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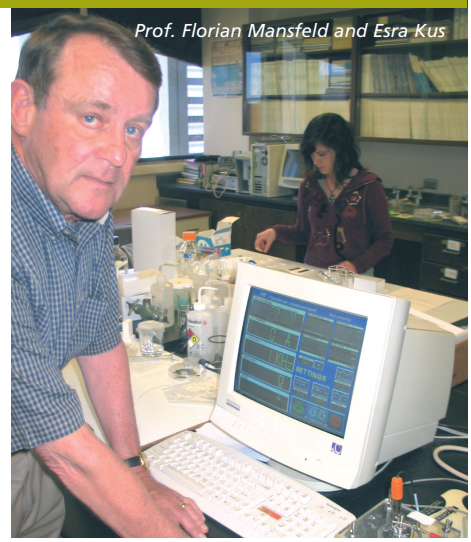


Prof. Florian Mansfeld and Esra Kus

Bacterium takes a shine to metals

Organism protects copper as well as some paints, Mork Department team reports

Exposed metal surfaces are highly vulnerable to corrosion, but paint or other protective coatings can interfere with some uses, as well as add significant costs. Now, a comprehensive series of experiments suggests a new form of protection: bacteria.

Researchers at the Corrosion and Environmental Effects Laboratory of (CEEL) of the USC Viterbi School have been working on analyzing the ability of an organism called Shewanella oneidensis MR-1 (hereinafter MR-1) to protect a number of metals.

The director of CEEL Prof. Florian Mansfeld and PhD candidate Esra Kus have been collaborating with Prof. Ken Nealson of Department of Earth Sciences. The team made a preliminary presentation at a Denver conference last month, and will make a more detailed one in Mexico in October.

The concept of "Microbiologically Influenced Corrosion Inhibition" (MICI) has been discussed in the scientific literature since 1997. Researchers at the University of Connecticut, University of Southern California and the University of California at Irvine had reported corrosion inhibition in their earlier studies by means of other organisms and regenerative biofilms.

The protector: electron microscope image of MR-1 bacteria.

In a study in 2001 Mansfeld and Nagiub reported microbiologically influenced corrosion inhibition for Al2024, mild steel, cartridge brass and stainless steel when bacteria contaminated an artificial seawater solution containing growth medium. They also showed that a bacterium of the same genus as MR-1, S. algae, prevented pitting of aluminum and some steel.

