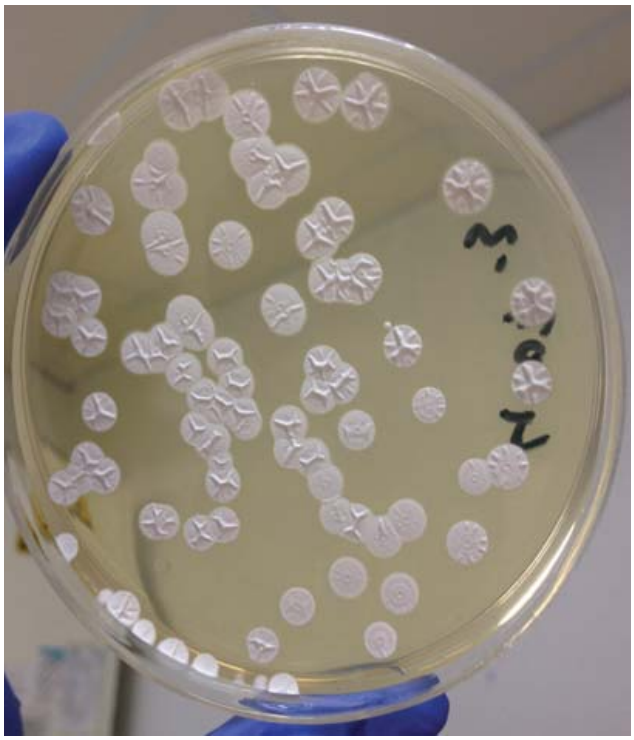

Figure 2. Growth of the isolate USC-4002 found to be closely related to *Laceyella sacchari* on a Tryptic Soy agar plate following the adhesion assay.

concluded that the concentration of bacteria and spores were just below the range to cause respiratory disease compared to other agricultural environments (such as sugar cane and sawmills) in other countries[28]. However, a pilot study, carried out in September to October 1989 around Queensland sugar mills, concluded that workers in these environments presented with symptoms related to certain occupational lung disease[29]. In a more recent study Brinkmann *et al*.[30] investigated selected overheated substrates commercially available for public use in sub-tropical Queensland in Australia and detected the presence of thermoactinomycetes (Figure 1). Subsequent molecular identification of the isolates confirmed their close relationship to previously reported allergenic *Thermoactinomyces* vulgaris and *Laceyella sacchari*. The isolates were also found to display adhesion ability and cytotoxicity to human lung cells (Calu-3 (ATCC®) HTB-55™). Figure 2 illustrates the growth of the isolate USC-4002, found to be closely related to *Laceyella sacchari*, on an agar plate following the adhesion assay indicating a high degree of adherence as non-adherent cells would have washed away during the completion of the assay. These findings might encourage further in-depth studies and continuous screening of EAA causing thermoactinomycetes and thermophilic actinomycetes in tropical and sub-tropical regions to prevent occurrence of the Farmer's Lung and similar diseases in Australia[31]. Furthermore, monitoring farm hygiene and air quality will contribute towards protection of agricultural workers and farm animals. These preventative measures are essential for the maintenance of healthy farm environments via ensuring the elimination of EAA causing bacteria.

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Biographies

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Mixed community biofilms and microbially influenced corrosion



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Metals are used in most marine infrastructures for energy extraction and production. Metal corrosion is a serious concern, due to the environmental, safety, and replacement costs associated with it. Microbially influenced corrosion (MIC) contributes to the overall corrosion process, through several chemical, electrochemical and biochemical

mechanisms, particularly in the presence of microbial biofilms. In this short article, we discuss briefly recent advances in MIC research, comparing corrosion in single species and mixed species biofilms, and outline possible strategies for biofilm and corrosion control.

The direct loss from corrosion in 1994 to the US industry was approximately 4% of the Gross National Product (GNP), with the highest cost in the power industry. A recent study estimates that the cost of corrosion in China is about 3.3% of the GNP, with the transportation and electronics industries bearing the highest costs. Older reports from Australia estimate a cost around 1.5% of the GNP, highlighting how corrosion control measures will contribute to national economy[1–3].

While most materials are affected by corrosion and weathering processes, it is a concern for metals due to their ubiquitous applications and their importance in advanced technology infrastructures such as oil rigs and processing plants. The corrosion of metals in marine environments is much faster than in freshwater and particularly problematic due to the possible release of crude and processed oil in a sensitive environment, which might affect marine ecosystems.

Corrosion of metals in seawater depends primarily on the charge imbalance in the electrical double layer at the metal/water interface; the presence of impurities that start pitting corrosion; and the co-occurrence of different metals, which create sites for galvanic corrosion. However, it is well established that microorganisms also play an important role in the corrosion process, known as microbially influenced corrosion (MIC). Mechanisms invoked to explain MIC include the formation of differential aeration cells caused by oxygen respiration; production of corrosive agents such as sulfide by sulfate-reducing bacteria (SRB) and organic and/or inorganic acids; metal-deposition; hydrogen embrittlement; the metal-binding effect of extracellular polymeric substance (EPS), and inactivation of corrosion inhibitors, with no specific mechanism playing a major role[3]. Most MIC mechanisms hypothesise that SRB play the major role in MIC, yet corrosion clearly also occurs in sulfate-free, anaerobic and even aerobic environments, although at lower rates. This highlights that many other microorganisms might contribute to MIC in mixed microbial communities.

Most recent strategies for corrosion inhibition draw from current experience in medical biofilm removal and focus on dispersal/inhibition, rather than lethal treatments. This opens new avenues for sustainable corrosion control in the shipping and oil and gas industries. In the following, we will outline a few aspects of MIC in biofilms and seawater, and the related research prospects.

Single species biofilms

Research on simple systems, such as monospecies biofilms, has revealed the existence of multiple MIC mechanisms. In fact, biofilms can either protect the metal surface from corrosion or enhance the corrosion rate, depending on the species considered. For example, *Pseudomonas aeruginosa* was shown to accelerate the corrosion of different grades of duplex steel and nickel-free stainless steel[4]. The same species promoted corrosion of nickel-copper coatings yet inhibited corrosion of nickel-zinc coatings[5]. Biofilms formed by *Vibrio neocaledonicus* appeared to inhibit corrosion of carbon steel in artificial seawater[6], while *Chlorella vulgaris* accelerated stainless steel corrosion in seawater[7]. The dual roles of biofilms in MIC were recently reviewed[8], and it was suggested that these variable effects might depend on biofilm matrix overproduction in monospecies biofilm or removal of oxygen through aerobic respiration.

Using these monospecies systems, some of the MIC mechanisms that have been demonstrated or hypothesised include adsorption or chelation of metals by proteins and the formation of an anaerobic/aerobic interface, which results in surface deterioration and depletion of the passivation layer, respectively. Work on electroactive bacteria, such as *Geobacter* sp.[9] and *Shewanella* sp.[10] has shown that direct electron transfer from the metal to the cells in the biofilm can enhance corrosion rates. The extracted EPS from iron-oxidising bacteria can either inhibit or enhance corrosion effects, depending on its concentration, age, and the presence of active enzymes[11]. Further, some of these differences in whether an organism is protective or corrosive could also be related to differences in medium composition and environmental conditions, which strongly affect the physiological activity of the microorganisms involved. SRB biofilms show enhanced corrosion under starvation conditions and this has been linked to their use of elemental iron as energy source in the absence of an organic energy source[12]. Similar results were observed for *P. aeruginosa* biofilms on carbon steel[13], where the starved sessile cells switched to elemental iron as an electron donor. Further, deaeration methods change the outcome of biocorrosion for stainless and mild steel as well as titanium[14]. It is also possible that the source and quality of the steel plays a role in these differences, due to the presence of impurities, which initiate the corrosion process. Overall, these and other studies on monospecies biofilms suggests that MIC is common in microorganisms and one should look beyond SRB to explain MIC in sulfate-depleted environments. They also highlight the multiplicity of MIC mechanisms and the need for standardisation of approaches to readily compare results across different studies[14].

Mixed biofilms

While research on monospecies biofilms has revealed much about the roles and mechanisms of microorganisms with respect to corrosion, there are relatively few habitats that are comprised of a single bacterial species. Indeed, most habitats would be comprised of hundreds or thousands of species of microorganisms and this would certainly be true for steel structures in marine habitats. However, studies of multispecies biofilms are complex, with higher levels of variation (thus requiring increased replication). The challenges here are that most studies of complex biofilms have investigated natural communities, which are highly variable with season and environmental conditions (e.g. nutrient concentration, temperature, etc). In contrast, fewer studies on defined communities that enable more detailed mechanistic studies have been reported.

In contrast to monospecies systems, mixed microbial communities change with time, and this should be accounted for when studying MIC. For example, the succession of microorganisms during colonisation and the community diversity observed on concrete materials indicate that the community decreases in diversity as MIC proceeds and the pH decreases. A similar study on mild steel in the marine environment showed that iron-oxidising bacteria are early colonisers and other bacteria join the community later, which can accelerate the MIC process[15]. Our work[16] showed a marked difference between mixed microbial communities from seawater before and after biofilm formation on stainless steel coupons (Figure 1), which is similar to what observed in oral biofilm development, where there is a reproducible pattern of colonisation. The different succession patterns observed in concrete and steel suggest that the formation of a mixed community associated with MIC is a complex phenomenon, and it may be that the early colonisers are also habitat-modifying organisms that produce the niches appropriate for the corroding organisms. For example, the formation of a biofilm under aerobic conditions can nonetheless result in anaerobic pockets where the anaerobes thrive. Similarly, the activity of community members can change nutrient availability, alter nitrogen and sulfate species as well as provide a spatially structured environment for microorganisms to interact through the exchange of metabolites. It is our opinion, supported by some experimental evidence, that such community systems can

