

exploite the microorganisms responsible for suppression in biotechnological venture as soil-borne diseases are only partially controlled by the pesticides available. Some forms of disease-suppressiveness may be due to non pathogenic fungi (*Phialophora*, *Fusarium*, *Trichoderma*) or to root colonizing fluorescent pseudomonads. The latter are an important factor of the suppressive capacity of soils against black root rot caused by *Thielaviopsis basicola*. These soils extend over a distinct geological area of 22 km² near Payerne, Switzerland. The actual knowledge will be discussed.

Microbiological processes in urban wastes management

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Among the multiple processes which can take part in a waste management system, several are controlled by microorganisms; these include landfill disposal, which will undergo a slow biological oxidation, either aerobic or anaerobic; composting, i.e. optimized, aerobic and mainly thermophilic decomposition of solids; biological depuration (activated sludge or microbial beds), allowing the aerobic decomposition of dissolved organic pollutants; methanogenic digestion, i.e. anaerobic decomposition of suspended and dissolved organic matter allowing recuperation of biogas. Further processes could be involved, including the microbial oxidation of gases and volatile compounds occurring in the soil layers covering a landfill or in soil filters. The choice of a biological process in waste management should take into account the importance of recycling organic matter for humus regeneration in soils, the possible material and energy recuperation, as well as the negative effects of conventional systems on the environment. Two strategies including biological steps are discussed: one is centered on an optimized landfill disposal, with possible recuperation of gas and later of stabilized humus; the other implies intensive digestion of the organic fraction of waste combined with sewage sludge.

Modified 'Chalmer', a new medium for the enumeration (of the total) lactic acid bacteria among competitive flora

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The commercialized media used at present for the enumeration of the lactic acid bacteria have two defects:

- a) They are inhibitory for some species due to the presence of substrates like acetic acid, sodium acetate, etc.
- b) They permit growth at certain nonlactic acid bacteria like *Bacillus* or *Micrococcus*, not differentiated by characteristic colony types.

The modified Chalmer medium contains no inhibitors for the gram +ve flora and allows distinction of lactic from nonlactic flora, by characteristic colony types which do not need additional confirmatory tests as the confirmation rate is 100%. Its repeatability is very satisfactory.

It can be used for the detection and enumeration of the total lactic acid bacteria in acidified dairy products and fermented meat products.

Measurement of virus inactivation in the environment

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Survival of viruses in the environment is an important factor for human and animal health. We were specially interested in the question whether sludge fertilizer still contains infectious virus, and to what extent sludge treatment may reduce this risk. We measured inactivation of the model virus bacteriophage f2,

which in many respects resembles enterovirus and shows extraordinary heat stability. The virions were adsorbed to electro-positive filters, sandwiched between two inert filter membranes with pores smaller than the virus diameter, and exposed within filter holders with openings instead of inlet and outlet connections. After exposition, the surviving fraction was eluted and determined by plaque counting.

Aerobic thermophilic predigestion of sludge at 60°C led to a virus titer reduction of $5.9 \pm 1.15 \log 10$ units per h, thermophilic anaerobic digestion at 52–55°C reduced 0.54–3.28 log 10 units per h, whereas mesophilic anaerobic digestion at 35°C inactivated only between 0.53 and 2.33 log 10 units per day.

Similar inactivation studies with human and animal viruses, i.e. rota- and parvoviruses are in progress.

Rapid enumeration of *Escherichia coli* type 1 in water and foods by a membrane filter method

Arbeitsgruppe Mikrobiologie der Lebensmittelkontrolle Nordwestschweiz (corresponding author: T. Burki, Labor für Lebensmittelhygiene, Aarau)

A membrane filter method for the enumerating of *Escherichia coli* type 1 in water and foods is described. After a preincubation period of 2–4 h at 37°C or over night at 20°C on trypticase soy agar the membrane is transferred to ECD agar for an additional 12–24 h incubation at 44°C. *Escherichia coli* colonies are identified by a positive indole test performed directly on the membrane.

Rapid enumeration of microorganisms in foods by the direct epifluorescent filter technique (DEFT)

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Membrane filtration and epifluorescence microscopy were used for the direct enumeration of bacteria in meat- and dairy products, vegetables, pastries and drinking water. The different homogenized food samples could be filtered after employing varying surfactants and enzyme mixtures.

Fresh and pasteurized products showed good correlation between the direct and the colony counts.

Limulus-amebocytes-lysate (LAL) tests with foods

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Three different micromethods of the LAL-test, i.e. the capillar, microplate and chromogene test for the rapid determination of the amount of gram-negative bacteria in perishable foodstuffs were compared with respect to their practical applicability.

All three micromethods showed comparable and reproducible results when applied to pure cultures or to various fresh foods such as lettuce, minced meat, poultry carcasses, etc., and also to natural, carbon-free mineral water. A relatively good correlation existed between the endotoxin contents and the number of colonies of gram-negative bacteria down to the amounts of 10^3 – 10^4 g⁻¹. At low colony counts, unknown interference factors which may originate from the products, have to be taken into account.

Microbial desulfonation of multisubstituted naphthalene sulfonic acids

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Sulfonated aromatics, e.g. dyestuffs and their metabolites, are observed to be about 10% of the organic pollutants of the river

Rhine and are generally considered to be recalcitrant. Knackmuss' group, however, has managed to obtain biodegradation of a few naphthalene sulfonic acids as carbon sources for growth (degradation rate about 1 mkat/kg of protein) and the first reaction is desulfonation.

