SESSION F:

Environmental microbiology: bioavailability, ecotoxicity, diversity and population dynamics.

SYSTEMS ECOTOXICOLOGY: FROM TRANSCRIPTOMICS, PROTEOMICS AND METABOLOMICS TO PHENOMICS

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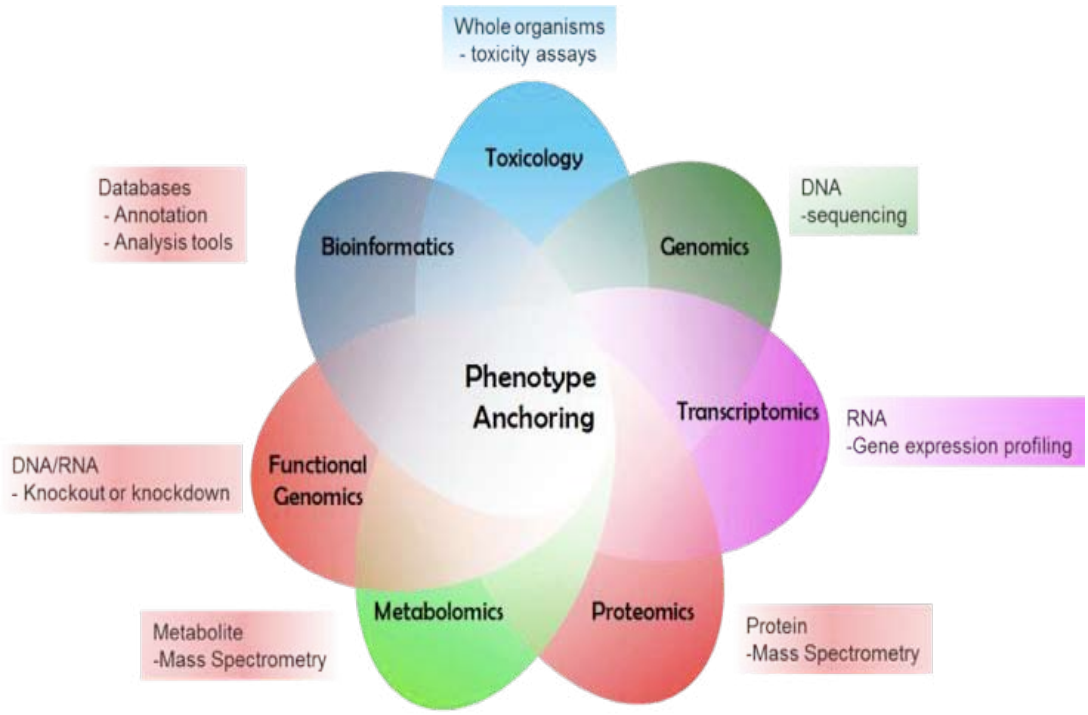
Classic ecotoxicology has focused upon a bottom-up approach to understand stressor effects in which a few genes, proteins, or biochemical reactions are studied at a time. The invention of new technologies in the last decade has enabled the analysis of whole transcriptome (gene transcripts), proteome (proteins) and small cellular molecules (metabolite profiling) resulting in whole system approaches and the field of systems biology. By complementing the traditional approach with the system biology it is possible to define the genetic, protein, and biochemical reactions as integrated and interacting networks of an organism. The consequent integration of the molecular phenotype (transcriptome, proteome and metabalome) to the physical and biochemical traits of an organism (phenomics) provides a holistic understanding of the effects of and the response to a toxicant. Also, importantly, it allows prediction of linkage of mechanistic impacts at the molecular level to impacts at the individual, population or ecosystem level.

This talk will present some systems ecotoxicology studies, wherein the earlier mentioned techniques have been exploited, ranging from mammalian cells, non-model vertebrates and invertebrate, to algae exposed to various toxicants.



ELECTROSTATIC INTERACTIONS BETWEEN NANOPARTICLES AND BACTERIA: HOW A SIMPLE MODIFICATION OF THE AMES TEST REVEALED MUTAGENICITY OF TIO_2 NANOPARTICLES?