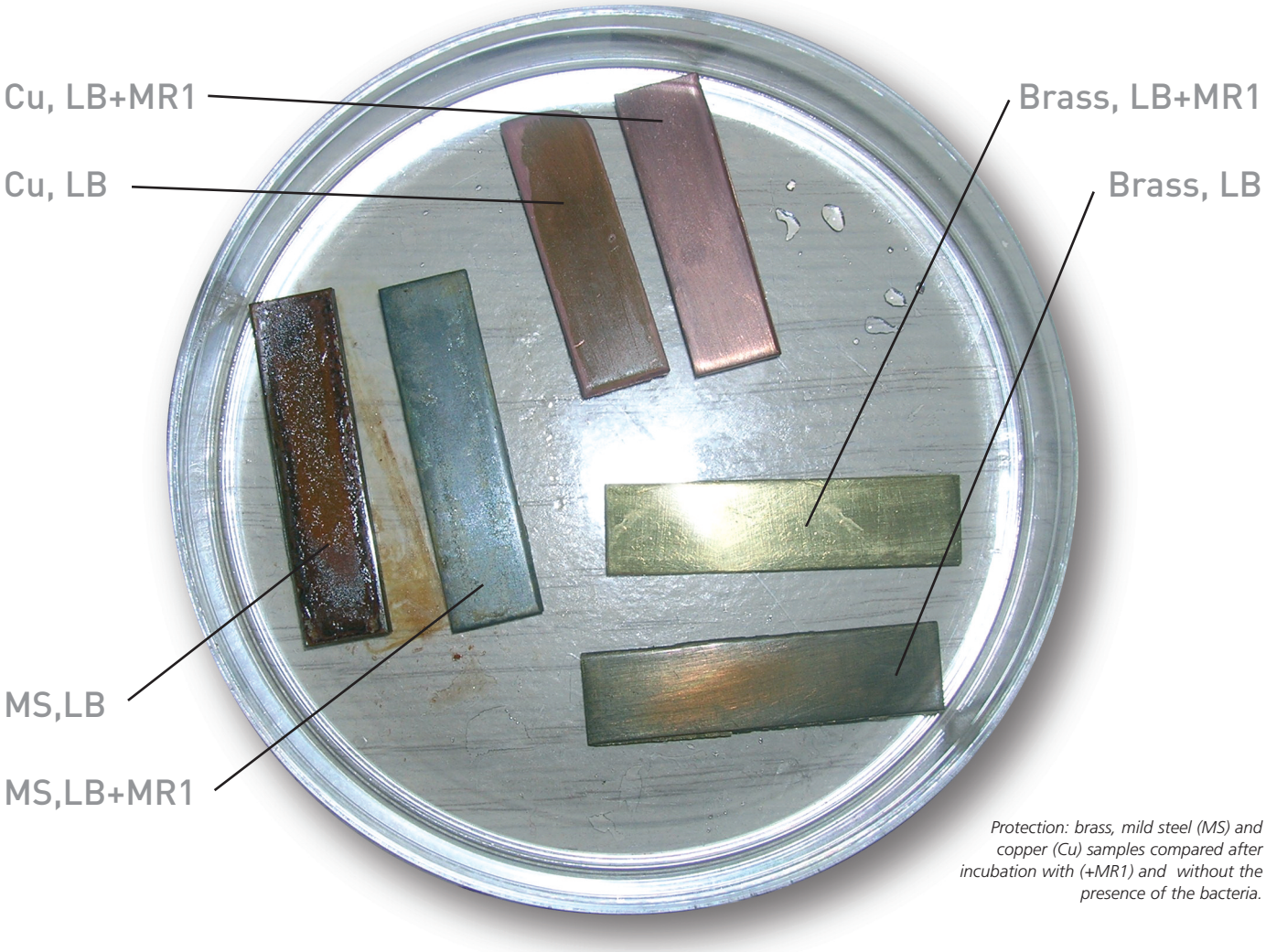
MR-1, first discovered by USC Professor Ken Nealson of the USC department of Earth Sciences, who is a co-author on the study is a remarkable organism that can incorporate metal into its metabolism, "inhaling certain metal oxides and compounds in one form, exhaling them in another," according to Denver presentation. MR-1 has previously been used to precipitate uranium out of contaminated water. And "it can grow almost anywhere and does not cause disease in humans or animals," they note.

And it can protect metal

The experiment performed at CEEL was simple. Matched pairs of samples of five metals - aluminum 2024, zinc, mild steel, copper, and brass - were prepared. One sample set of each pair was incubated in a growth medium containing MR-1; the other in a sterile bath of the same growth medium, containing neither MR-1 nor any other organism.

For a week corrosion rates were monitored, both visually and by measuring electro-chemical impedance (resistance to conducting alternating current.) Because electrical effects play a role in many forms of corrosion, higher AC impedance is associated with increased corrosion resistance.

The results were clear-cut. For all the materials, impedance increased with exposure to bacteria, and the longer the metals were exposed, the more resistant they became. For the aluminum alloy, for example, researchers observed a drastic increase of the impedance. By the end of the week the control samples showed obvious visual pitting, while the ones with MR-1 colonies were unscathed. The pattern of impedance varied from metal to metal. Aluminum showed drastic reduction in resistance to



Protection: brass, mild steel (MS) and copper (Cu) samples compared after incubation with (+MR1) and without the presence of the bacteria.

electrical currents in all frequencies. Brass and, particularly copper, showed nearly as dramatic an effect - readings indicated active corrosion in the control samples, but a large reduction in the MR-1 samples. The copper MR-1 samples, in fact, showed a profile similar to that demonstrated by copper covered with a protective polymer plastic film.

The patterns for steel and zinc were much less marked, but still significant, as was the difference in the metals' appearance (see photo, above).

Protection: brass, mild steel (MS) and copper (Cu) samples compared after incubation with (+MR1) and without the presence of the bacteria.

The next step, according to Mansfeld, is to figure out exactly what is going on and determine where and how the presence of bacteria is altering the corrosion equation. To do this, the group will be making molecular scale analysis of bacteria/metal interfaces, and looking to determine what the properties of MR-1 biofilm are, as well as why the pattern of interaction differs from metal to metal.

