energy. The following possibilities could be envisaged:

- Use of the biomass as single cell protein, fertilizer or compost (after processing). These applications would be restricted to biomass from waste degradation processes without toxic organic or metallic impurities.
- Production of biogas (methane) by anaerobic digestion of sewage sludge. Such biogas plants are often combined with municipal sewage treatment plants (for instance in Zürich-Werdhölzli). 1 kg of biomass (dry weight) yields 500 l of biogas (~ 70% CH_4, 29% CO_2 and 1% H_2; heat of combustion: 5500–6500 kcal/m^3). The biogasification of industrial sewage sludge would also result in a significant decrease of the remaining sludge volumes to be deposited in landfills or incinerated.
- Extraction of interesting cell components from pure cultures: polyhydroxy butyrate, enzymes, nucleic acids etc.
- In the future, the production of interesting chemicals by genetically modified microorganisms grown on waste substrates instead of the usual C- and N-sources could be of interest.
- A number of ideas and methods for the processing and application of biomass or modified biomass have recently been patented by scientists of Bayer Ltd[7].

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Bacterial growth on 1,2-dichloroethane[1*]

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Summary. 1,2-Dichloroethane (5 mM) served as the only carbon and energy source for bacterium DE2, a gram-negative, oxidase-positive, motile rod. The specific growth rate μ of strain DE2 on 1,2-dichloroethane was 0.08 h^{-1}. A NAD-dependent 2-chloroacetaldehyde dehydrogenase activity and a 2-chloroacetate halidohydrolase activity were detected in extracts of cells grown on 1,2-dichloroethane.

1,2-Dichloroethane or ethylene dichloride is one of the highest volume chemicals produced in the world with an estimated annual production of 13 million tons[3]. About 10% of the compound, which is mainly used as an intermediate in the production of vinyl chloride and other chemicals, is released into the environment[3]. Oxidation of the compound in the atmosphere is believed to result in the formation of the mutagens 2-chloroacetaldehyde and formylchloride and of 2-chloroacetic acid[4–6]. The same degradation products were detected in mice and rats fed with 1,2-dichloroethane[7,8]. Despite its importance as an industrial and environmental chemical, the microbial degradation of 1,2-dichloroethane has not been studied thus far. Here we present evidence for the quantitative degradation of 1,2-dichloroethane by the bac-

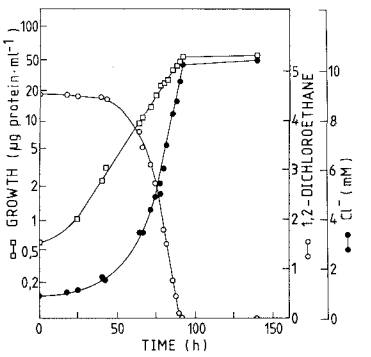
terial strain DE2, an organism isolated from an enrichment culture with 1,2-dichloroethane as the only carbon source[9].

Materials and methods. Strain DE2 and the mixed culture it was isolated from were grown at 30 °C on a rotary shaker in 300 ml Erlenmeyer flasks sealed with gas-tight stoppers. Mineral salts medium[10] supplemented with vitamins[11] and 5 mM 1,2-dichloroethane was used. Larger amounts of cells were prepared in continuous culture using a 1-l glass fermenter[10]. It was verified that spontaneous dehalogenation of 1,2-dichloroethane did not occur under the growth conditions used.

Cell extracts were prepared in 20 mM potassium phosphate pH 7.2 as described[10]. Dehalogenation rates were determined by following the release of protons[12]. Resting cells at pH 7.5 and 37 °C were used for measuring the dehalogenation of 1,2-dichloroethane (1 mM) while the dehalogenation of 2-chloroacetate (7 mM) was performed with crude cell extracts at pH 9.0 and 30 °C. The incubation mixture for measuring 2-chloroacetaldehyde dehydrogenase activity contained in a volume of 1.0 ml: 0.1 mmol of Tris-sulfate pH 9.0, 0.5 µmol of NAD, cell extract (up to 40 µg of protein) and 10 µmol of 2-chloroacetaldehyde to start the reaction which was run at 30 °C. The reaction rate was followed by measuring the increase in absorbance at 340 nm of the reaction mixture. Catalytic activity is expressed in katals (kat), 1 kat corresponding to the amount of activity catalyzing the transformation of 1 mol of substrate per sec.

Protein and chloride were quantitated as previously described[10]. 1,2-Dichloroethane was determined by gas-chromatography on a porapak P column[10]. It was obtained from Fluka (Buchs, Switzerland). Its identity and purity were checked by mass-spectrometry, by nuclear magnetic resonance and by GC-analysis.

Results and discussion. The enrichment of a mixed culture utilizing 1,2-dichloroethane from soil that had been exposed to chlorinated hydrocarbons over a period of several years has been described[9]. The isolation from this mixed culture of a pure culture growing on 1,2-dichloroethane was complicated by the fact that the 1,2-dichloroethane degrading organism did not grow on solid media. It was possible, however, to obtain a pure strain by terminal dilution of the mixed culture in liquid medium with 1,2-dichloroethane. The isolate was a vitamin-dependent bacterium, designated strain DE2. Strain DE2 was a motile, gram-negative, oxidase-positive rod which grew in liquid culture with the following compounds as the sole carbon and energy source: ethanol, acetate, 2-chloroethanol, 2-chloroacetate, glycol, glycolate, succinate and 1,2-dichloroethane. Strain DE2 did not form colonies on nutrient agar or on other media solidified with agar, silica gel or gelatin. Uniform morphology upon inspection by phase contrast- and electron-microscopy as well as the absence of growth on nutrient agar were routinely used as criteria for the purity of cultures of strain DE2. When kept at room temperature, liquid cultures of the organism remained viable over a period of at least 6 months.