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Titanium dioxide has been used commercially since the early 1900s and is currently the most widely used white pigment in the world, with a total annual production of 4.5 million tons [1]. TiO_2 provides whiteness and opacity to many consumer products such as paints, plastics, inks, papers, coatings, ceramics, and textiles. To date, majority of the TiO_2 used industrially is micrometer sized. But, with the development of nanotechnology, TiO_2 nanoparticles (NP-TiO_2) are more extensively produced and find wider applications due to their unique physicochemical properties compared to the bulk TiO_2. The percentage of manufactured TiO_2 as nanoparticles has been estimated as 2.5% in 2009 and a completely converted industry by 2025 in USA [2].

The booming demand for NP-TiO_2 has spurred significant public alarm about its possible adverse effects especially genotoxicity. It was occurring in 2006, after the IARC reclassified TiO_2 from Group 3 to Group 2B carcinogen. NP-TiO_2 were recently listed by the OECD as one of the priority nanomaterial for immediate testing. Several studies have been done to determine the carcinogenic potential of NP-TiO_2. However, these studies have given very controversy results. Some authors found that NP-TiO_2 are genotoxic, whereas some other, like Warheit et al [3] and Pan et al [4], which both used the classical Ames Mutagenicity test on *S. typhimurium*, found that these nanoparticles are not genotoxic.

In this study, we showed that the conventional Ames Mutagenicity test is not adapted to study the nanoparticle genotoxicity. The culture medium used in this test (rich medium - pH close to nanoparticle isoelectric point) promoting the nanoparticle aggregation and thereby minimizing the possible interactions between nanoparticles and test cells. Based on these considerations, the Ames test was modified and made more effective for the detection of the nanoparticle genotoxicity. Thus modified, the Ames test revealed genotoxicity of two manufactured NP-TiO_2 (Aeroxide P25 and Nanoamor) and a TiO_2-based nanomaterial used in sunscreens.

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NEW INSIGHTS INTO MICROBIAL MECHANISMS OF NITRIFICATION IN ACID SOILS

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Recent studies of ammonia-oxidizing archaea (AOA) suggested their significant contributions to global nitrogen cycling. AOA are widespread in terrestrial ecosystems with possibly unique mechanisms for nitrification, better adaptation to low-pH pressures, and strikingly lower ammonia requirements compared with ammonia-oxidizing bacteria (AOB). Previous perceptions that microbial ammonia oxidation in acidic soils was minimal and entirely meditated by autotrophic bacteria and occasionally by heterotrophic nitrifiers, have been dramatically challenged. Relative contributions of AOA and AOB to autotrophic ammonia oxidation have been reported to vary in different soils, but ammonia substrate availability, which was largely restricted under acidic conditions, seemed to be the key driver. Theoretically predicted ammonia concentrations in acidic soils below the substrate threshold of AOB and remarkably high ammonia affinity of AOA raised the supposition that AOA could represent the dominant ammonia-oxidizing group in ammonia-limited acidic environments. Recently, the functional dominance of AOA over its bacterial counterpart and autotrophic archaeal ammonia oxidation in acidic soils has been compellingly confirmed by DNA-stable isotope probing (SIP) experiments [1] and the cultivation of an obligate acidophilic thaumarchaeaon, Nitrosotalea devanaterra [2]. In this paper, we present the currently available knowledge concerning the history and progress in our understanding of the ammonia-oxidizing microorganisms and the mechanisms of nitrification in nutrient-depleted acidic soils, propose the possible mechanisms shaping the distinct niches of AOA and AOB, and thus strengthen the assumption that AOA dominate over AOB in the ammonia oxidation of acidic soils.

