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Microbes in crystalline bedrock. Assimilation of CO_2 and introduced organic compounds by bacterial populations in groundwater from deep crystalline bedrock at Laxemar and Stripa

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MICROBES IN CRYSTALLINE BEDROCK. ASSIMILATION OF CO_2 AND INTRODUCED ORGANIC COMPOUNDS BY BACTERIAL POPULATIONS IN GROUNDWATER FROM DEEP CRYSTALLINE BEDROCK AT LAXEMAR AND STRIPA

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Assimilation of CO_2 and introduced organic compounds by bacterial populations in groundwater from deep crystalline bedrock at Laxemar and Stripa

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ABSTRACT

The assimilation of CO_2 and of introduced organic compounds by bacterial populations in deep groundwater from fractured crystalline bedrock has been studied. Three depth horizons of the subvertical boreholes KLX01 at Laxemar in southeastern Sweden, 830-841 m, 910-921 m and 999-1078 m, and V2 in the Stripa mine, 799-807 m, 812-820 m and 970-1240 m were sampled. The salinity profile of the KLX01 borehole is homogeneous and the groundwaters had the following physico-chemical characteristics: pH values of 8.2, 8.4 and 8.5; Eh values of -270, no data and -220 mV; sulphide: 2.3, 11.0 and 5.6 μM; CO_3^{2-}: 104, 98 and 190 μM; CH_4: 26, 27 and 31 μl/l and N_2: 47, 35 and 18 ml/l, respectively. The groundwaters in V2 in Stripa were obtained from fracture systems without close hydraulic connections and had the following physico-chemical characteristics: pH values of 9.5, 9.4 and 10.2; Eh values of +205, +199 and -3 mV; sulphide: 0, 106 and 233 μM; CO_3^{2-}: 50, 57 and 158 μM; CH_4: 245, 170 and 290 μl/l and N_2: 25, 31 and 25 ml/l, respectively. Biofilm reactors with hydrophilic glass surfaces were connected to the flowing groundwaters from each of the 3 depths with flow rates of approximately 3 x 10^{-3} m sec^{-1} over 19 days in Laxemar and 27 to 161 days in Stripa. There were between 0.15 to 0.68 x 10^5 unattached bacteria ml^{-1} groundwater and 0.94 to 1.2 x 10^5 attached bacteria cm^{-2} on the surfaces in Laxemar and from 1.6 x 10^3 up to 3.2 x 10^5 bacteria ml^{-1} groundwater and from 2.4 x 10^5 up to 1.1 x 10^7 bacteria cm^{-2} of colonized test surfaces in Stripa. Assuming a mean channel width of 0.1 mm, our results imply that there would be from 10^3 up to 10^6 more attached than unattached bacteria in a water conducting channel in crystalline bedrock. The assimilations of $^{14}CO_2$, ^{14}C-formate, 1,2,3-^{3}H-acetate, U-^{14}C-lactate, U-^{14}C-glucose and L-4,5-^{3}H-leucine by the Laxemar and Stripa populations were demonstrated with microautoradiographic and liquid scintillation counting techniques. The measured CO_2 assimilation reflected the *in situ* production of organic

carbon from CO_2. Assimilation of formate followed that of CO_2 and indicated the presence of bacteria able to substitute formate for CO_2 e.g. methanogenic bacteria. The presence of sulfate reducing bacteria (SRB) is suggested by the observed assimilation and respiration of lactate by up to 74 % of the bacterial populations. The recorded uptake of acetate and glucose indicates the presence of heterotrophic bacteria other than SRB. Upto 99 % of the populations assimilated leucine which showed that major fractions of the populations were viable. Incubation in air compared to N_2 indicated that portions of the studied populations were obligate anaerobes as their ability to assimilate the added compounds was sensitive to oxygen. The results show that the use of several different compounds for assimilation experiments, reduces the risk for false conclusions about the viability and the metabolic activity of the deep groundwater populations. The Stripa results implies that deep groundwater bacteria have a CO_2 assimilating potential that may have a profound influence on the groundwater chemistry, through its action on the carbonate system.