Formation of bacterial protein (□), degradation of 1,2-dichloroethane (○), and liberation of chloride (●) by bacterium DE2 growing on a mineral salts plus vitamins medium with 5 mM 1,2-dichloroethane as the sole carbon and energy source.

As shown in the figure, strain DE2 grew exponentially on 5 mM 1,2-dichloroethane with a specific growth rate of 0.08 h^{-1}. The substrate was used completely and the chlorine atoms were quantitatively released as Cl^-. No growth was observed in control experiments without substrate. No degradation products of 1,2-dichloroethane other than Cl^- were detected in supernatants from growing or resting cells. The recovery of 1,2-dichloroethane plus Cl^- in the growth medium varied between 90% at the beginning of the experiment and 98% at the end. The relatively low recovery of the substrate in the early phase of batch cultures was due to the distribution of the substrate between the liquid phase and the gas phase of the growth vessels. As the utilization of 1,2-dichloroethane proceeded, the liquid phase was replenished and the recovery improved. 1,2-Dichloroethane was dehalogenated by resting cells at a rate of about 0.5 mkat (kg protein)$^{-1}$. This amounts to 15% of the activity required to support the observed growth rate. The dehalogenating activity of intact cells was completely inhibited by KCN (0.5 mM) and by $HgCl_2$ (0.2 mM). Various attempts to detect a 1,2-dichloroethane-degrading activity in cell extracts from 1,2-dichloroethane-grown cells were unsuccessful. However, the extracts contained an NAD-dependent 2-chloroacetaldehyde dehydrogenase activity of 8.0 mkat (kg protein)$^{-1}$ and a 2-chloroacetate halidohydrolase activity of 33.5 mkat (kg protein)$^{-1}$. The presence of these enzyme activities suggests that the degradation of 1,2-dichloroethane by strain DE2 is initiated by an oxidation yielding the unstable intermediate 1,2-dichloroethanol[7]. 1,2-Dichloroethanol decomposes spontaneously to hydrochloric acid and 2-chloroacetaldehyde. The latter compound is

thought to be oxidized to 2-chloroacetate, which is dehalogenated to glycolate[13], a metabolite readily utilized as a carbon source by strain DE2 as well as many other bacteria.

*While the present paper is a short communication, the editors include it here as a useful appendage to the preceding review papers.

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Short Communications

The microbial oxygenation of the benzylisoquinoline alkaloid laudanosine[1]

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Summary. The microbial transformation of the benzylisoquinoline alkaloid laudanosine by a strain of *Pseudomonas putida* gives a metabolite in which O-demethylation of 1 methoxyl group of ring C, and introduction of 1 ketonic oxygen at C_9 and 1 phenolic oxygen at ring C have occurred. Also, O-methylcoripalline is formed in this transformation.

Laudanosine (**1**) is a minor opium alkaloid with the benzylisoquinoline skeleton. The chemical and biological importance of benzylisoquinoline alkaloids has stimulated many studies on the regiospecific oxygenation and the oxidative cyclization reaction[3,4]. The chemical oxidation of laudanosine with lead (IV) acetate or Fenton's reagent (Fe^{II}-H_2O_2) gives only O-methylcoripalline (**2**) and veratraldehyde (**3**), resulting from 'benzylic fission' between carbons 1 and 9. Neither cyclization to other alkaloids nor further functionalization of the benzylisoquinoline skeleton have been noted[5].

Here we report the results of the microbial transformation of laudanosine with a strain of *Pseudomonas putida,* isolated from a biological waste water treatment plant by an enrichment technique in the presence of laudanosine as the only carbon and energy source. This organism was grown on Raymond and Davis[6] liquid mineral salts medium with 0.5‰ (w/v) laudanosine. Under these culture conditions the formation of a compound absorbing at 395 nm at pH = 7 was detected.

For transformation experiments the *Pseudomonas putida* cells were inoculated into 750 ml flasks containing 0.5‰ laudanosine in 150 ml mineral medium. The flasks were incubated with shaking at 30 °C for 96 h.

The metabolites were isolated as follows: extraction of the culture broth with n-butanol gave a residue which was chromatographed on silica gel (R = 100). 15% of O-methylcoripalline (**2**) (based on the initial amount of laudanosine) and the yellow metabolites A, 6%; B, 12% and C, 6% were isolated. In the mass-spectrum of B were apparent peaks at m/e = 373 (M^+), 354, 207 and 193. This suggested that compound B was derived from laudanosine by loss of 1 carbon atom and 4 hydrogen atoms and introduction of 2 oxygen atoms. One of these oxygen atoms was part of a carbonyl function, as shown by the band at 1695 cm^{-1} in the IR (nujol).

The NMR-spectrum of B in $CDCl_3$ showed the N-methyl hydrogens as a singlet at 3.40 δ and the hydrogens of 3 methoxyls as singlets at 3.75 δ (3 hydrogens) at 4.15 δ (6 hydrogens). This suggested that the 1 carbon loss resulted from cleavage of 1 methoxyl group. Furthermore, 4 aromatic hydrogens were present. Two of them were singlets at 6.42 δ and 7.72 δ and 2 were doublets centered at 7.10 δ and 7.65 δ with J = 7 cps, suggesting an ortho arrangement. Since laudanosine has 5 aromatic hydrogen atoms, these data suggested that the additional oxygen atom in B could be a nuclear hydroxyl. An indication of the structure of B came also from the observation that in its