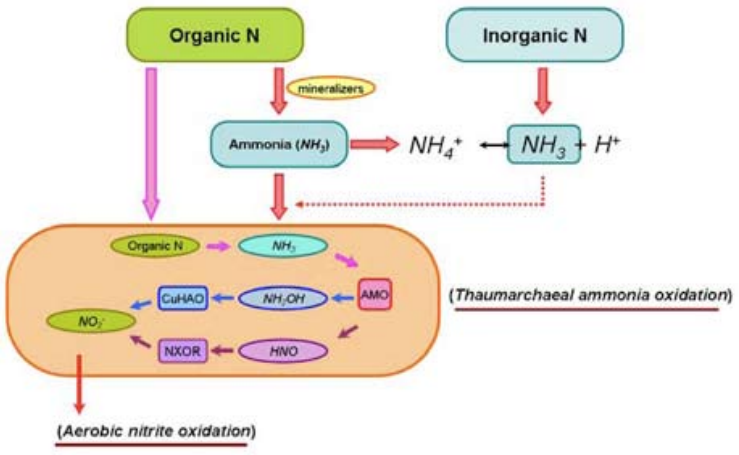

Fig. 1. Possible ammonia sources for thaumarchaea and proposed pathways of thaumarchaeal ammonia oxidation based on genomic analysis of N. maritimus [3].
AMO: Ammonia monooxygenase; CuHAO: periplasmic multicopper oxidase; NXOR: nitroxyl oxidoreductase.

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CONTROL OF THE ROUTES FOR MICROBIAL FORMATION OF THE HYDROXYCARBONATE FEII-FEIII (GREEN RUST) AND MAGNETITE BY IONIC AND NONIONIC POLYMERS

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It is well known that iron oxide reduction by *Shewanella spp.* bacteria promotes the formation of FeII bearing minerals, such as the mixed FeII-FeIII hydroxysalt green rusts (GRs), in anaerobic conditions. Although the formation of microbially promoted GRs is widely demonstrated, the mechanisms and factors governing the GR precipitation as the main (major) secondary iron mineral at the expense of other products in lab-scale investigations or environmental systems are largely unknown. As GR is an effective reductant for several contaminants the mechanism controlling its formation merit investigation, from both the environmental and engineering points of view. Several factors such as cellular material (i.e. autoclaved cells and/or bacterial polymers), synthetic anionic polymers or oxyanions have been identified to control GR mineralization as secondary mineral at the expense of other products such as magnetite. The arrangement mode of the heterogeneous aggregates resulting from the interactions between bacterial cells, iron oxide particles and polymers was suggested to influence the routes of formation of secondary iron minerals by limiting the diffusion of reactive species and thus creating favorable microenvironment for GR formation. In these aggregates, the electron transfer from cells to iron oxides is supported by organic electron shuttles. On the other hand, anionic polymers, colloidal and aqueous silicates were found to also influence the nature of the secondary iron minerals through the stabilization of the GR crystals. These results indicate clearly that the bacterial cells drive indirectly the nature of the secondary FeII-bearing mineral.

Moreover, they give new insights into the understanding of the mechanisms of « biogenic » mineral formation based on the electron transfers from bacteria towards iron oxides. Finally, this work contributes to our understanding of the processes leading to green rust formation in environmental systems, such as soils or aquatic systems biofilms (Fig. 1), in which a very high cell density can be found at a micro-scale level, associated to exo-cellular polymers and natural silica mineral composites.

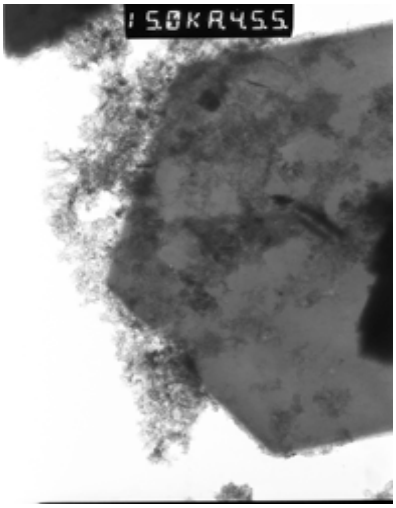

Fig. 1. Photography by transmission electronic microscopy of a crystal of green rust issued from a ferruginous biofilm material incubated in anaerobic conditions.

SPATIAL DYNAMICS IN BIOFILMS ARCHITECTURES

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Most of what makes microbial cells in a biofilm different from their planktonic counterparts is their multicellular spatial organization. Beyond the static description of the 3D structure, biofilm formation is a dynamic cyclic process including different steps from the initial attachment to surface to the dispersion of cells. Numerous studies have shown that this biological process involves a coordinated spatio-temporal expression of adhesion, motility, matrix or cell death genes in response to micro-environmental conditions and signaling molecules. As the understanding of biofilm complexity increases, simple descriptive architectural studies are being replaced by those in which the three-dimensional organization of the biofilm is related to other information, such as species composition, relationship with substrate or physiology. The analysis of such structure/function relationships has considerably evolved during the last decade, in line with technical advances in microscopy. The developments in confocal laser scanning microscopy (CLSM), coupled with the creation of new fluorescent molecules and protein reporters allowing the labelling of specific matrix components or cell states, have deepened our knowledge of biofilms. These new tools allow researchers to explore non-invasively the dynamic architectural and physiological evolution in the three-dimensional structure of biofilms.