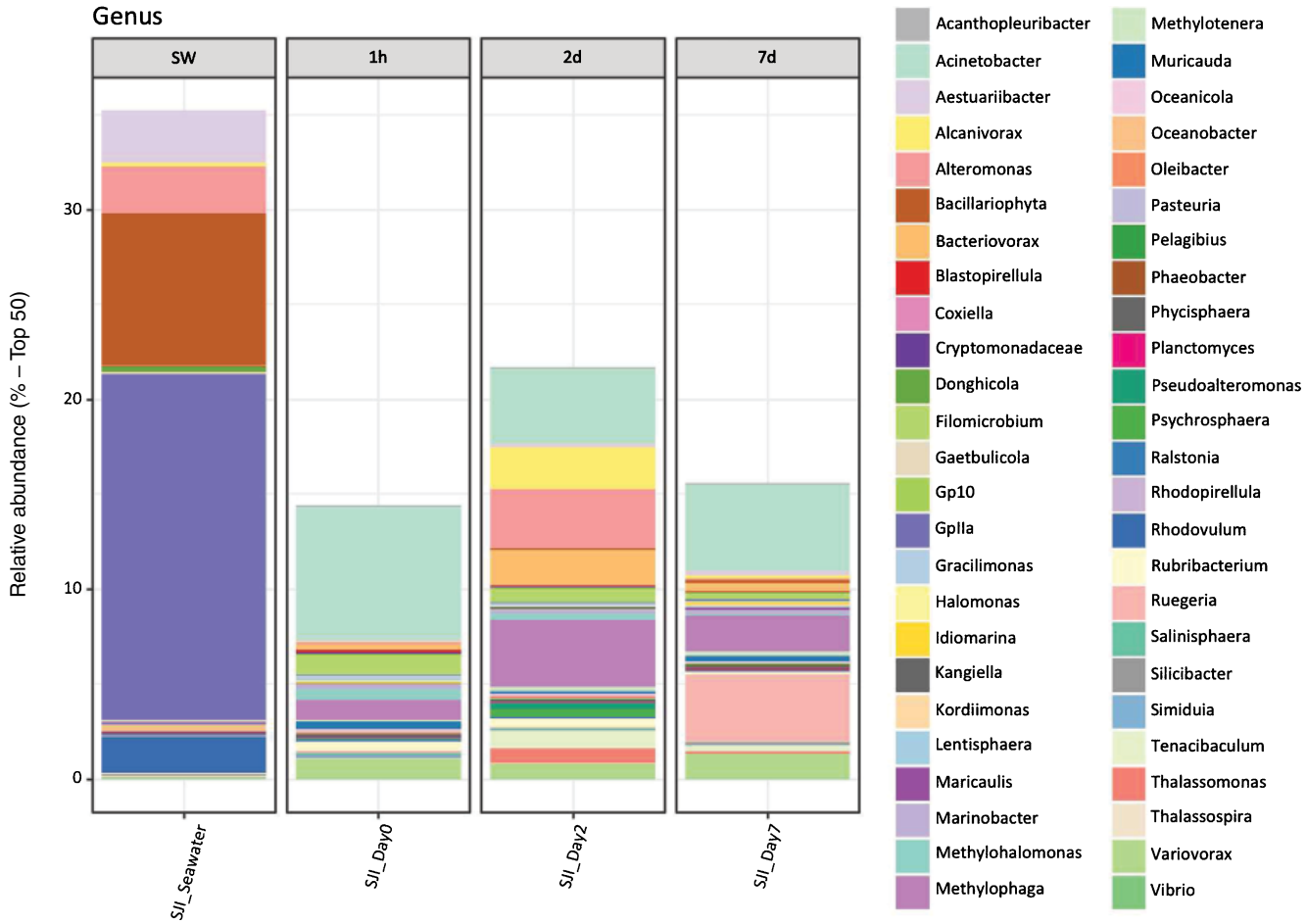

Figure 1. Comparison of microbial communities in seawater and in biofilms formed on UNSS32750 stainless steel coupons (reproduced with permission from Jogdeo *et al*.[16]).

be used in the laboratory to incorporate this high level of diversity to study how microorganisms function at the system level to cause corrosion.

Research on the microstructure and microbial ecology of mixed microbial communities and biofilms has helped to increase our understanding of MIC and its underlying mechanisms. Recent studies on the biofilm matrix[17] have shown how the matrix can concentrate corrosive metabolites at the interface, thus accelerating local corrosion rates. Examples include sulfide accumulation in SRB[18] and the formation of SRB biofilms on inorganic sulfides deposited on steel[19]. The matrix can also facilitate the concentration of quorum-sensing effectors, and thus accelerate cell–cell communication-based gene expression responses[20]. Finally, biofilm electroactivity has provided further theoretical background to explain MIC in terms of direct electron transfer between the metal surface and biofilms, particularly under starvation conditions[21]. The type of metal used can also select for different communities, where we have observed strikingly different communities on low-grade stainless steel and superduplex stainless steel, most likely due to the presence of metals that are toxic to microorganisms (unpublished data). The complex microbial ecology of marine biofilms elicits multiple corrosion routes, which are affected by iron oxidisers, iron reducers, methanobacteria, fermenters and other specialised microorganisms (Figure 2)[22].

Control of biofilms associated with MIC

One of the highly sought-after goals of MIC studies is to develop effective control measures. These can either be in the form of ways to keep the corroding microorganisms in check or to develop advanced surveillance methods that can readily give operators better information on *in situ* corrosion rates. For example, biocide enhancers such as D-tyrosine and D-methionine increase activity of tetrakis hydroxymethyl phosphonium sulfate (THPS) and can help to delay biofilm formation[23], thus maintaining an intact passivation layer, which reduces the corrosion rate. Further, hydrogen peroxide inhibits microbial colonisation but did not increase abiotic corrosion, and the addition of MgO_2 slows down SRB growth, thus inhibiting corrosion on carbon steel[24]. Virulent phage have been also proposed to target microorganisms associated to MIC in mixed microbial communities, although there are few demonstrations of this in practice.

Microbial composition is not the only MIC determinant. The biofilm microstructure seems also important, as patchy biofilms do not protect from corrosion in saline media, while a uniform, homogenous biofilm layer does appear to protect[25]. The presence of inorganic deposits can protect bacteria from the external environment and thus decrease the activity of biocides in real seawater[26].

Sub-lethal biofilm treatments

While conventional treatments for MIC site sanitation use highly toxic agents, such as glutaraldehyde and benzalkonium chloride, MIC control strategies typically use sub-lethal concentrations of chemical agents that disperse biofilm and minimise the extent of corrosion. This approach was first developed for biomedical equipment and only recently extended to MIC. However, no commercial

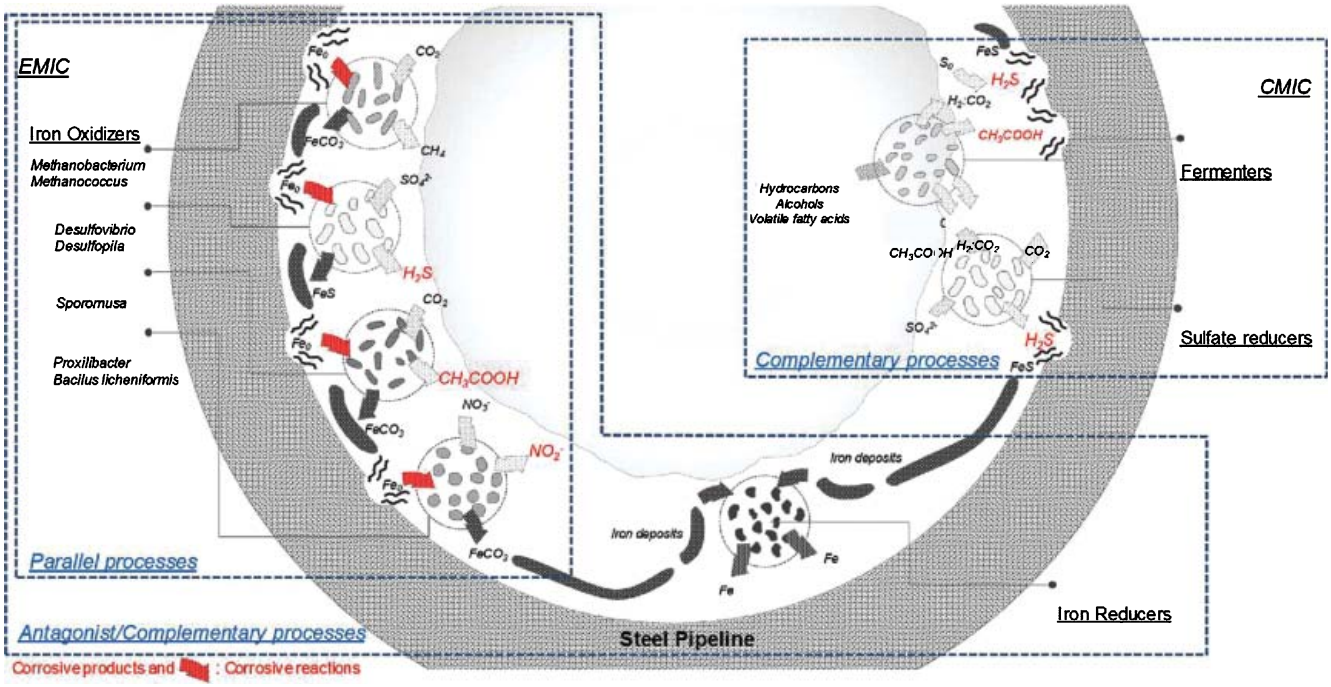

Figure 2. MIC mechanisms in mixed microbial communities (reproduced with permission from Vigneron *et al*.[22]).

product is available yet. For example, 100 ppm D-methionine decreased corrosion of monospecies *Desulfovibrio* biofilms by 50% without any effect on the planktonic cells, indicating the mitigation is due to biofilm dispersal only[27]. Similar effects were observed for mixed species biofilm consortia from an oilfield[12]. Additionally, a mixture of amino acids has been shown to partially inhibit MIC in mixed biofilms[23], presumably by altering community metabolism such that the corrosive metabolites were not produced or did not accumulate. MIC can be enhanced by the presence of microbially produced mediators, such as flavins, which enhance electron uptake from the metal surface[28]. Thus, removal of these mediators might help in mitigating MIC. Other MIC control strategies use surfactants[29] and mixed-type inhibitors carbazole derivatives to inhibit SRB biofilm formation[30]. Ideally, biofilm inhibitors should be applied at the metal surface, and engineered to allow slow release over long periods of time. Alternatively, the inhibitors could be triggered for release in response to the presence of corrosion-related organisms, which could be achieved by metabolic, physiological or physical conditions (e.g. low pH) specific for corroding organisms. Thus, there are a range of mild biofilm control approaches that could be used to delay or reduce corrosion rates, although they remain to be demonstrated *in situ*. Some of the challenges around such approaches are related to the cost (due to the large volume/surface to be treated) and method of application. For example, a coating would be most likely to localise activity where it is needed, but is subject to loss of function due to damage to the coating and depletion of active compounds over time[31].

Summary and Conclusions

There is a considerable appreciation for the roles of microorganisms in mediating corrosion processes in conjunction with abiotic mechanisms. However, further studies on how microbial biofilm communities form on, and contribute to, the corrosion of metals and other materials, are needed. The challenges partly lie in the complexity of the problem, with multiple organisms working together, each influencing their neighbours as well as the surface in question. Then there is the challenge of understanding the microbe-surface nexus which is further complicated by batch variations of materials and compositional differences. However, the introduction of reproducible community systems, coupled with solid interdisciplinary collaboration and advanced analysis approaches may help to further elucidate how microorganisms manage to modify their environment in such a dramatic fashion.