We decided to test whether the sulfur rather than the carbon in 7 'non-biodegradable' sulfononaphthalenes (2–4 substituents) was available to microorganisms. Sulfur-limited batch enrichments were inoculated with washed material from industrial sewage plants. Substrate-dependent growth with substrate disappearance was observed with each substrate.

Strain Z63 utilized SO_4^{2-} or e.g. 2-amino-5-hydroxy-7-sulfononaphthalene with a growth yield of about 3 kg of protein/mole of S (degradation rate about 20 $\mu\text{kat/kg}$ of protein). The observations, that the sulfur but not the carbon of these compounds is readily available to microorganisms from native environments, lead us to believe that desulfonation and ring-degradation reactions occur naturally with different specific activities in different organisms.

Microbial degradation of benzenesulfonic acid and its derivatives

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Sulfonated aromatic compounds (e.g. metabolites of dyestuffs) are observed to be major pollutants of rivers and lakes. We have enriched for organisms that are able to utilize as sole source of carbon and energy for growth benzenesulfonate, its 2-amino-, 4-amino-, 4-hydroxy-, 4-methyl- or 4-carboxy-derivative. Pure cultures (18) were isolated. All were bacteria.

One organism, OS-1, isolated to utilize 2-amino-benzenesulfonate, also metabolized benzenesulfonate and 4-methyl-benzenesulfonate. The specific growth rates on these substrates were 0.11, 0.19 and 0.07 h^{-1} , respectively. Each substrate was utilized quantitatively with growth yields of about 5 g of protein/mole of C. Sulfite tended to accumulate towards the end of growth and was then oxidized to sulfate which accumulated stoichiometrically.

Cell extracts of strain OS-1 were prepared and NAD(P)H-dependent substrate disappearance and sulfite release were observed. Specific activities in non-optimized assays were 0.1 and 0.3 mkat/kg of protein for benzenesulfonate and 2-amino-benzenesulfonate, respectively.

ELISA test application on food intoxication at CHUV (Centre Hospitalier Universitaire Vaudois)

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The use of ELISA test (recommended by Prof. Dr H. Fey from the University of Bern), confirmed the presence of staphylococcus enterotoxins strains in prepared dishes served at CHUV, which have been suspected to be the cause of food intoxication. 88 samples have been analyzed according to the standard criteria meaning the research of *Staphylococcus*, the *Salmonella* and the *Clostridium perfringens*. 10 samples have been revealed as highly infected by staphylococcus coagulase positive. 4 samples contained the strains which showed, due to ELISA test, the capacity of producing a great quantity of enterotoxin D.

On the other hand, the strains produced from the nose of some CHUV kitchen's employees with the same lysotype as the strains produced by the infected food, showed also the capacity of producing a great quantity of enterotoxin D.

Anaerobic degradation: catabolism of xylene under denitrifying conditions

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The microbial degradation of m-xylene (1,3-dimethylbenzene) under denitrifying conditions was studied in a perfusion column filled with river sediment material. This column represented a typical river water/ground water infiltration system. After several months of adaption, as much as 0.3 mM m-xylene at a flow rate of 2.4 cm/h was completely degraded in a column with a total length of 26 cm. Using radiolabeled substrate, 80% of the [^{14}C] m-xylene was mineralized to $^{14}\text{CO}_2$. The conversion of m-xylene was coupled with a reduction of NO_3^- to NO_2^- . No rapid metabolism of m-xylene was observed upon substitution of NO_3^- by either oxygen or sulfate. Studies to elucidate the mechanism of the anaerobic m-xylene metabolism are in progress. The crucial step in the anaerobic degradation appears to be the introduction of a functional group followed by a conversion to an intermediate such as a phenol.

Surface proteins and Western Blot analysis of *Listeria monocytogenes*

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Most clinical isolates of *Listeria monocytogenes* (Lm) belong to only two serotypes, i.e. 1/2b and 4b, which does not allow sufficient discrimination for epidemiological studies. Disc SDS PAGE of Lm after light sonication showed about 50 different bands, including eight major bands. All the 17 known serotypes presented similar patterns. A 160 kd protein had a shorter migration for serotypes 4ab, 6a, 6b. Differences in intensity have also been found in 25 kd doublet. Serotype 1/2c is characterized by a dense band at 36 kd. Most of the bands found on the SDS-PAGE react with rabbit anti 4b antiserum at Western Blot analysis. With this technique, four major immunodominant components were found, with apparent mol. wt of respectively 40 kd, 50 kd, 70 kd and 95 kd.

In conclusion, SDS-PAGE of surface proteins in Lm might be a useful tool for epidemiological studies.

New Antibiotics and Resistance

DNA homology of a transferable *Clostridium difficile* resistance determinant with transposon TN551

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Clostridium difficile, described as a causative agent of antibiotic associated pseudomembranous colitis, shows resistance to many antimicrobial agents. Resistance to either clindamycin/erythromycin (CC^r/Ery^r) or tetracycline was found to be transferable to susceptible *C. difficile* strains. Our present results suggest that the two resistance determinants are located on the chromosome. Further results, achieved by DNA-DNA hybridization experiments, indicate DNA homology of the CC^r/Ery^r determinant with transposon Tn551 which is found in *Staphylococcus aureus*. Tn551 belongs to a group of related transposable elements coding for resistance to macrolide, lincosamide and streptogramin B in a variety of gram positive bacteria. — Experiments are in process to determine whether the CC^r/Ery^r determinant may