TREATMENT OF ARSENIC CONTAMINATED MINING WATER USING BIOFILMS

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Arsenic is a common trace-level constituent of gold-quartz vein deposits in mining regions. This toxic metalloid is often present in waste material, water and soil near gold mining areas. The discharge of mining drainage water containing arsenic contributes to its dispersion in the environment. The resulting contamination of surface waters, groundwater and sediments is a matter of great public concern due to the deleterious effects of arsenic on human health. In water, arsenic exists mostly as trivalent arsenite (As (III)) and pentavalent arsenate (As(V)), both forms being toxic to living organisms. Most of the existing treatment processes are effective only on As(V) which forms anionic complexes and is therefore more easily converted into solid waste, unlike As(III) which forms mobile neutral complexes. As a consequence, the removal of arsenic from water requires a preliminary oxidation step. The chemical oxidation of As(III) by O_2 is very slow (Jekel [1]), and the use of oxidant chemicals entails high operation costs (US-EPA [2]). This cost aspect could be overcome by using biological treatment with As(III)-oxidizing bacteria (Zouboulis and Katsoyiannis [3]). Arsenic oxidation capabilities of bacteria are known for a long time, since Green [4] isolated a bacteria belonging to *Achromobacter* in cattle dip in South Africa. Numerous studies reported isolation of heterotrophic and autotrophic bacteria able to oxidize arsenic (Philips and Taylor [5], Osborne and Ehrlich [6], Abdrashitova et al. [7], Battaglia-Brunet et al. [8]).

The present study deals with a mining water drainage resulting of the digging of an exploration gallery in a gold-arsenopyrite mineralization (cherts and quartz veins). Its flow-rate varies between 10 and 30 $m^3\ h^{-1}$ and the arsenic concentration between 10 and 500 $\mu g\ L^{-1}$ for a pH close to 6.5. The water contains also high concentrations of iron (between 2 and 15 $mg\ L^{-1}$). Both arsenic and iron concentrations are higher than the French quality standards (which are set to 100 $\mu g\ L^{-1}$ for arsenic and 3 $mg\ L^{-1}$ for iron in this kind of context), and thus, require a treatment to be removed from the effluent before discharging it in the environment. In the earlier part of the study, Battaglia-Brunet et al. [9] identified a biological As(III)-oxidizing activity in diverse micro-environments along the water stream, from the source to the discharge point. From those results, we investigated the possibilities of using this natural phenomenon for the removal of arsenic in a passive on-site treatment process based on three successive steps: (i) biological oxidation of As(III) by indigenous bacteria biofilm in a fixed-bed reactor filled with pozzolana, (ii) precipitation of iron in the form of hydroxides, (iii) adsorption of As(V) onto iron hydroxides (and/or co-precipitation of As(V) with iron).

Small-scale laboratory experiments showed that the indigenous bacterial population promoted As(III) and Fe(II) oxidation in conditions close to those of the site, i.e. temperature, water composition and oxygen availability. The immobilization of the bacteria on pozzolana by the development and the maintenance of a biofilm increased oxidation rates compared to natural conditions. These results were confirmed by long-term on site pilot-scale experiments. As and Fe removal was significant and their concentrations at the pilot outlet decreased under the discharge standards. The critical operation parameters determined in the study will be used to design a treatment unit based on the biological passive process described above.