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Biographies

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Scott A Rice completed his PhD in 1996 from the University of Tennessee, USA, after which time he took up a post-doctoral position at The Centre for Marine Bio-Innovation at UNSW. More recently, he has taken a position at The Singapore Centre for Environmental Life Sciences Engineering and the School of Biological Sciences at Nanyang Technological University. His interests are in microbial communities, with a focus on their function and formation.

Thiocyanate biodegradation: harnessing microbial metabolism for mine remediation

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Thiocyanate (SCN^-) forms in the reaction between cyanide (CN^-) and reduced sulfur species, e.g. in gold ore processing and coal-coking wastewater streams, where it is present at millimolar (mM) concentrations[1]. Thiocyanate is also present naturally at nM to µM concentrations in uncontaminated aquatic environments[2]. Although less toxic than its precursor CN^-, SCN^- can harm plants and animals at higher concentrations[3], and thus needs to be removed from wastewater streams prior to disposal or reuse. Fortunately, SCN^- can be biodegraded by

microorganisms as a supply of reduced sulfur and nitrogen for energy sources, in addition to nutrients for growth[4]. Research into how we can best harness the ability of microbes to degrade SCN^- may offer newer, more cost-effective and environmentally sustainable treatment solutions[5]. By studying biodegradation pathways of SCN^- in laboratory and field treatment bioreactor systems, we can also gain fundamental insights into connections across the natural biogeochemical cycles of carbon, sulfur and nitrogen[6].

Thiocyanate: a common wastewater contaminant

Thiocyanate (SCN^-) is a common contaminant associated with a range of industries, and is typically found at its highest concentrations in the wastewater of gold and silver mines as a result of the use of CN^- as a lixiviant during ore processing[7]. Thiocyanate is also commonly a component of coal coking wastewater alongside phenol and CN^-[8]. These typically voluminous waste streams pose a serious environmental hazard due to their persistence and toxicity. Although a number of chemical SCN^- degradation techniques exist, they are often inefficient and expensive[9]. Alternatively, many mines choose to store contaminated tailings indefinitely in dam structures, and re-use the SCN^- contaminated water during ore processing. However, the presence of SCN^- in this re-used water is known to impact gold extraction efficiency negatively[10], as well as to impede the metabolism of biomining microorganisms[11]. Improved SCN^- treatment systems, therefore, offer an opportunity improve the sustainability of mining processes globally.

Diversity of thiocyanate-degrading microorganisms

Thiocyanate offers a rich source of growth nutrients and energy to microorganisms, in the form of reduced sulfur and nitrogen, and a number of microbial species are known to be capable of SCN^- degradation[4]. Importantly, these species do not belong to a distinct phylogenetic group, and the presence/absence of SCN^--degrading potential is often even strain specific. This complicates their identification using phylogenetic markers, such as the 16S rRNA gene. Much of what is currently known has, therefore, been achieved through culturing experiments. These studies have revealed diverse metabolic traits associated with SCN^- degradation, where chemolithotrophs utilise the reduced sulfur as an energy source[12–14], and heterotrophs utilise the nitrogen as a growth nutrient[15–17]. Despite SCN^- biodegradation being widely regarded as an aerobic process, one bacterium (*Thialkalivibrio thiocyanodenitrificans*) was found to be able to couple this process to nitrate reduction[14], opening up the possibility of anaerobic SCN^- degradation.

Experimentation on culturable strains has also allowed the elucidation of two distinct pathways of SCN^- degradation. These pathways proceed via two intermediates, carbonyl sulfide (COS) or cyanate (CNO^-), catalysed by one of two distinct types of SCN^- hydrolases (SCNase)[18,19], or by an SCN^- dehydrogenase (TcDH) enzyme[20,21], respectively. The resulting COS and CNO^- intermediate chemical species are then available for degradation by enzymes associated with β-carbonic hydrase[22] or cyanate anhydrase[23]. Both of these SCN^- biodegradation pathways result in the release of reduced sulfur (S^{2-} or S^0), NH_4^+ and CO_2.

The advent of high-throughput sequencing, and the rise in genome/metagenome and proteome/metaproteome sequences, has yielded vast databases of protein and DNA sequences. Significantly, protein sequences for the three known SCN^--degrading enzymes are available, which allow a deeper look into the distribution of SCN^--degrading enzymes. The three-subunit SCNase enzyme, originally isolated in *Thiobacillus thioparus* THI115[18], is the most widely identified enzyme in protein databases, with 416 sequences identified as the γ-subunit in the NCBI nr database. These sequences primarily belong to the Actinobacteria, due to a bias towards full genome sequences of the medically significant *Mycobacterium* genus. They also contain sequences belonging to a number of thiobacilli, sulfur-oxidising γ-proteobacteria (including a number of Chromatiales) and α-proteobacteria (*Methylobacterium* spp. and *Sphingomonas* spp.). The alternative SCNase and the TcDH have far fewer closely related sequences in the NCBI non-redundant protein sequence database. This SCNase has representatives encoded in a number of thiobacilli, *Afipia* spp. and sulfur-oxidising γ-proteobacteria. The TcDH, originally purified from two *Thioalkalivibrio* spp.[21], appears to be closely related to other *Thioakalivibrio*, and more distantly related to proteins of unknown function in *Thioploca ingrica*, Nitrospirae and *Hydrogenobacter thermophilus*. The comparatively few metaproteome and metagenome studies, targeting SCN^--contaminated systems, limit our understanding of the true scope of the distribution of these enzymes.

Thiocyanate biodegradation triggers complex microbial community interactions

Although SCN^- degradation is undertaken by a limited number of bacterial species (or strains), wider implications can result for the whole microbial community due to the roles its constituent elements can play as biological energy sources or growth nutrients. As a result, interesting and potentially useful symbiotic or dependent relationships can develop between SCN^- degraders and non-SCN^- degraders (Figure 1).

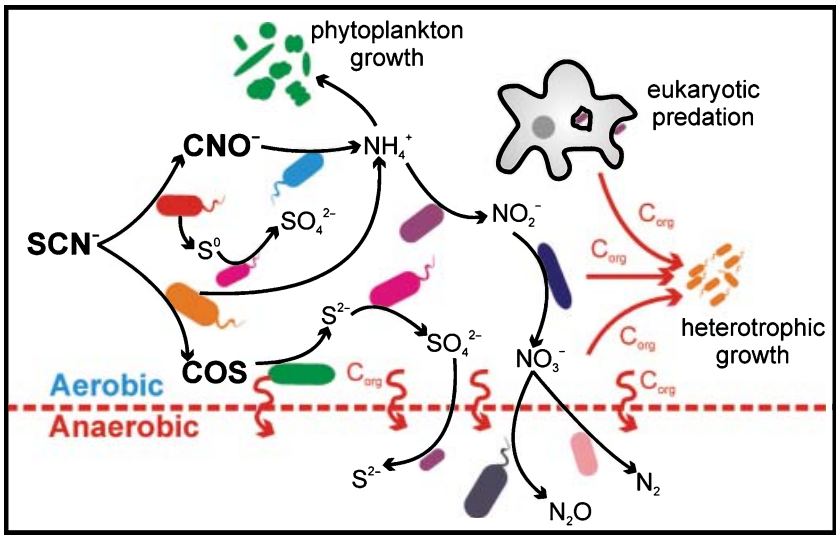

Figure 1. Thiocyanate biodegradation can trigger a complex series of interactions within a microbial community revolving around the cycling of carbon, nitrogen and sulfur. The initial degradation is primarily an aerobic process mediated by sulfur-oxidizing chemolithotrophs capable of CO_2 fixation. These primary producers are able to supply recycled organic carbon (C_{org}) to a diverse population of heterotrophic bacteria. The released NH_4^+ is available as a growth nutrient to the community or an energy source to nitrifying bacteria.

Thiocyanate-degrading bioreactors are often dominated by SCN^--degrading and sulfur-oxidising strains of the thiobacilli[24,25]. Although autotrophic, these bacteria have also been found to dominate in systems fed with a labile carbon source[26,27]. Their dominance is a testament to the strong selective pressure that SCN^- exerts on a microbial community. The presence in these systems of a number of sulfur-oxidising taxa that do not genetically encode for known SCN^--degrading enzymes suggests a fraction of the reduced sulfur is shared within the community. The eventual liberation of NH_4^+ from SCN^- degradation also opens up potential cross-feeding relationships. Several SCN^--treating systems were found to sustain populations of nitrifying bacteria, utilising NH_4^+ as an energy source and lacking the ability to degrade SCN^-[24,26]. The subsequent nitrate can also supply an electron acceptor for denitrification in anoxic regions of these systems[26]. The supply of NH_4^+ as a nitrogen-containing nutrient likely provides the primary source of nitrogen for growth of microbial communities inhabiting these systems[25]. This nitrogen can also support phytoplankton growth under phototrophic conditions[28]. These symbiotic relationships have the potential to be harnessed for the effective removal of nitrogen from wastewater following SCN^- degradation.

As SCN^- degradation is able to support dominant populations of chemolithotrophs, it has the potential to exert a large influence on carbon cycling in SCN^- treatment systems. Indeed, the SCN^--degrading thiobacilli and *Thioalkalivibrio* are known to utilise the Calvin-Benson-Bassham cycle to fix CO_2[21,26]. In the absence of a labile carbon source, the sulfur- and nitrogen-oxidising chemolithotrophs can provide the primary source of carbon in the system. Our own work, for example, found that a SCN^- treatment system operated without labile carbon input, and was dominated by chemolithotrophs, sustaining SCN^- degradation and a considerable diversity of heterotrophic microorganisms[25]. This study also implicated a role for bacterivorous Amoebazoa in preying on the microbial community and closing the microbial loop in the system.

Implications for thiocyanate-degrading biotechnology

Microbial communities capable of SCN^- degradation offer an opportunity to treat large quantities of wastewater effectively. Such systems, adopting various designs, have already been deployed with success at field scale[24,29,30]. Much research is now going into understanding these systems at the molecular scale, and developing more efficient processes. Understanding the aforementioned cross-feeding relationships within SCN^- treatment systems are vitally important to this process improvement. A good example is the revelation, achieved through a genome-resolved metagenomics approach, that even in organic carbon-fed systems, the community can be dominated by SCN^--degrading autotrophic bacteria[26]. Our own work has proven experimentally that SCN^--degrading bioreactors can be operated in the absence of organic carbon amendments[25]. This autotrophic SCN^- degradation approach, combined with nitrification and denitrification, has been deployed at pilot scale to treat contaminated groundwater at Stawell Gold Mines in Victoria (Figure 2). This process is able to completely degrade the influent SCN^- (300–400 mg L^{-1}), resulting in sulfate and N-containing products. By harnessing various metabolic niches the initially released NH_4^+ is nitrified to nitrate and subsequently removed by denitrification. In addition to this

Figure 2. A pilot-scale engineered approach to the biological treatment of SCN^--contaminated groundwater at a Victorian gold mine. The bioreactors contain autotrophic and heterotrophic microbial communities capable of degrading SCN^-, complete nitrification and denitrification. Photo taken by M. Watts.

engineered approach, we reported for the first time the ability to promote *in situ* SCN^- biodegradation in contaminated gold mine tailings water held in large open air storage facilities[9]. This process was promoted through phosphate nutrient addition alone, and offers a passive approach for the treatment of large quantities of contaminated material. Our future work aims to better resolve the active metabolic interactions within the microbial communities in these *in situ* and *ex situ* approaches. This will help us to promote beneficial symbiotic relationships within the system.