While MR-1 itself may not be the metal protector of the future, it may well suggest an agent that can be, Mansfeld says. The research will be presented at the 210th Meeting of the Electrochemical Society in Cancun, Mexico l October 29-November 3, 2006.

At the previous meeting of the group, the 209th, Mansfeld received the De Nora Award. In his award address he discussed the concept of MICI, showing how different bacteria can protect different materials from corrosion. He also described bacteria producing electricity in the bacterial batteries and fuel cells.

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Nonylphenol ethoxylates (NPEO)

Part two of two

Ecological and toxicological properties and consequences of the EU risk reduction strategy for technical applications

Dr. Simone Hoffmann-Dörr and Dr. Andreas Willing
Cognis Deutschland GmbH & Co. KG, Department of Product Safety & Regulations, Henkel Str. 67, D-40551 Düsseldorf, Germany

** For several reasons the situation in the US is different, i.e. under the specific US conditions NPEO is not seen as of environmental concern*

÷ Directive 2000/60/EEC introduces provisions for pollution reduction measures at Community level. Based on the list of priority substances in Annex X of the Directive, the Commission will propose quality standards and emission controls, including emission limit values two years after adoption of the list. For certain "priority hazardous substances" the emission controls shall aim at the cessation or phase-out of discharges, emissions and losses within 20 years. Nonylphenols are classified as "priority hazardous substances". The first list of priority substances including NP was adopted on 11 June 2001.

	Regulation
EU Commission	*2003/53/EC ◊* *NP, NPEO may not be used with more than 0.1 % in a product for :* • *Industrial and institutional cleaning* • *Domestic cleaning* • *Textiles and leather processing* • *Emulsifier in agricultural teat dips* • *Metalworking (except controlled, closed systems) ±* • *Manufacturing of pulp and paper* • *Cosmetics and personal care* • *Co-formulants in pesticides and biocides* *775/2004/EC amending Annex I to regulation 304/2003/EC (export and import of dangerous chemicals)* *NP, NPEO are prohibited for* • *washing and cleansing agents*
OSPARCOM	*PARCOM Recommendation 92/8, for:* • *Cleaning by 1995* • *Industrial processing by 2000*

Table 2: Documentation of NPEO risk reduction by regulation

In addition, voluntary industrial agreements have contributed to NPEO risk reduction (see table 3).

	Voluntary phase out	
	Cleaning	*Industrial processing*
Belgium	*By 1995*	*By 2000*
Netherlands	*By 1988*	
Germany	*By 1986*	*By 1992* *According to the recommendation by VSI** and VKIS±±, use of NP/NPEO in metalworking fluids should be restricted to 0.1%*
Austria	*By 2000*	
Denmark	*By 1987 (SPT)*	*By 2000*
Sweden	*By 1998*	*By 2004*
Norway	*By 2000*	*By 2000*
Finland		
Spain	*By 1998 (ADTA)*	
Greece	*No use*	*No use*
UK	*In 1976* *By 1990* *(wool scouring)*	*By 1998* *By 2002* *(waste water flocculants)*

Table 3: Documentation of NPEO risk reduction by voluntary industry agreements

◊ Directive of the European Parliament and of the Council amending for the 26th time Council Directive 76/769/EEC relating to restrictions on the marketing and use of certain dangerous substances and preparations (nonylphenol, nonylphenol ethoxylate and cement)

± A definition for closed systems can be found in EU Directive 2001/59/EC, Annex 7B, point 5.

*** Verband Schmierstoffindustrie e.V. (German lubricant association)*

±± Verbraucherkreis Industrieschmierstoffe (German association of industrial lubricant users)

As a consequence of the implementation of the risk reduction strategy, the amount of NPEO used for industrial applications decreased during the last years in Europe. For example, in Germany the amount of NPEO used as additives in metalworking fluids diminished from 800t in 1997 to 300t in 2002 [4].