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BACTERIAL OXIDATION OF ARSENIC IN POLLUTED SOILS: ROLE OF ORGANIC MATTERS

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Microbes play a major role on the behavior of metals and metalloids in soils. Arsenic speciation, in particular, is related to the activity of bacteria able to oxidize, reduce or methylate this element, and determines mobility, bioavailability and toxicity of As. Arsenate (AsV) is less toxic and less mobile than arsenite (AsIII). Bacterial As(III)-oxidation tends therefore to reduce the toxicity of arsenic in soils and its risk of transfer toward underlying aquifers. It is well known that organic matter influences abiotically the speciation of arsenic and thus its mobility in soils. Previous results suggest an effect of organic matter on the kinetics or efficiency of bacterial As(III)-oxidation in presence of oxygen, thus in conventional physico-chemical conditions of a surface soil. The objective of the present project is to quantify the influence of organic matters on the bacterial speciation of arsenic in polluted soils. Moreover, the biogeochemical consequences of this phenomenon on the mobility and ecotoxicity of this metalloid will be studied. The first task of this program is the systematic investigation of the influence of different types and concentrations of organic matters on the activity of As(III)-oxidizing pure strains. Influence of simple substrates (aspartate, succinate) and complex substrate (yeast extract) on As(III)-oxidation kinetics has been studied. For each experiment, the bacterial growth and the expression of genes involved in the speciation of arsenic, *i.e*. *aio* and *ars* genes, has been monitored. A direct perspective of this work will be to perform experiments with humic and fulvic acids (complex organic matter commonly found in soils), and with water-extracted organic matter from polluted soils. Then the As(III)-oxidation activity of bacterial communities extracted from contaminated soils will be followed. These assays should allow the screening of conditions which will be applied in subsequent experiments with several real contaminated soils, including a former mining site, impacted industrial sites, and a forest soil heavily contaminated after arsenical ammunitions storage.

This work is co-funded by BRGM and ADEME (convention TEZ 11-16).

CONTROL OF THE HYDROXYCARBONATED GREEN RUST STABILITY, A TRANSITORY PHASE FROM FE(II) OXIDATION BY AZOSPIRA ORYZAE

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One of the major constraints to remove nitrate from water by the organotrophic denitrification is the availability of the organic electron donor. Lithotrophic denitrifying bacteria could be an alternative as they gain electron from inorganic donors. Thus anaerobic iron-oxidizing nitrate-reducing bacteria are capable of coupling the oxidation of Fe^{2+} to dissimilatory reduction of NO_3^- to N_2 [1]. Various secondary iron minerals can be produced during this process and the mechanisms of their formation are worth studying because they could influence the denitrification. Recently, green rust (GR) – a layered Fe(II)-Fe(III) double hydroxides – was identified as an intermediate compound during the oxidation of Fe(II) by the nitrate-reducing Acidovorax sp. strain BoFeN1 [2]. Our work was dedicated to investigate *Azospira oryzae* in different media containing ferrous iron, nitrate, and various cell densities. The results showed that a high cellular concentration led to hydroxycarbonated GR formation stable in time (Fig. 1), whatever the media.

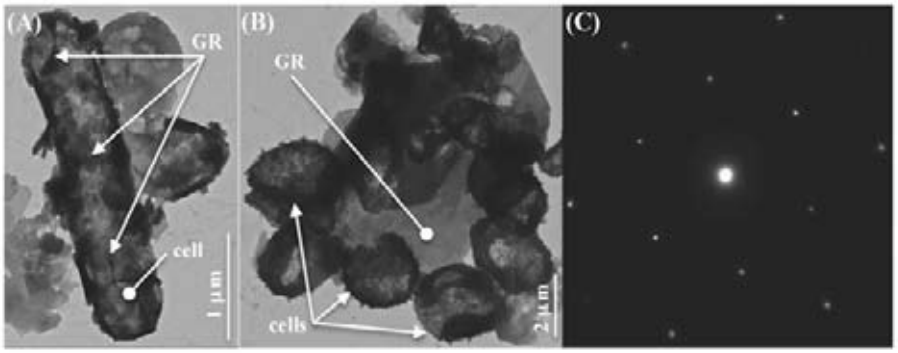

Figure 1. Transmission electron micrographs of GR produced by the anaerobic iron-oxidizing nitrate-reducing bacterium Azospira oryzae: (A) cells covered by GR crystals, (B) cells grouped around GR particles. The electron diffraction pattern (C) is composed of spots distributed on a hexagonal lattice. The cell parameters deduced from the diffraction pattern are a = b = 3.154 Å and c = 22.326 Å in agreement with the hydroxycarbonated GR structure [3].

However, this intermediate mineral is metastable at low cellular concentration, its fate depending on the chemistry of the medium and can be transformed to a more thermodynamically stable mineral: magnetite/goethite.