Collectively, the advances in our understanding of the metabolisms underpinning SCN^- biodegradation allow for better designs and approaches to harnessing this microbial potential. Biodegradation of SCN^-, therefore, is offering a route to improving the water efficiency of industrial processes such as gold mining on a global scale.

In conclusion, much has been learned at the molecular scale about biodegradation of SCN^- since this metabolic trait was first reported in *T. thioparus*[12], including the enzymes responsible and the community wide biogeochemical impacts. The advent of 'multi-omics' approaches is allowing us to probe these processes *in situ* in SCN^--biodegrading treatment systems. The few studies utilising these techniques have spurred significant process improvements, in addition to revealing fundamental insights into SCN^- biodegradation and the subsequent metabolic cycling of its breakdown products. These insights, although gained from engineered systems, can help inform the global cycling of SCN^-, CNO^- and COS and better constrain the role of microbial communities in the wider carbon, nitrogen and sulfur cycles. Given the limited number of systems investigated to this depth, more work is needed to appreciate fully the diversity and complexity of microbial communities degrading SCN^-.

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Biographies

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Mathew P Watts is a Postdoctoral Research Fellow in geomicrobiology at the School of Earth Sciences, The University of Melbourne. His primary research project focuses on understanding and developing a biological approach to thiocyanate remediation at a gold mine in Victoria. His general interests in the field extend to how microbial metabolic activities impact the form and fate of metals and organics in the environment.

Activated sludge foaming: can phage therapy provide a control strategy?

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Foaming in activated sludge systems is a global problem leading to environmental, cosmetic and operational problems. Proliferation of filamentous hydrophobic bacteria (including the Mycolata) are responsible for the stabilisation of foams. Currently no reliable methods exist to control these. Reducing the levels of the filamentous bacteria with bacteriophages below the threshold supporting foaming is an attractive approach to control their impact. We have isolated 88 bacteriophages that target members of the foaming Mycolata. These double stranded DNA phages have been characterised and are currently being assessed for their performance as antifoam agents.

The activated sludge process

The activated sludge process is a robust and proven system for treating domestic and industrial wastewater and is used globally[1]. It relies on a specialised community of microbes organised into structures called flocs, which metabolise organic nutrients and remove inorganic nitrogen and phosphorus compounds so that the treated effluent can be discharged safely into a receiving body of water without leading to eutrophication from growth of toxic *Cyanobacteria*[1].

These systems are no longer considered as wastewater water disposal systems, but as valuable sources of purified water for reuse and useful chemicals. Despite their popularity most suffer from the problem of foaming where a brown foam layer develops on the reactor surface and leaves in the treated effluent[2].

What is foaming?

Foaming, which increases plant operating costs, reduces effluent quality and acts as a source of opportunistic human pathogens, is a flotation event, requiring three components; air bubbles, surfactants and hydrophobic particles (bacterial cells), which act to stabilise it (Figure 1). With only air bubbles and surfactants, an unstable foam forms, and is often seen in start-up, where abundances of hydrophobic bacteria are below the threshold supporting foam formation[3]. With insufficient levels of surfactants, air bubbles collapse and a greasy surface layer, a scum, forms, consisting of hydrophobic bacteria. There are no reliable control measures to deal with an already established foam, but any proposed strategy should target the hydrophobic bacterial cells, since control of the other two is impractical.

It is now clear that a diverse range of bacteria are responsible for foaming episodes[4–8]. Theoretically, any sufficiently hydrophobic cell can stabilise this foam, but surveys suggest that the unbranched actinobacterial filamentous organism '*Microthrix parvicella*' and the right angled branching mycolic acid producing filaments placed in the Mycolata (include members of the genus *Gordonia*, *Nocardia, Rhodococcus, Tsukamurella, Skermania* and related members)[5,7] are the main culprits (Figure 2). Being strongly hydrophobic, these organisms escape the plant bulk liquid to the air liquid interface, often carrying biomass or sludge with them, and there attach to the liquid air films of the bubbles, preventing liquid drainage from them and hence stabilise them.

Foaming control

The conventional way to deal with existing foams is commonly non-selective using bactericidal chemicals, where organisms other than those causing foaming are likely to be harmed. Others include changing aeration rates, or reducing sludge ages hoping that foaming organisms, assumed to be slow growing, are washed out. Unfortunately other desirable bacteria are also lost. All

Figure 1. Foam covering the surface of an aeration basin and walkways.

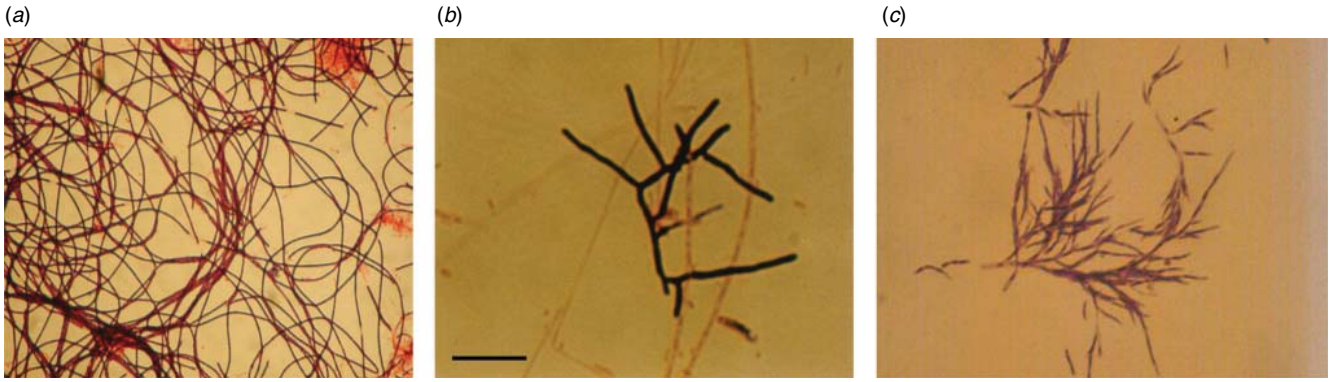

Figure 2. Gram stain of (*a*) '*Microthrix parvicella*', (*b*) *Gordonia amarae* like organism (GALO) and (*c*) *Skermania piniformis. Gordonia amarae* like organism (GALO) and *Skermania piniformis* are right angled branching mycolic acid producing filaments and belong to the Mycolata.

reflect our inadequate understanding of the microbial ecology of foams.

What is needed is a specific control strategy, which is environmentally safe and importantly only removes the nuisance organisms[9,10]. Bacteriophages (or phages), viruses that specifically target only their bacterial hosts, and are naturally occurring and self-dosing, seem especially attractive. They are used clinically to treat infectious bacterial diseases, where the causative organism is antibiotic resistant[11]. As they infect their hosts, they replicate and upon lysis, release often hundreds of new phages that then infect other host cells.

The general experience has been that wherever bacteria are present, phages able to lyse them will also be present. Consequently, phages lytic for members of the foaming Mycolata should be plentiful in activated sludge. Thomas and colleagues[9], demonstrated that phages, some polyvalent, are isolated readily from activated sludge plants, capable of killing their Mycolata hosts under laboratory conditions. What we know of phage/host population dynamics suggest that their presence would not lead to the total loss of their bacterial hosts. Such outcomes would be disastrous, since Mycolata play important roles in metabolising recalcitrant xenobiotics there. Strategically the aim is to reduce Mycolata numbers below individual threshold levels needed for stable foam formation. This requires identifying which are the causative organisms. FISH probing provides the tools to screen foam samples, and while their true level of biodiversity, is not known, FISH data suggest a limited number of foaming bacteria are common in plants.

What have we achieved?

The advent of Next Generation DNA Sequencing (NGS) has revolutionised our understanding of phage genomics and allowed us to screen those attractive for phage therapy, avoiding those possessing virulence or toxin genes. We have isolated 88 double stranded DNA phages that seem suitable for further study. These include phages against foaming *Gordonia*[12,13], *Rhodococcus*[14–16], *Nocardia*[17], *Skermania*[18] and *Tsukamurella*[19,20]. While most are monovalent, polyvalent phages are clearly more attractive, since most foams contain more than one Mycolata member. Not surprisingly, sequencing reveals that all are highly novel at the DNA level, but share the same genomic arrangements. They have all been screened against foaming Mycolata hosts using a simple foaming apparatus[21]. Almost always their foaming abilities were reduced to the point where no foam was detected as previously described[13,22].

Where next?

The next step is to scale up the system. Before this is warranted, it is important to determine their host specificities and burst sizes *in situ*, their persistence times in full scale plants, the host cell threshold values for foam production and how much inoculum is required. Equally, the location for introducing the phages into the system is likely to be important. These parameters are plant specific, and so will need to be determined on an individual basis. Whether these phages are involved in gene transfer between host cells (transduction), and whether they acquire, as a consequence, antibiotic resistant genes and hence pose a possible threat upon release into the environment, will need investigation. In addition, the possibility of the bacterial strains developing phage resistance will need to be investigated and one possible solution would be to add multiple phages for the same host as multiple mutations is less likely.

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Microbiologically influenced corrosion in floating production systems

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Microbiologically influenced corrosion (MIC) represents a serious and challenging problem in Floating, Production, Storage and Offloading vessels (FPSOs), one of the most common type of offshore oil production facilities in Australia. Microorganisms can attach to metal surfaces, which under certain conditions, can result in corrosion rates in excess of 10 mm per year (mmpy) leading to equipment failure before their expected lifetime. Particularly, increasing water cut (ratio of water vs. total fluids produced), normally resulting from the age of the assets, results in an increased risk of MIC. This paper provides an overview of causative microorganisms, their source of contamination and the areas within FPSOs that are most prone to MIC. Although mitigation practices such as chemical treatments, flushing and draining and even cathodic protection are effective, MIC can still occur if the systems are not properly monitored and managed. A case study is presented that describes the microorganisms identified in a FPSO operating in Australia suspected of having MIC issues.