Toxicogical properties of nonylphenol ethoxylates

Human risk assessment of NPEO

Risk is defined in general as the probability that an adverse health effect (hazard) occurs at a given exposure level. Thus, hazard as well as exposure assessment is needed for risk characterisation. Human exposure with a substance can result from inhalation, dermal contact and/or ingestion. The highest concentration of the substance that causes no adverse effect in animal experiments is defined as NOAEL (no observed adverse effect level). Comparison of the NOAEL with exposure data allows the determination of a so-called margin of safety (MOS). The higher the MOS, the higher the level of safety associated with that exposure route. As it is known from experience, a MOS of 100 is sufficient to account for uncertainty in extrapolating from animals to humans and for varying sensitivities among the human population [5].

Hazard assessment

Available hazard data on NPEO were summarised in a report of the Cosmetic, Toiletry and Fragrance Association (CTFA) [6]. From a subchronic oral study with rats, a NOAEL (no observed adverse effect level) of 40 mg/kg body weight/day can be derived. The adverse effect observed at higher concentrations was a dose-related increase in liver-to-body weight ratio. However, the haematologic parameters and the absolute liver weights remained unchanged. In reproductive toxicity tests with rodents, NPEO (with 10 or 30 moles EG) did neither affect maternal health nor development of the offspring [7, 8]. A NOAEL for maternal toxicity and teratogencity of 50 mg/kg body weight was derived from a study with NPEO (with 9 moles EG). Above this concentration significant decrease in weight gain of the dams, increase in pre-implantation loss, and enhanced skeletal anomalies of the pups was observed [8].

With regard to the NPEO degradation product nonylphenol, renal effects have been reported in repeated dose studies with rats ([9] and IUCLID dataset [10]). From a 90-day study, a NOAEL of 50 mg/kg body weight/day was derived [8]. Kidneys were also affected in dams that were applied NP by gavage within the scope of a teratogenicity study [10], resulting in a NOAEL for maternal toxicity of 75 mg/kg body weight/day. Nonylphenol is classified as toxic to reproduction (category 3, R62 - R63) in Annex 1 of Directive 67/548/EEC, implying that there is strong suspicion for developmental toxicity in absence of signs of maternal toxicity.

Exposure and metabolism

With regard to NPEO, exposure can arise from food, environment and technical applications. Hence, all routes of exposure (inhalation, dermal contact, and ingestion) have to be considered.

In the EU human health risk assessment (2002) for nonylphenol [11], the estimated exposure due to inhalation of NP during pesticide application was calculated to be 21 µg/human/event. The modelled dermal exposure was estimated to be 32 µg/human/event. According to data provided by industry, pesticide formulations contain 5% of NPEO, which has a NP residue of approximately 0.04%. Since the NPEO concentration in pesticide formulations is much higher than the concentration of NP, human exposure to NPEO most probably exceeds that of NP by a multiple.

Depending on the route of exposure, the amount of absorption of the substance and thus the bioavailability can vary. For this reason it is important to consider as well the toxicokinetics and the metabolism of the substance resulting from different exposure routes. As demonstrated in in vitro studies, NPEO are poorly absorbed by skin (less than 1% within 48 hours) [6]. For comparison, the dermal absorption of NP was assumed to be 10% in the EU risk assessment [11]. No information is available on bioavailability of NPEO after inhalation or oral uptake. After intravenous injection (representing 100% bioavailability) of radioactive labelled NPEO to rats the substance was completely metabolised, and all the radioactivity was excreted by 48 hours via faeces and urine [12].For both NP and NPEO, it has to be considered that rapid metabolism bases on the first pass effect in the liver and hence only occurs after oral intake. Therefore, a higher systemic bioavailability of NP/NPEO has to be expected after

inhalation or dermal exposure compared to oral intake. In 91 human blood samples which were examined for NP and NPEO, nonylphenol was found in 16 samples at a maximum concentration of 16 ng/g serum, whereas no NPEO was detected at all [13].

Human risk

Although NPEO exposure seems to exceed NP exposure by far, only NP is found in human blood. In relation with animal studies that suggest a rapid metabolism of NPEO, it can be assumed that NPEO toxicity predominantly derives from metabolic products such as NP. The hazard data available for NP and NPEO for repeated dose toxicity and reproduction toxicity are in the same order of magnitude and hence support this hypothesis.