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1 INTRODUCTION

In recent years there has been an increasing interest in microbiology of fractured rock. One reason is that many countries are seriously considering using fractured rock as the final repository for nuclear waste, at depths ranging from few tens of meters for low and intermediate waste, up to more than a kilometre for high level waste. There is considerably less information and experience on depths below a few hundred meters than at shallow depths (Ghiorse & Wilson 1988; West *et al.*, 1982, 1985). A more detailed analysis of microbial interactions with nuclear waste reveals complex and difficult research areas (West *et al.*, 1985), made more difficult by the classical problem of sample disturbance and contamination during the drilling of boreholes. Further, the investigations have to be aimed at assessing the long term safety of nuclear waste disposal, where time scales range from hundreds to several millions of years due to the very long half-lives of many radionuclides. There are currently large national research programs in Canada, Switzerland and Sweden for the study of radionuclide transport in crystalline rock. In addition an international field research programme organized by OECD/NEA in the Stripa research mine has entered its final phase.

Pedersen and Ekendahl (1990) investigated the distribution of bacteria in groundwater from 16 different levels in 5 boreholes in crystalline bedrock down to at most 860 m in the Laxemar-Äspö study area for the forthcoming hard-rock laboratory in SE Sweden (Gustafsson *et al.*, 1991; Stanfors *et al.*, 1991). An average of 2.6×10^5 bacteria ml^{-1} groundwater and 3.5×10^5 bacteria cm^{-2} surface exposed to flowing groundwater for 10 days was found, and enrichment cultures indicated the presence of autotrophic bacteria with capability to grow on C-1 compounds and also heterotrophic dissimilatory sulfate-reducing bacteria (SRB). Most probable number assays confirmed up to 5.6×10^4 SRB ml^{-1} groundwater in the Laxemar-Äspö area.

Dissolution and transport by the groundwater is the most important

dispersion mechanism for radionuclides eventually released from the waste. The documented presence of bacteria (Pedersen & Ekendahl 1990) can influence the transport of such radionuclides. Free-living bacteria constitute mobile suspended particles which may have a radionuclide sorbing capacity higher than that of the surrounding rock (Beveridge & Fyfe, 1985; Pedersen & Albinsson, 1991; Strandberg *et al.*, 1981). The radionuclide transport will then go faster with, than without, bacteria. If, on the other hand, the majority of the bacteria are growing in biofilms on fracture surfaces, there may be a retardation of the transport. Finally, bacterial production of complexing agents and other metabolites can affect speciation and thus mobility of radionuclides independent of whether the bacteria are attached or not.

The relevance of different mechanisms for radionuclide transport by bacteria can only be evaluated with knowledge about the ecology and the physiology of the bacterial populations that may inhabit a nuclear waste repository and its surroundings. Identification of their nutritional strategies is an important step in this understanding. Autotrophic bacteria may provide organic matter for heterotrophic organisms if hydrogen and CO_2 are available for the autotrophs, e. g. acetogenic bacteria (Fuchs, 1986, Wood & Ljungdahl, 1991), methanogenic bacteria (Belayaev & Ivanov, 1983; Belayaev *et al.*, 1983; Godsy, 1980; Olson *et al.*, 1981) and several of the sulfate-reducing bacteria (SRB) (Faque *et al.*, 1991). Fermentative or respiratory utilization of geological deposits of organic material are other possible explanations as to why heterotrophic bacteria have been found in deep geological formations (Chapelle *et al.*, 1987, 1988; Hicks & Fredrickson, 1989; Pedersen & Ekendahl, 1990).