The production of oil and gas is of major importance to the stability of the world's energy supply. With the depletion of onshore reserves, offshore exploration and production of oil and gas has increased sharply. In particular, Western Australia's oil and gas industry is a vital component of Australia's national economy, producing over 70 per cent of Australia's natural gas, crude oil, and condensate[1]. A Floating, Production, Storage and Offloading vessel (FPSO) is currently the production facility of choice for oil accumulations uneconomic for fixed installations like platforms with around 70 FPSOs in operation worldwide, including seven in Australia (Figure 1). FPSOs carry all necessary production and processing facilities, tanks for storage of oil recovered from the reservoir as well as oil offtake systems. Typically the production fluids are separated into crude oil, formation water and gas. FPSOs are secured on site by mooring and station keeping systems and are connected to production wells in the seabed via subsea flowlines and flexible risers.

FPSO are designed to have an expected service life of 5–20+ years depending on reservoir size. For many FPSOs existing trading tankers have been converted to a FPSO so that the marine systems are already >10 years old. For FPSOs in operation, maintaining the asset as fit for service is critical. Water production increases with time as the oil in the reservoir is produced. As the assets get closer to the end of their service life, there is more need for managing corrosion[2]. In addition, improving facility economics can involve tie-in of new oil accumulations to existing ageing FPSOs[3]. This can bring new challenges to operations due to the mixing of water chemistries and oil with different properties.

In particular, microbial contamination and consequent microbiologically influenced corrosion (MIC) represents a serious problem to FPSO operations, which can incur significant costs, and be difficult to control[4,5]. The number of FPSOs that have been in operation for significant time has increased worldwide and the issues are usually common to all operators. However, conditions in Australia can be different from other oil and gas producing regions thus particular challenges can arise.

Microorganisms in petroleum reservoirs and FPSOs

Due to the *in situ* presence of substantial amounts of electron donors and electron acceptors for bacteria growth, diverse populations of microorganisms with a wide range of physiological and metabolic activities have been found in oilfield systems[6]. Microorganisms inhabiting petroleum reservoirs are typically capable of surviving in environments with high temperature, pressure and

Figure 1. Floating Production Storage and Offloading (FPSO) vessel operating in Australia.

salinity. Oil reservoirs with a water leg at temperatures less than 80°C are likely to have been biodegraded *in situ*[7]. Microorganisms commonly identified in these environments include sulphate-reducing prokaryotes (SRP), iron-reducing bacteria, fermenting bacteria and methanogenic archaea[8]. The majority of these microorganisms are strictly anaerobic. Largely, the availability of electron donors governs the type of metabolic activities within oil field ecosystems[9]. Typically, sulphate and carbonate are the most common electron acceptors present which suggests that the most significant metabolic processes occurring *in situ* are sulphate reduction, methanogenesis, acetogenesis and fermentation. In particular, the widespread presence of SRP in crude oil and formation waters and the concomitant production of H_2S have been extensively linked to deleterious processes in the oil and gas industry such as reservoir souring of crude oil systems and corrosion[10]. However, all of the aforementioned microbial groups have been previously associated with corrosion via different mechanisms[5]. To limit corrosion and microbial activity, produced water is typically treated with chemicals including corrosion and scale inhibitors, biocide and oxygen scavengers.

Source of microbial contamination of FPSOs

The presence of microorganisms in oilfield systems is usually the result of contamination by any number of mechanisms including from a biodegraded reservoir, contaminated mud during well-drilling operations, the use of raw seawater during subsea and topsides flushing operations, re-circulating waste oil from bilges back into the process as well as seawater injection into the oil reservoir for pressure support. However, several microorganisms retrieved from petroleum reservoirs are potentially indigenous to these ecosystems. Likewise, subsea flexible flowlines comprising an inner castellated or ribbed stainless steel carcass can become infected with microorganisms and provide a large surface area for sessile bacterial growth with estimates in the order of 2×10^{13} cells per m^2 (A. Polomka, Confidential Report, 2015). This provides a ready supply of bacteria to the FPSO as well as biogenic H_2S.

Typically, a FPSO is installed initially on a single field and provided the reservoir is consistent across the field, production will be the same. However, in several cases oil production from various reservoirs is co-mingled into one FPSO. In this case, different water chemistries are mixed with subsequent risk of scale formation and additional nutrients and microorganisms, which could result in an increased risk of MIC. Bacteria present at the front end will spread through the entire system including the back end oil offtake system in the water phase. Likewise, a FPSO can combine seawater injection with production that can potentially result in souring (sulphide production) and MIC in the facility thus requiring biocide injection into the reservoir. Values of >3000 ppm of H_2S

have been detected in the gas exiting the high-pressure separator on board the facility under these scenarios thus requiring H_2S scavenger chemical dosing to control the risk of sulphide stress cracking of carbon steel[11].

Major areas of MIC threat

The vast majority of MIC on FPSOs occurs on carbon steel components. Although MIC of corrosion-resistant alloys (CRAs), e.g. stainless steels, can occur in aerobic environments (e.g. seawater utility systems), hydrocarbon-processing trains on FPSOs are anaerobic and MIC of CRAs is not an issue. Souring of the reservoir is a common outcome of facilities that conduct produced water reinjection. This generally results in a higher than anticipated H_2S content in well, process and rundown streams.

Topsides process: Any carbon steel process piping, dead leg (a length of pipe which is rarely or never used) or vessel with free water contacting the surface, nutrients and a microbial consortia is prone to MIC. Increasing water cut results in increased nutrient levels and water wet carbon steel with very high sessile bacteria densities and high resultant corrosion rates. Topsides corrosion rates greater than 10 mmpy have been detected with no or inadequate control of MIC (e.g. full wall penetration of 12 mm thick produced water piping in 12 months). Maximum corrosion rates have been observed in the 30–50°C temperature range. Examples of MIC in topside facilities are shown in Figures 2, 3.

Cargo tanks: MIC can occur at the bottom of oil cargo tanks where anaerobic bacteria can thrive in the water layer that collects in the bottoms of the tanks and the tank coating has broken down or been damaged. MIC corrosion rates in the order of 1 mmpy under normal operation can occur but can be higher if excessive produced or seawater levels are allowed to sit in the tanks underneath the crude oil (A. Polomka, personal observation).

Slops tanks (cargo and bilges waste water handling system): Biogenic H_2S generation can be a concern with the inert gas vented to atmosphere. Corrosion can be further complicated if the tank has heater tubes to aid emulsion breaking as a galvanic component of the corrosion is possible. Corrosion rates, resulting from a combination of galvanic and MIC, in the order of 6–9 mmpy can be observed if untreated (A. Polomka, personal observation).

Crude offtake system: Although the crude offtake system typically contains less than 0.5% basic sediment and water (BS&W), MIC risk increases for pump room and offtake piping as offtake frequencies decrease and water settling out of the crude is left sitting in low points for longer. In addition, this can lead to internal coating breakdown. Regular flushing of the offtake system with raw seawater can markedly increase MIC rates due to the addition of trace oxygen and nutrients. Corrosion rates in the order of 3–4 mmpy have been seen with regular seawater flushing after each offtake (A. Polomka, personal observation).

Mitigation of MIC

Control of acid gas (CO_2 and H_2S) corrosion of carbon steel in oil and gas production requires the continuous dosing of an organic filming corrosion inhibitor at the ppm level upstream of the carbon steel. For MIC control, batch or shock dosing of a biocide (e.g. 250 ppm for 4 hours weekly) is used to limit MIC in the topsides process[12]. As part of this treatment any dead leg needs to be flushed to drain during the treatment to control MIC in the dead leg.

On some facilities, de-sulphated (via ultrafiltration and sulphate removal membranes) customised water flood (CWF) is used to maintain reservoir pressure while limiting souring and corrosion due to the activity of SRP. Removal of sulphate should dramatically change the microbiome of production facilities. In addition, since

Figure 2. Microbiologically influenced corrosion of high pressure separator horizontal baffle.

Figure 3. Microbiologically influenced corrosion of crude line to cargo storage (0.5% water cut).

FPSOs process oil from different reservoirs (dissimilar formation water chemistries and reservoir temperatures) differences in the microbial communities inhabiting these systems are also expected from site to site[6].

For crude rundown into the cargo tanks, a continuous dose of biocide e.g. 200–500 ppm based on the BS&W percentage present in the oil is carried out. Target BS&W levels of <0.5% are normal. In slops tanks generating H_2S, MIC control involves draining of the tank, application of batch dose biocide based on volume of water in tank (treat and soak) and repeat after in-boarding of water or time based depending on H_2S levels. MIC control in slops tanks involves the use of sacrificial anodes (cathodic protection) to maintain surface potentials more negative than –900 mV vs silver/silver chloride reference electrode (Ag/AgCl) to prevent MIC and galvanic corrosion as well as regular biocide for biogenic H_2S control.

Case study: identification of microorganisms in an Australian FPSO

MIC monitoring has traditionally been done via the serial dilution or most probable number (MPN) techniques. These techniques may only detect a small proportion (1–10%) of the natural community. More recently techniques measuring adenosine triphosphate and adenosine monophosphate (ATP/AMP) levels and molecular microbiological methods including next generation sequencing (NGS) have been implemented which provide better insights into the microbial community and their activity in the system[13,14].

Background: Produced water samples were gathered from a FPSO in operation in Australia that experienced recurring corrosion issues, for microbiological characterisation and MIC assessment. The FPSO processes oil, produced water and gas from a biodegraded reservoir. Produced water from this facility is known to have limited concentrations of sulphate. Temperature at the sampling location was 60°C. In addition, samples from a corroded surface (corrosion products on the metal piece) were also collected. Samples were inoculated in various culture media for the detection and enumeration of several metabolic groups. In addition, culture-independent 16S rRNA gene sequencing (Illumina MiSeq) was used to identify microbial populations and their relative proportion (%) in the samples using primers 341F and 806R for the detection of both bacteria and archaea.

Results: The most active populations detected via culture-dependent methods corresponded to methanogens and fermenting microbes with capabilities to reduce sulphur and thiosulphate into hydrogen sulphide (sulphidogenic species). Sulphate-reducing bacteria were not detected. DNA sequence analysis showed that methanogenic archaea were the predominant populations in the system. Specifically, the archaeal taxa *Methanobacterium*, *Methanothermobacter thermoautotrophicus*, *Methanothermococcus*, *Methermicoccus shengliensis* and *Methanoculleus* dominated in produced water and even more notably in corroded equipment. The dominant bacterial populations identified in the samples were *Thermovirga* and *Thermoanaerobacter* although populations including *Thermosipho, Thermothoga*, *Petrotoga*, *Kosmotoga* and *Anaerobaculum*, were also identified, albeit at

low proportions. In agreement with cultivation analysis, SRP species were not identified via DNA analysis.