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GROWTH AND EXTRACELLULAR ENZYME PRODUCTION OF FUNGI ON CADMIUM, CHROMIUM, COBALT, LITHIUM AND MANGANESE CONTAINING CULTURE MEDIA

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Impacts of metals on the growth and enzyme production of fungi were tested with Cd- (0-10 mg kg^{-1}), Co- (0-100 mg kg^{-1}), Cr- (0-100 mg kg^{-1}), Li- (0-100 mg kg^{-1}) or Mn- (0-400 mg kg^{-1}) containing culture media. ABTS [2,2'-azino-bis(3-ethylbentzthiazoline-6-sulfonic acid)] was used as an indicator of oxidative enzymes, mainly laccases and/or peroxidases, in culture media.

Fungi are important in the cycling of carbon and other nutrients in the well-balanced soil ecosystems [1]. Metals may decrease the ability of fungi to grow and produce extra- and intracellular enzymes, which are useful when degrading the xenobiotics from polluted soils e.g. for bioremediation purposes. Therefore it is important to understand the responses of fungi to different metals as pollutants. Cd, Cr, Co, Li and Mn are metals which are found as pollutants in the mining and industrial sites and their surroundings [2]. Little is known about impacts of Cd, Cr, Co, Li and Mn on the growth and oxidative enzyme production of different taxonomical groups of fungi.

All selected fungi, namely *ascomycetes Alternaria sp*., *Chaetomium sp*., *Epicoccum sp*., *Fusarium sp*., *Trichoderma harzianum* and *basidiomycetes Agrocybe praecox*, *Pleurotus pulmonarius*, *Phlebia radiata*, *Physisporinus rivulosus*, *Stropharia rugosoannulata*, were able to grow with all tested metals (Cd, Cr, Co, Li and Mn). The growth of *basidiomycete A. praecox* was tolerant to Mn, Cr and Li. The colour zone was formed with tested basidiomycetous fungi with all five metals indicating the unabated production of oxidative enzymes. The five tested ascomycetous fungi did not show any colour zone formation with Mn, Cd, Li and without metals. *Alternaria sp., Chaetomium sp.*, *Epicoccum sp.* and *Trichoderma harzianum* showed colour zone formation with Co and Cr indicating enzyme production. Each of the tested fungus responded individually, which means that metal pollution may change the balance between different fungi in soil.

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MICROENVIRONMENT CHARACTERISTIC AND MICROBIAL COMMUNITY IN ACTIVATED SLUDGE FLOCS OF DIFFERENT PARTICLE SIZE

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The activated sludge was comprised by many flocs with different particle size. Interdependent microorganisms in each floc are principal subjects during biodegradability of organic matter in wastewater treatment plant (WWTP).

Microenvironmental characteristics in floc of different particle size are closely related to the microbial community structure in corresponding floc. However, the complex interplay between microenvironment variation and microbial responses within activated sludge flocs is yet poorly understood due to the limitation of analyzing methods.

In this study, the internal microenvironment and microbial community in activated sludge flocs (ASF) were detected by microelectrodes technique and Fluoresence in situ hybridization (FISH) method. The aims of this study were to explore the characteristics of microenvironment and relationship between microenvironment and microbial structure in flocs with different particle size level. The flocs collected from a WWTP in Beijing were divided into five levels (< 61 μm for L1, 61-96 μm for L2, 96-160 μm for L3, 160-250 μm for L4, and >250 μm for L5).Results showed that the concentrations of dissolved oxygen (DO), NH_4^+ and NO_3^- were distinctly different in ASF with different particle size. The distribution of DO, NH_4^+ and NO_3^- could penetrate the whole floc when the floc particle size was 50 μm. The obvious variation was exhibited in floc with particle size of 250 μm, and the decrement rates of DO, NH_4^+ and NO_3^- concentration reached to 54.92%, 3.40% and 90.77%, respectively. The distribution and contents of ammonia-oxidizing bacteria and nitrite-oxidizing bacteria showed that their contents reduced gradually along the depth of bigger flora, whereas they distributed evenly from surface to core in the smaller flocs (Figure 1). The microenvironmental characteristics and microbial structure in different size sludge flocs presented relatively stable in a stable process of sewage treatment.