In the EU human health risk assessment (2002) for nonylphenol [11], the margins of safety for repeated dose toxicity and reproductive effects were estimated. The conclusion was reached that MOS are very low for some exposure scenarios, including occupational exposure, local environmental exposure and combined exposure. These very low MOS gave rise to concern for repeated dose toxicity (target organs: kidney and liver) and reproductive effects. Therefore, need for further information and/or testing and for risk reduction measures was pointed out in the risk assessment.

The Canadian Environmental Protection Act (CEPA) also published a risk assessment on nonylphenol and its ethoxylates (1999) [14]. The relatively low MOS calculated for some products led to the conclusion that the assessment should be refined in order to determine the need for measures to reduce public exposure to both NP and NPEO.

In summary, a risk for human health with regard to nephrotoxic, hepatotoxic or reprotoxic effects can not be excluded for specific exposure scenarios. Risk reduction measures concentrate on diminishing the human exposure to NPEO and NP. Use restrictions and monitoring of pollution that were initiated to reduce environmental risk are likely to have a beneficial impact on human risk reduction as well.

Endocrine disrupting properties of NPEO

Endocrine disruptors are substances that interfere with the hormone system, disrupting its natural balance. Therefore, endocrine disruptors can adversely influence the reproductive success of a species, e.g. by changing the natural sex ratio (turning male fish into female).

The impact of potential endocrine modulators for humans can be assessed by deriving a hygiene-based margin of safety (HBMOS) which integrates exposure scenarios and endocrine potency. Such a HBMOS was assessed for nonylphenol [15]. The assessment based on a worst case exposure assumption of 2 µg/kg body weight and day which was obtained by evaluating the information of the Existing Chemicals Programme of the EU. Assuming that NP is twice as potent as p-tert-octylphenol (which was used in the in vivo test for oestrogen potency), a HBMOS of 125 was derived. Since the HBMOS is >1, no oestrogenic effects are expected for humans at the estimated dietary intake level.

Based on structural similarities between NPEO and steroid hormones (ring system), NPEO is also often suspected to be an endocrine disruptor. It has indeed been shown in vitro that NPEO can interfere with the hormone system. However, compared to hormones (oestrogen) the effect is rather weak, i.e. much higher (several orders of magnitude) concentrations of NPEO are required to observe hormone-like effects. The only weak endocrine effect of NPEO is reflected by the fact that the Predicted No Effect Concentration (PNECendocrine) of 10-20 µg/L is at least an order of magnitude higher than the PNEC for aquatic toxicity (PNECaquatic toxicity = 0.33 µg/L). In other words, the aquatic organisms will die due to the general toxicity of the substance before the endocrine disrupting properties become apparent.

Supplementary remarks

Environmental classification according to the EU-Directive for labelling of chemicals as "dangerous for the environment" (92/69/EEC):
On the basis of an evaluation of all available data on ecotoxicity and biodegradability CESIO recommends to classify NPEO as follows:

- Alkylphenol ethoxylates branched, C8-9, 3-10 EO: N, R-51/53
- Alkylphenol ethoxylates branched, C8-9, >10 EO: R-52/53

German water pollution class: According to the VwVwS of May 1999 [16] NPEO are classified as water endangering (WGK 2).

Conclusion

Biodegradation of NPEO leads to the formation of recalcitrant degradation intermediates of relatively high aquatic toxicity. According to the EU risk assessment the PEC/PNEC quotient is 2.4, i.e. the current exposure situation of NPEO in Europe gives rise to environmental concern. Furthermore, certain exposure scenarios give also rise to human concern. As a consequence, the EU Commission has recommended to implement a risk reduction strategy, which -among other measures- foresees strict emission controls for industrial applications using NPEO as surfactant. In the long run a phase-out of NPEO is envisaged and some industry associations have already pro-actively taken measures to phase-out NPEO, e.g. the European detergent industry (AISE) by a voluntary commitment.

With regard to the application in metalworking fluids a limit value of 0.1 % has been set for NPEO by EU Directive 2003/53/EC, except for use in closed systems. Based on the definition of closed systems given in 2001/59/EC it may be difficult to demonstrate compliance with 2003/53/EC in practise. Therefore, substitution of NPEO with less problematic alternatives may be the more straight-forward way.

For specific industrial applications, e.g. emulsion polymerization, well performing and cost-efficient substitutes for NPEO are already available, which will help industry to make their processes more sustainable.

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