Discussion: The low abundance of SRP appears to be associated with the lack of sulphate in produced waters. The abundance and predominant activity of methanogenic archaea and fermenting-sulphidogenic microbes possesses a risk of MIC. Methanogens have been described as corrosive microorganisms due to their hydrogenotrophic (hydrogen scavenging) capability that cause cathodic depolarisation thus accelerating corrosion. Direct electron extraction from iron by methanogens has also been postulated as an important MIC mechanism[5]. In addition, the dominance of active fermenting-sulphidogenic microbes suggests the presence of intermediate forms of sulphur in produced water such as elemental sulphur (S_0) and thiosulphate ($S_2O_3^{2-}$). These microorganisms can contribute to corrosion via sulphide generation and acid production[15]. There are also reports of deposits in pipelines harbouring mainly methanogens and sulphur/thiosulphate-reducing bacteria that have been associated with localised corrosion[16,17]. These results highlight the importance of studying and monitoring microbial populations other than SRP (SRP are commonly associated with corrosion of oil production equipment) since these microorganisms can potentially contribute to corrosion, particularly in systems exposed to production fluids with low levels of sulphate where such conditions are usually deemed low risk for MIC.

Conclusions

Carbon steel is a common material of construction of FPSO's due to its commercial attractiveness. However, carbon steel is subject to MIC in offshore oil and gas production at even low water cuts (<0.5%) under the right combination of nutrient availability, microbial populations and operating conditions supporting microbial activity. Control of MIC via regular batch or shock dosing of biocides and in tanks by continuous biocide dosing and cathodic protection is used to prevent leaks. Monitoring of bacterial numbers, speciation and activity is required to validate management strategies or to validate alternative control options. Molecular microbiological methods (MMM) are critical to enable biocide assessment for MIC control and to elucidate possible MIC mechanisms.

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Biographies

Dr Laura L Machuca leads research and industry projects on microbiological influenced corrosion (MIC) at the Curtin Corrosion Centre (Curtin University). She is an environmental microbiologist and a corrosion specialist whose research and teaching activities focus on the interaction of microbes with metals and the role of microbes in deterioration processes particularly, those relevant to the oil and gas and marine industries.

Anthony Polomka is a consulting materials and corrosion engineer with over 30 years' experience in the upstream oil and gas industry. Anthony has worked directly for Santos Ltd, Woodside Energy Limited and BHPBilliton Petroleum and consulted to a number of other operating companies and engineering contractors in the areas of materials selection, coatings, cathodic protection, production chemicals, failure analysis and operations corrosion management.

Development of a laboratory test for microbial involvement in accelerated low water corrosion

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Microbially influenced corrosion (MIC) is a general term for when microbes affect material corrosion processes. The rapid corrosion that can occur due to MIC can cause significant dangers and costs for owners of relevant assets in relation to predicting structural safety, design of new structures and maintenance. Verification and/or prediction that a structure may be subject to MIC is not straightforward and, when metal surfaces are involved, it requires a series of metallurgical, microbiological and chemical tests. A useful part of this testing can be laboratory-based studies of microbial consortium samples from the environment of interest. However, there are no standard guidelines for how to perform such tests. Here we report the results of a preliminary study of laboratory corrosion simulations with biomass from a marine metallic corrosion event and show that simple changes in the test conditions can alter the rate of corrosion and the composition of microbial consortia during the test.

Accelerated low water corrosion (ALWC) is increasingly being recognised as a form of MIC, which occurs on metal structures in the marine environment[1–3] (Figure 1*a*). ALWC occurs in ports and harbours worldwide and is often associated with orange patches on steel surfaces at the low-tide water level (see Figure 1*a*, *b*). The orange patches are a combination of iron oxide-rich corrosion products and microbial biomass. The exact details of the corrosion mechanisms and microbial processes involved in ALWC are still not well understood but are believed to be mediated by a combination of microbial sulfate reduction and sulfur oxidation and there is good evidence that electrochemistry is centrally involved. Currently, there is limited guidance available for those wanting to determine if microbially-induced ALWC is present at a particular site. Some of the suggested tests include visual surveys for orange patches and holes in sheet piling, ultrasonic thickness measurements and tests for the presence of sulfate reducing bacteria (SRB) and/or their by-products such as iron sulfide. A possible addition to these tests could be to use microbial samples from field corrosion studies, for corrosion simulations in the laboratory or at some other suitable test location. These ALWC tests should be able to be carried out with relevant microbial communities, basic equipment and initiated in the field by technical staff.

Laboratory-scale corrosion testing to ascertain MIC/ALWC requires standard test procedures (including sample collection and storage) and operational conditions, since they are critical for generating comparative test outcomes and they do affect microbial community composition, as has previously been shown[4–10]. Some important test factors include the simulation medium composition, including nutrients and dissolved oxygen supply, physicochemical aspects of redox potential and pH, and the biomass used as inoculum. Thus, with a view to widespread application in the corrosion industry, we used samples of naturally occurring ALWC orange patches as an inoculum and a relatively simple laboratory set-up to study the effect of the selected microbes and the impact of several environmental conditions on corrosion of metal coupons.

Samples of an orange patch were taken from a steel sheet pile wall suffering ALWC located in a port in southeastern Australia. The test set-up involved solutions of seawater and nutrients in 500 mL Schott bottles incorporating suspended marine grade steel coupons, and the homogenised orange patch as inoculum (see Figure 1*c*, *d*). The range of experimental variables included oxygen availability, nutrient addition and filtering of the seawater. After

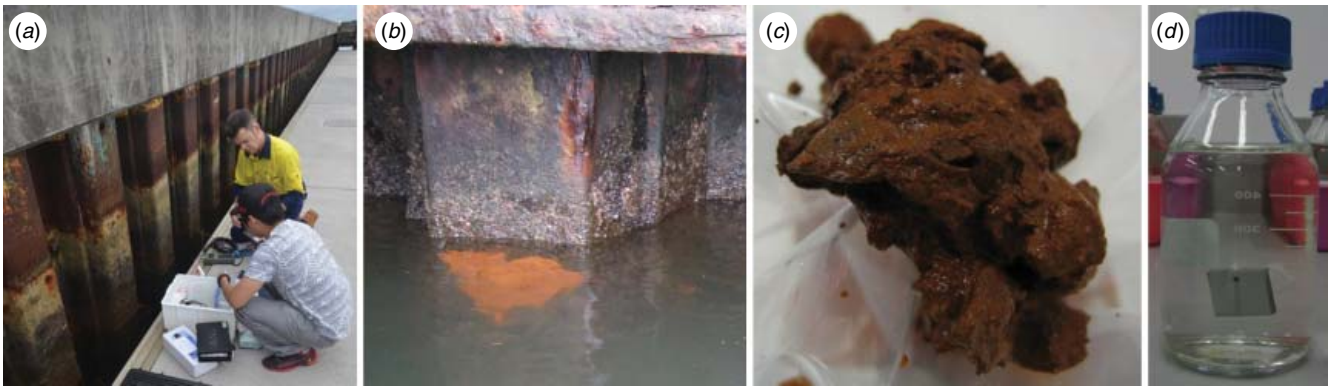

Figure 1. (*a*) Photo of steel sheet piling, the type of which can be subject to ALWC, (*b*) example of steel sheet piling with patch of orange bloom, (*c*) collected orange patch material (= corrosion products and microbial biomass), and (*d*) test set-up prior to inoculation, including suspended marine grade steel coupon.

immersion for 60 days at laboratory temperature, the metal coupons were removed and the extent and nature of corrosion was determined by surface contour changes (Bruker Contour GT-K1 3-D optical profilometer), scanning electron microscopy (Zeiss SUPRA 40VP-25-38) and mass loss (Mettler Toledo MS205DU mass balance). Samples of the planktonic suspensions representing the different test conditions were evaluated for microbial communities by Illumina MiSeq metabarcoding of the V6-V8 part of the 16S rRNA genes.

The corrosion rates of the coupons in the tests containing microbial inocula and additional nutrients (two test set-ups) were 100–125 µm yr^{-1}, which are about 3 times that of coupons from the solutions lacking inoculum. We concluded that the microbes present in the orange patch have the ability to increase corrosion rates. While the greatest corrosion rates were observed when nutrients were added to the test solutions (glucose and yeast extract, after 15 and 44 days), teasing out the exact reason for this is not straightforward. These nutrients can act as a corrosion inhibitor[7] and also cause changes in microbial community composition and dominant microbial metabolisms, which also can affect corrosion. Oxygen availability was also found to affect corrosion, where the corrosion rates of nominally aerobic tests (bottles with ~100 mL air headspace, with holes drilled in lids and weekly manual agitation) were about 3 times greater than for the nominally anaerobic tests (the liquid completely filled the tightly sealed, non-agitated bottles).

A total of 100 different bacterial genera were identified in all the test setups with the number of unique bacterial taxa found in each of the test solutions varying from 15 to 37. Overall the phylum *Proteobacteria* (73.9%) made up the majority of the bacteria identified, with phylum *Bacteroidetes* (13.2%) the second most dominant bacterial phylum. The two test set-ups with the highest corrosion rates contained seawater, nutrients and inoculum, and they selected for unique upper taxonomic level microbial populations comprising the following groups in the Bacteria:

- *Proteobacteria* (63%)
  - Class Deltaproteobacteria: 24% (comprised of different Families)
  - Class Gammaproteobacteria: 35%
- *Bacteroidetes* (17%)
  - All Class Bacteroidia
- *Tenericutes* (7.5%)
  - All Class Mollicutes
- *Firmicutes* (8%)
  - All Class Clostridia
- *Lentisphaerae* (5%)
  - All Class Lentisphaeria.

In the Archaea, one high corrosion test set-up contained Candidate Division WCHD3-30 from Phylum *Parvarchaeota* (48.1%), but very little is known of this group.

Phylum *Tenericutes* were present only in the two tests with the highest corrosion rates, implying a key role in ALWC. High abundances of Class *Deltaproteobacteria* were also found in the two highest corrosion rate tests. Class *Deltaproteobacteria* contains many SRB, which have a noted role in MIC and ALWC. The SRBs that we identified were from several different families, largely *Desulfobulbaceae*. There is plenty of scope for multivariate analyses of the microbial community (diversity and evenness) with phenotypic aspects including corrosion rate, oxygen and nutrient availability and these studies will statistically tease out linkages between specific microbes and function.

We showed that a simple, laboratory test set-up can potentially be used to predict and identify ALWC. In addition, the results obtained demonstrate how changing the test conditions can affect both the magnitude of corrosion that takes place and the microbial community that develops. The test arrangements used in this work were deliberately chosen to be rudimentary so as to mimic a practical application. Some of the areas of further study include oxygen effects, changes in the community with time, sessile versus

planktonic communities and the timing/types/levels of nutrient addition. Reducing the test volumes (from 500 mL), increasing the throughput, and making the test available in an interpretable form to the industry are future practical directions. From a fundamental perspective, we are delving into the functional aspects of the microbial groups that were specifically selected in the high corrosion tests and exploring the microbes that grew on the coupons.