In this study, we have assayed the total numbers of bacteria in the groundwater and on surfaces exposed to slowly flowing groundwater from two boreholes called V2, characterized during the geochemical investigations of the Stripa groundwaters (Nordstrom *et al.*, 1985) and KLX01, characterized during the geochemical investigations for the hard rock laboratory (Gustafsson *et al.*, 1988). The nutritional responses of the unattached and attached bacterial populations were studied by measurement of their assimilation of CO_2 and different radiolabeled organic compounds and their production of CO_2 from lactate using microautoradiographic (MARG) and liquid scintillation counting (LSC) techniques. Assimilation of CO_2 was used to assay the *in situ* production of organic carbon from CO_2. Assimilation of formate indicated the presence of bacteria able to substitute formate for CO_2, eg. methanogenic and acetogenic bacteria. Acetate, lactate and glucose assimilation demonstrated the presence of heterotrophic bacteria and assimilation of leucine showed that the populations studied were viable. Respiration of lactate indicated the presence of bacteria capable of anaerobic catabolism.

2 MATERIALS AND METHODS

2.1 DESCRIPTION OF STUDY SITES

2.1.1 Laxemar

This investigation area is a part of the Precambrian bedrock in SE Sweden where the Småland granites predominate the older, Sveocokarelian complexes (Gustafsson *et al.*, 1988, 1989). The borehole KLX01 was situated in the centre of a major block of the Laxemar area and is sub-vertical. It was core drilled to 702 m in 1987 and continued to 1078 m during summer 1990, the core having a diameter of 76 mm. The drilling water used was groundwater from a 100 m deep percussion drilled hole. It was marked with fluorescein (Aldrich, West Germany, order no. 16,630-8) as a fluorescent drilling water tracer. Hydraulic tests were performed in the borehole in connection with the drilling operations. Large quantities of groundwater were then drawn to estimate the water capacity of the borehole. This operation simultaneously cleaned the borehole and the fractures from drilling water and mud. The examined depths were closed off with packers, made of inflatable 76 mm rubber tubes (length: 1 m), in sections of 5 to 10 m, or more when necessary to achieve a satisfactory flow rate of approximately 50 - 100 ml min^{-1}. The sampling depths studied were 830-841 m, 910-921 m and 999-1078 m.

2.1.2 Stripa

The Stripa mine has been a deep underground research facility since 1976 when the iron ore was mined out. The ore consisted of a quartz-banded haematite and occurred in a lepatite formation. Adjacent to the lepatite is a large body of medium-grained granite through which borehole V2 runs. It is a subvertical shaft with a diameter of 76 mm and runs from one of the

deepest drifts of the mine, 410 m, down to a depth of 1240 m. It was drilled to 860 m in 1977 and continued to 1240 m in 1981. A number of sampling depths of this artesian borehole were closed off with packers made of inflatable 76 mm rubber tubes and connected to the drift with 6 mm teflon tubings. The sampling depths used in this study were 799-807 m, 812-820 m and 970-1240 m below ground. There were approximately 2 fractures per m of the borehole, sealed or coated mainly with chlorite, epidote and calcite (Nordstrom *et al.*, 1985). Because of silicate weathering, the pH of the groundwater approaches 10.

2.2 WATER AND GAS ANALYSIS

2.2.1 Laxemar

An integrated mobile field laboratory (Wikberg *et al.*, 1987) was used for water sampling, analysis and incubation of samples. The pH, Eh, the concentrations of $N\text{-}NO_2^-$, $N\text{-}NO_3^-$, $N\text{-}NH_4^+$, $P\text{-}PO_4^{3-}$, SO_4^{2-}, S^{2-}, CO_3^{2-}, the conductivity and the drilling water fluorescent marker were analyzed in the field lab. $N\text{-}NO_2^-$ and $N\text{-}NO_3^-$ (SIS 028132, Swedish Standard Commission), $N\text{-}NH_4^+$ (SIS 028134) and $P\text{-}PO_4^{3-}$ (SS 028126) were analysed spectrophotometrically. SO_4^{2-} was measured with ion-chromatography, S^{2-} with a iodometric method (SIS 028115), HCO_3^- by endpoint titration (SS 028139). A borehole probe continuously registered temperature, pH, Eh and the sulfide concentration at the sampling depth as described by Wikberg (1987). An important condition for the analysis of the groundwater composition was that the continuously measured redox potentials were stabilized (gold, platinum and glassy carbon electrodes). Water had to be pumped from each sampling depth for about 2 weeks to fulfil this requirement. This meant that about 2 m^3 of groundwater was drawn out of the fracture system at each depth during the weeks before sampling.