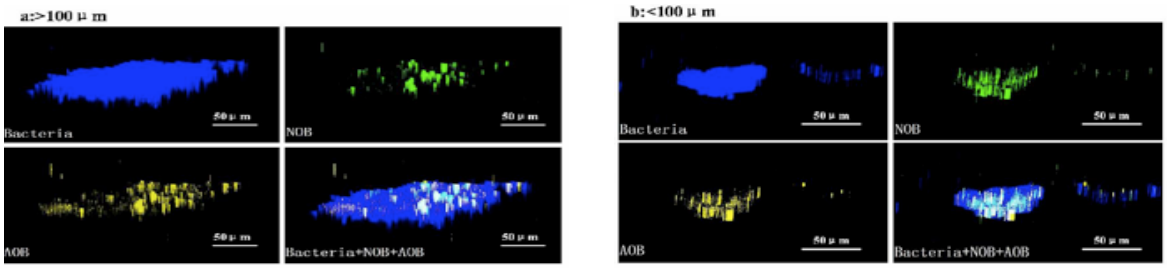

Figure 1 FISH images of functional bacteria in activated sludge. Bacteria that were dyed DAPI displayed blue, the green pixels in images represented NOB, and the yellow pixels represented AOB. The combination of images was 3D projects in LAS-AF soft. (a:>100 μm, b:<100 μm)

SEM/EDX MONITORING THE BIAEROSOL PARTICLES COLLECTED BY ANDERSEN SAMPLER IN A WASTEWATER TREATMENT PLANT

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Within wastewater treatment plant, the sites that contain moving mechanical equipment for water aeration are the regions with the highest emission of bioaerosols which have become of increasing concern and research interest in recent years. The risks associated with exposure to aerosols may not only be related to microorganism species and concentration but also to the size of aerosolized particles. Small particles can be very easily carried by the wind to distances ranging from a few hundred meters to several kilometers. The structure characteristics and the chemical components in aerosols with different particle size caused by rotating brushes in an Orbal oxidation ditch were assessed by scanning electron microscope together with energy dispersive X-ray spectroscopy (SEM/EDX). Air samples were collected by an Andersen sampler at different distances from the rotating brushes and different heights above the water surface. The Andersen sampler can separate airborne particles by collecting them in stages with holes of different sizes, providing information about cell density and particle size. Cells, spores and a large amount of inorganic particles could be observed through SEM photos. Most of the particles presented in the stage with particle size of 2.1-3.3 μm. The structure and chemical components in the aerosols presented site-related variability. The cells and spores often emerged at the sampling site closer to the water surface and near the rotating brushes, whereas most of the insoluble particles were detected from 40m downwind the rotating brushes.

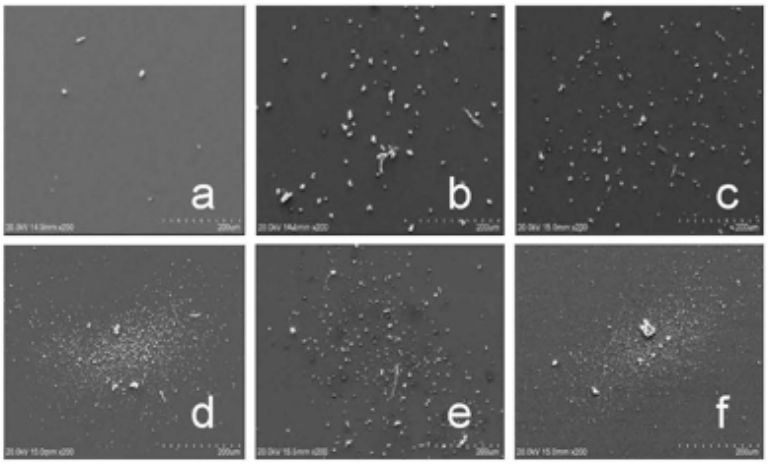

Figure The SEM photos of aerosols collected from the WWTP a: Stage 1 of Andersen sampler; b: Stage 2 of Andersen sampler; c: Stage 3 of Andersen sampler; d: Stage 4 of Andersen sampler; e: Stage 5 of Andersen sampler; f: Stage 6 of Andersen sampler.

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