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Biographies

Scott Wade is an Associate Professor in the Faculty of Science, Engineering and Technology at Swinburne University of Technology. He undertakes research, across the spectrum of fundamental to applied, on the microbiological, metallurgical and environmental aspects of microbiologically influenced corrosion.

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Report from ASM 2018

Kate Seib

Chair of the Local Organising Committee for ASM 2018, Brisbane, Queensland

The sunshine state did not disappoint, and provided some wonderfully warm weather for the 552 delegates that attended the Australian Society for Microbiology's 2018 Scientific Meeting and Trade Exhibition at the Brisbane Convention and Entertainment Centre, 1–4 July.

The 2018 Australasian Mycological Society (AMS) Scientific meeting was also held in conjunction with ASM2018 on Wednesday 4 July, and continued on Thursday 5 July. Overall, we had a fantastic scientific line-up with a range of leading speakers from Australia and around the world. Microorganisms affect every aspect of life on Earth, and the topics covered by our speakers reflected the diversity of microbiology and the central themes of Environmental Microbiology, Clinical Microbiology and Microbial Pathogenesis represented within our society.

The conference commenced with the annual Public Lecture on Sunday afternoon, presented by Nicholas Graves, Professor of Health Economics at the Institute of Health and Biomedical Innovation (IHBI), Queensland University of Technology (QUT). Professor Graves is currently the Academic Director for The Australian Centre for Health Services Innovation (AusHSI) and the Centre of Research Excellence in Reducing Healthcare Associated Infections (CRE-RHAI) at QUT. His research brings economics to the study of healthcare and he presented some thought-provoking insights into the use of economic methods for decision-making in healthcare settings.

The conference was then officially opened with a welcome from the ASM President, Professor Roy Robbins-Brown, and the annual ASM awards ceremony. On behalf of the whole society, I would like to congratulate all our 2018 Award Winners, who gave some wonderful presentations during the conference, highlighting their contribution to, and passion for, microbiology.

The award winners and their presentation titles are as follows:

ASM Jim Pittard Early Career Award for distinguished contributions in any area of Australian research in microbiology by scientists in early stages of their career.

Sam Manna (*Murdoch Children's Research Institute*) – Variation in the capsular polysaccharide locus of *Streptococcus pneumoniae* isolates from low and middle-income countries in the Asia.

ASM Frank Fenner Award for distinguished contributions in any area of Australian research in microbiology by scientists in a formative stage of their career.

Makrina Totsika (*Institute of Health and Biomedical Innovation, Queensland University of Technology*) – My journey with *E. coli* and urinary tract infections: 15 years, 3 continents, 6 universities and lots of fun on the way.

Anton Peleg (*Alfred Hospital/Monash University*) – Bacterial drivers of neutrophil behaviour during an *in vivo* infection.

ASM Lyn Gilbert Award for major contributions in any area of diagnostic laboratory microbiology in Australia or internationally by ASM members/fellows.

Pat Blackall (*University of Queensland*) – The dilemma and the joy of diagnostic veterinary bacteriology.

ASM David White Excellence in Teaching Award for excellence in the teaching of, and/or innovation in the teaching of microbiology in Australia.

Prue Bramwell (*RMIT University*)

ASM Nancy Millis Student Awards, which provide the opportunity for one student member from each ASM State Branch to attend and give an oral presentation.

VIC: **Pramod Subedi** (*La Trobe University*) – Elucidating the Scs redox pathway and its role in copper tolerance in Salmonella.

NSW/ACT: **Kenya Fernandes** (*University of Sydney*) – Cryptococcus and the Swiss army knife of virulence.

TAS: **Zoe Bartlett** (*University of Tasmania*) – Surveying *Bacillus cereus* sensu lato in Tasmanian dairy environments and dairy products to inform food safety risk assessments.

WA: **Nicole Bzdyl** (*University of Western Australia*) – Folding your way to greater pathogenicity; the role of cyclophilins in *Burkholderia pseudomallei* virulence.

QLD: **Carrie Coggon** (*The University of Queensland*) – Presence of inhibitory antibodies in patients with *Escherichia coli* urosepsis.

SA/NT: **Erin Brazel** (*University of Adelaide*) – Overcoming antimicrobial resistance – exploiting zinc intoxication to restore antibiotic efficacy.

ASM Distinguished Service Award for outstanding service of, or contributions by, individuals to the Society.

Richard Bradbury (*CDC Parasitology Reference Diagnostic Laboratory*)

Stephen Graves (*Australian Rickettsial Reference Laboratory*)

ASM Honorary Life Membership Award, the highest membership recognition given by the Society.

Julian Rood (*Monash University*)

Chris Burke (*University of Tasmania*)

The next highlight of Sunday evening was the Bazeley Oration, presented by Professor Dennis Burton. The Bazeley Oration is supported by the Commonwealth Serum Laboratories (CSL) to recognise significant achievements in the field of vaccines. Professor Burton is Chairman of the Department of Immunology and Microbial Science, The Scripps Research Institute, La Jolla, USA. He delivered an informative and engaging talk about the interplay of antibodies and the highly mutable virus HIV.

The following three days of the conference featured a diverse and stimulating series of scientific sessions. Of note were the fantastic presentations given by our Plenary Speakers, who described some their recent work in the areas of bacterial biofilms (Professor Fitnat Yildiz – University of California, Santa Cruz), cost effective microbial diagnoses (Dr Susan Sharp – Kaiser Permanente, Portland), bacterial pathogenesis and glycobiology (Professor Michael Jennings – Institute for Glycomics, Griffith University), immunological insights to staphylococcal infection (Associate Professor Victor Torres – New York University School of Medicine), diversity of archaea (Dr Anja Spang – Netherlands Institute for Sea Research) and medical mycology (Professor Karl Kuchler – Medical University, Vienna).

Another highlight of the conference was the Rubbo Oration held on Tuesday evening, which is supported by the Rubbo Trust to recognise outstanding contribution to the field of microbiology. This year, the awardee was Paul Young, Professor of Virology and Head of the School of Chemistry and Molecular Biosciences at the University of Queensland. Professor Young spoke about 'a Virologist's Adventures in Wonderland' and the various stages of his career in microbiology. He also gave us some insights into his research that has focused on developing diagnostics, therapeutics and vaccines for the flaviviruses. The Rubbo Celebration that followed was held in the Sky Room, with South Bank and the Brisbane Wheel as a backdrop. This celebration provided a great opportunity to interact with colleague and friends, while also listening to the DJ, and having some silly photos taken at the photo booth.

The symposium speakers all delivered excellent talks that highlighted the quality and diversity of research and clinical studies being performed in Australia by people at all career stages, including Students, Early/Mid-Career Researchers, and Senior Researchers. Topics covered in the various symposia and workshops included: clinical diagnostics; antimicrobial resistance; vaccine and therapeutic development; public health and one health; tropical, regional and point of care medicine; genomics; microbial evolution; marine, wildlife and livestock microbiology; bacterial pathogenesis and regulation; viral pathogenesis; medical mycology; fungal ecology and evolution; as well as communication, education and history. As in past years, ASM2018 also hosted a series of events specifically targeted at people in the early stages of their careers, including the Nancy Millis student and ECR breakfast and lunch. These events always provide a great opportunity for students and ECRs to interact with senior scientists in their field, and get some tips and tricks of the trade.

The Sunday and Monday night poster sessions and trade exhibitions gave everyone some time to interact and it was fantastic to see so many people engaging with the poster presenters. Approximately 150 posters were presented over the two nights, 80 of which were presented by students. The quality of these presentations was extremely high, and although it was extremely difficult to judge, six students were selected as poster prize winners.

Karen Kong (*City University of Hong Kong*) – Combating multidrug-resistant bacteria by phages equipped with sRNAs.

Amy Pham (*University of Queensland Diamantina Institute*) – The prevalence of inhibitory antibodies in an Australian cystic fibrosis cohort.

Miljan Stupar (*University of Queensland*) – Molecular characterisation of PPVP, a key effector molecule within the LirA regulon of *Mycobacterium tuberculosis*.

Alma Wu (*Westmead Institute for Medical Research*) – Functional diversity of toxin-antitoxin systems in antibiotic resistance plasmids in Enterobacteriaceae.

Xiaomei Zhang (*University of New South Wales*) – Identification of serovars' specific genes for typing the five most prevalent Salmonella serovars in Australia.

Marina Zupan (*The University of Adelaide*) – Elucidating the Zn(II)-binding mechanism of the pneumococcal protein AdcAII.

ASM2018 was officially closed on Wednesday by the incoming ASM President, Professor Dena Lyras. This was followed by a series of

workshops organised by the ASM Special Interest Groups, which focused on genomics, clinical serology and molecular biology, culture media, and Eukaryotic microbes. EduCon 2018 also started immediately after the main ASM2018 Conference, and focused on contemporary and exciting ways to engage students and teach microbiology at all levels.

Finally, I would like to thank all the people that made ASM2018 such an enjoyable event. This includes all our speakers and delegates for their contribution to the event, and our trade sponsors whose ongoing support of ASM is essential to the success of our conference. I'd also like to thank all those involved in the Organising Committee for their hard work - the Scientific Program Chairs, Nick West and Adam Taylor, as well as all the other committee members, Amy Jennison (Sponsorship), Jacqueline Harper (Workshops), Christopher Day (Abstract coordinator), Aimee Tan (Social coordinator), Makrina Totsika, Freda Jen, Alvin Lo, Manisha Pandey, Ian Peak, Erin Shanahan and Tsitsi Diana Mubaiwa (Student/ECR Rep). Similarly, the ASM National Executive (Roy Robbins-Brown, Jon Iredell, Dena Lyras, Cheryl Power and Jack Wang, Rebecca Le Bard) have provided invaluable support to the organisation and smooth running of the conference, along with the National Scientific Advisory Committee and the Theme Leaders (Linda Blackall – Environmental Microbiology; Heidi Drummer – Virology; Tom Riley – Clinical Microbiology; Mark Schembri – Molecular Microbiology; Deborah Williamson – Public Health Microbiology), the Special Interest Groups, the #2018ASM communication ambassadors and ASN Events (especially Kara Taglieri the ASM National Office Manager).

I look forward to catching up with everyone at next year's ASM2019 conference to be held in Adelaide at the Adelaide Convention Centre from 30 June to 3 July.

