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Compound specific stable isotopes fractionation analysis (CSIA) is an useful approach in studies on the mechanisms involved in dichloromethane degradation by aerobic methylobacteria

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Dichloromethane (CH_2Cl_2, DCM) is a highly toxic volatile and water solvent. Due to large quantities of industrial production ($3•10^5$ tons annually) and persistence, this solvent has become one of the most abundant anthropogenic environmental pollutants. The genotoxic and carcinogenic effects associated with DCM caused acute interest concerning its microbial degradation. Among the DCM-degraders several aerobic methylotrophic bacteria have been earlier isolated and characterized. In these aerobic methylobacteria, primary dehalogenation of DCM is catalyzed by cytoplasmic DCM dehalogenase (glutathione S-transferase) encoded by *dcmA* gene. Dehalogenation products are hydrochloric acid and formaldehyde, the latter being used for energy generation and biosynthesis. However the mechanisms of DCM transport into the cells remain unknown. To clarify this we determined $^{12/13}$C and $^{35/37}$Cl stable isotopes fractionation of CH_2Cl_2 during its mineralization by resting cells, cell extracts and purified preparations of DCM dehalogenases from the serine pathway *Methylobacterium dichloromethanicum* DM4 and *Methylobacterium extorquens* AM1 pME 8220. In addition to carbon stable isotope composition measurement technique, we developed method of estimation of $^{35/37}$Cl isotope composition in each of two positions

of DCM molecule based on abundance of three types of molecules which present 95% of all CH_2Cl_2 and contain different chlorine isotopes ($^{35}Cl_2^{12}C^1H_2$, $^{35}Cl^{37}ClC^1H_2$ and $^{37}Cl_2^{12}C^1H_2$). The difference between kinetic isotope effects obtained for both elements in enzymatic dehalogenation reaction and during DCM consumption by cell suspensions suggests the operation of diffusional DCM transport in these bacteria. On the other hand, no significant changes in $^{12/13}C$ isotope composition of produced CO_2 were observed for the cells mineralizing DCM. This effect presumably results from simultaneous oxidation of other intracellular substrates that accompanies DCM biodegradation. This suggestion is also verified by O_2 consumption and CO_2 production during DCM mineralization by degraders found to be more active than theoretically calculated for this process. Based on data obtained we concluded that an analysis of stable carbon and chlorine isotope composition might be rather useful in studies on the mechanisms involved in bacterial biodegradation of various chlorinated hydrocarbons as well as for the evaluation of the *in situ* environmental bioremediation from these pollutants.

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