New Fellows of the Australian Society for Microbiology

Amy Jennison
Benjamin Howden
Cynthia Whitchurch
Derek Sarovich
Erin Price
Gary Dykes
Geoffrey Coombs
Glenn Browning
Ian Carter
Jacqueline Harper
James Triccas
John Boyce
John Hamblin
Jonathan Iredell
Makrina Totsika
Namraj Goire
Priscilla Johansen
Renato Morona
Roy Hall
Sally Patridge
Stephen Turner
Steven Djordjevic
Suresh Mahalingam
Suzanne Garland
Tania deKoning-Ward
Thomas Ross
Trevor Lithgow
Vernon (Nigel) Kelly

ASM Summer Student Research Awards: 2018

Priscilla Johanesen

ASM Student and Early Career Microbiologist Engagement Coordinator

This year saw the introduction of the ASM Summer Student Research Awards, an initiative previously delivered by the Western Australia Branch, which was expanded to several other state branches. The ASM Summer Student Research Awards gives students the opportunity to complete a research project in a laboratory over the summer vacation period, providing students the invaluable experience of working in a real Microbiology research laboratory. Selection for the awards is a competitive process, with scholarship awardees provided with an allowance and student membership for one year upon completion of their project and submission of a report. One award is funded through National Office with additional awards offered, where possible, at a state branch level. This year the society awarded six students summer scholarships: Talitha Santini from Queensland; Don Ketagoda, Sonja Repetti, and Lauren Zavan from Victoria; and Hannah Grieg and Sarah Negus from Western Australia. ASM would like to congratulate all awardees. As highlighted by the students' abstracts (see below) the society is excited by the diverse array of research the students performed during the 2018 summer break and is looking forward to the future years of the program.

Optimization and preconditioning of microbial inocula and organic carbon substrates for the bioremediation of bauxite residue

Jack Wang[A], Giselle Pickering[B], Kimberley Warren[B], Maija Raudsepp[B,C] and **Talitha Santini**[B,D]

[A]School of Biomedical Sciences, The University of Queensland; [B]School of Earth and Environmental Sciences, The University of Queensland; [C]Department of Earth and Atmospheric Sciences, The University of Alberta; [D]School

of Agriculture and Environment, The University of Western Australia

Refining bauxite to produce alumina via the Bayer process produces a highly alkaline (pH 12–13), saline by-product (bauxite residue) that is inhospitable for the majority of plant and microbial life. Recent research has identified the viability of microbially driven bioremediation in neutralising the pH of bauxite residue. By augmenting the microbial community within bauxite residue and supplying a suitable organic carbon source, microbially driven bioremediation neutralises pH through the production of acidic metabolites via fermentation. This experiment aimed to determine the optimal combination of microbial inoculant and carbon substrate to produce the greatest and most rapid neutralisation of bauxite residue pH. The effect of residue preconditioning was also tested by transferring remediated residue to bioreactors with differing inoculants that had not been neutralised. It was shown that a combination of a mixed inoculum from a geochemically similar environment, and a simple sugar substrate (for example, glucose or fructose) produced the most rapid neutralisation of residue. This is attributed to the metabolic efficiency of degrading simpler monosaccharides and select species within the inoculum that drive neutralisation. Future research may focus on further exploring metabolite compositions in remediated residue and adding new functionality to the bioremediation process to achieve other remediation goals.

The effect of *Clostridium difficile* infection on the thymus

Don Ketagoda and Dena Lyras

Monash Biomedicine Discovery institute, Department of Microbiology, Monash University

Thymic atrophy and a loss in $CD4^+CD8^+$ T cells is a phenomenon that occurs in mouse models of infections with pathogens such as *Salmonella typhimurium*, *Trypanosoma cruzi* and Rhabdo virus. Previous work has suggested that thymic atrophy occurs in mice following infection with toxigenic *Clostridium difficile* strains. However, the observed thymic size reduction has not been quantified, and the effect on specific T cell populations during *C. difficile* infection (CDI) is yet to be investigated. To address this, we utilised a mouse model of CDI, in which mice were infected with toxin-producing *C. difficile* or an isogenic toxin mutant strain. Mice infected with toxigenic *C. difficile* exhibited increased weight loss, severe gut damage and inflammation, in contrast to mice infected with the non-toxigenic strain. Importantly, thymi collected from these mice showed a significant size reduction when compared to mice infected with the toxin mutant strain. Furthermore, there was a loss of $CD4^+CD8^+$ T cells in mice infected with toxigenic *C. difficile*, which was not observed in mice infected with the non-toxigenic strain. Collectively, these results indicate that CDI leads to a significant reduction in thymic size and a loss of $CD4^+CD8^+$ T cells in mice in a toxin dependent manner.

Identifying the genetic footprint of how microbial symbiosis became permanent: Searching for targeting signal in *Lepidodinium* genes implicated in enslaving secondary green plastids

Sonja Repetti and Heroen Verbruggen

School of BioSciences, The University of Melbourne

Plastid endosymbiosis, where photosynthetic organelles arise through microbial symbiosis, is arguably the most important process underlying the success and diversification of microbial photosynthetic eukaryotes. While the significance of endosymbiosis is accepted, events key to establishing such symbioses are less well characterised. Secondary endosymbiosis events involving green algal plastid donors are an excellent model system for identifying genes crucial for plastid establishment because there are three well-identified independent evolutionary events giving rise to the euglenophytes, chlorarachniophytes and the dinoflagellate *Lepidodinium*. My project aimed to find genes encoding plastid-targeted proteins and originating in the green algal endosymbiont in *Lepidodinium*, and search for a conserved plastid-targeting motif. This motif could then be used to identify a core set of genes for comparison across these separate evolutionary events. I identified 19 putatively plastid-targeted genes in *Lepidodinium*, but no conserved plastid-targeting motif was detected across these sequences. While this lack of conserved targeting motif is an interesting biological phenomenon inviting further investigation, it poses a significant challenge in determining which genes might be universally crucial to the host cell for 'enslaving' its microbial endosymbiont.

Characterisation of *Helicobacter pylori* outer membrane vesicles

Lauren Zavan and Maria Liaskos

Department of Physiology, Anatomy and Microbiology School of Life Sciences, College of Science, Health and Engineering, La Trobe University

All Gram-negative bacteria release outer membrane vesicles (OMVs) from their membrane as part of their normal growth. However, OMV biogenesis is not a well understood concept, and with differences in the methods of OMV production and subsequent analysis across research groups, this has resulted in inconsistencies within the OMV field. In this study, we demonstrate that the growth stage of *Helicobacter pylori* influenced the amount of OMVs produced in addition to their size and content. The progression of *H. pylori* growth resulted in a decrease in the size range of OMVs produced along with a reduction in the amount of their DNA, RNA and protein cargo. Collectively, our work suggests that bacterial growth stage is a previously unknown regulator of OMV number, size and content and that this may subsequently alter their biological functions.

Tropism of a novel alphavirus

Hannah Greig and Allison Imrie

School of Biomedical Sciences, University of Western Australia

Alphaviruses are the most clinically important endemic viruses in Australia and have a wide range of possible reservoir hosts and vectors. A novel alphavirus was isolated in 2011 from *Culex annulirostris* mosquitos in the Kimberley region of Western Australia. The virus, named Derby virus (DERV), clusters with the Old-World arthritogenic alphaviruses and is a close relative of Sindbis virus. Important features of DERV that are yet to be characterised, are its possible reservoir hosts and its potential to cause human infections. This study was conducted to determine the tissue tropism of DERV by using human and avian cell lines as a model of *in vivo* infection. There was evidence of DERV replication in the human epithelial and fibroblast cell lines, HeLa and HFF, and in the avian embryonic fibroblast cell line, DF-1. The human B and T cell lines, SKW and Jurkat, and PMA-induced U937 macrophages, were not susceptible to DERV infection. In combination with preliminary serological evidence these findings indicate that DERV may infect humans and birds.

Uncovering virulence related secondary metabolites in the human pathogen, *Talaromyces marneffei*

Sarah Negus[A], Mitali Sarkar-Tyson[B] and Yit-Heng Chooi[A]

[A]School of Molecular Sciences, University of Western Australia; [B]Marshall Centre for Infectious Diseases, School of Biomedical Sciences, University of Western Australia

Iron is a vital cofactor for a multitude of cellular process in all eukaryotes and some prokaryotes. Due to this, pathogens have had to evolve sophisticated strategies to ensure that they are able to obtain the required amount of iron for survival, including the production of secondary metabolites such as siderophores. Siderophores are relatively low molecular weight, ferric iron specific chelating agents that are produced by bacteria and fungi that are growing under low iron conditions. This project aimed to continue the research into targeting non-ribosomal peptide synthetase (NRPS) genes SidD and SidC within the pathogenic fungi *Talaromyces marneffei*, predicted to be involved in siderophore biosynthesis. By doing this, it allowed the further characterisation of the biosynthesis of these siderophores, as well as their effect on *T. marneffei* virulence. Two knockout strains were confirmed for ΔTmSidC and three knockout strains were confirmed from ΔTmSidD. Elevated levels of cell cytotoxicity seen at all hours measured within the LDH assay suggests that the cells used for the assay were not healthy and may have contributed to the lack of results. The experimentation completed in this scholarship project, while not providing extensive results, will provide a strong base moving forward with further research.

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Vale Associate Professor Sylvia Mary Kirov

By Emeritus Professor John Goldsmid and Dr Louise Roddam

Members of ASM will be saddened to hear of the recent death of Associate Professor Sylvia Kirov.

Sylvia graduated from University of Sydney (BSc Hons.) and completed her PhD at ANU with expertise in two disciplines; immunology and microbiology, before accepting a position at the University of Tasmania (UTAS) in 1975. Initially she joined the research team of Professor Ray Lowenthal in the Department of Medicine but, on the resignation of Dr Dick Tucker, who was appointed Director of the microbiology laboratory of the Royal Hobart Hospital, Sylvia joined the medical microbiology section of the UTAS Department of Pathology. Until that time, the microbiology section was mainly concerned with routine diagnostic work, assessment of new diagnostic laboratory methods and epidemiology. Sylvia brought to the department a research interest in such areas as microbial pathogenicity. She participated fully in the teaching of medical microbiology to medical, pharmacy and science students, with a special interest in *Aeromonas* and other enteric Gram-negative rods. She proved to be an outstanding teacher in bacteriology and virology and her enthusiasm and expertise attracted a significant number of students to undertake doctoral studies under her supervision. This was an important development and the research output in medical microbiology increased markedly; and more so when Professor Kon Muller became Head of Pathology.

Sylvia thus played an important role in the development and advancement of medical microbiology in Tasmania. Many of her postgraduate, Honours and PhD students went on to fill important academic posts, thanks to her tuition and guidance.

Sylvia was also an important contributor to microbiology through the ASM. Sylvia joined the ASM in 1971 as a BSc undergraduate. She held many roles in ASM over the years, serving time on the NEB/NEBQ, the research trust, two LOCs and NSAC as well as being an active member of SIGs, the state FASM advisor and a frequent convener and chair-person at ASM scientific meetings. In recognition of her significant contributions to the ASM, Sylvia was awarded an 'ASM Distinguished Service Award' in 2013.

As members of ASM, we are fully aware of her research talents and contributions and we are grateful for her considerable efforts to advance medical microbiology not only in Tasmania but for Australia as a whole.

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