A gas sampler (Torstensson, 1984) was adapted for the collection of undisturbed samples for gas analysis. Two cylindric tubes made of stainless steel were supplied with nitril rubber stoppers and evacuated. They were opened and closed at the sampling depth by penetration of the stoppers with hypodermic needles with a mechanical device controlled from ground. The total volume water sampled at each depth was 150 ml. The samples were brought to the surface with the pressure at the sampling depth sustained. The contents of He + H_2, CO, CO_2, CH_4, C_2H_2, C_2H_4, C_2H_6, O_2 and N_2 of the waters were extracted by degassing the samples at <40 Pa whereafter the total gas volume was collected in a burette with a

septum by step-wise pumping with mercury and compressed air. The gas composition was analysed with a Perkin Elmer gas chromatograph supplied with two columns (Porapak N 80 - 100 Mesh 4m +1/8" and Molesieve 5A 60-80 Mesh 2' + 1/8"), a thermal conductivity detector and a flame ionisation detector. Carbon monoxide and carbon dioxide were catalysed to methane before detection. The carrier gas used was argon.

2.2.2 Stripa

The pH and the Eh were measured *in situ* in the mine with a PHM Autocal pH meter (Radiometer), a GK2421C combined pH electrode and redox electrode PK1401. SO_4^{2-} was measured turbidimetrically with $BaCl_2$ (Franson 1985), S^{2-} with a iodometric method (Franson 1985) and the CO_3^{2-} contents were measured with a coulometer (Model 5011 CO_2 Coulometer) (Huffman, 1977). These procedures were repeated at a 30 day interval.

Gas pipettes (100 ml) were connected to the flowing groundwaters, left for 17 hours and closed. The contents of He + H_2, CO, CO_2, CH_4, C_2H_2, C_2H_4, C_2H_6, O_2 and N_2 in the waters were analysed as for the samples from Laxemar. This procedure was repeated at a 30 day interval. The carrier gas used was argon except for the hydrogen analysis when helium was used.

Oxygen was additionally analysed with the Winkler method (Carlberg, 1972) 26 June 1991. Briefly, winkler bottles, approximately 120 ml, were filled with water under 100% nitrogen atmosphere. 1 ml KI solution (KI 600 g/l + NaOH 320 g/l) and 1 ml $MnSO_4$ x H_2O (500 g/l) were added simultaneously with syringes with the needles placed at the sample bottle bottom. The bottles were stored at +4°C until analysis. At analysis, 1 ml 9 M H_2SO_4 was added to each bottle and titration was performed with 0.016 M $Na_2S_2O_3$, standardised against 0.01 M KIO_3. A 0.1 g ml^{-1} iodine indicator was used to detect the end point of the titration.

2.3 ATTACHMENT AND GROWTH OF BACTERIA

Attachment and growth of bacteria. Biofilm reactors (Pedersen 1982; Pedersen *et al.*, 1986) were connected to the flowing groundwaters from KLX01 and V2 at flows of approximately 1 x 10^{-3} m sec^{-1}. Microscope slides, 60 x 24 x 1 mm, were heated in a muffle furnace at 475 °C for 4 h and used as hydrophilic substrata for the attachment and growth of bacteria.

2.4 TOTAL NUMBER OF BACTERIA

2.4.1 Unattached bacteria in groundwater

Acridine orange stained direct counts (AODC) were used to determine the total number of bacteria. Nuclepore filters (pore size 0.2 μm, 13-mm diameter) were pre-stained with a sudan-black solution which was prepared by dissolving 25 mg sudan-black in 75 ml 99% ethanol and then diluted with 75 ml de-ionized water. The filters were thoroughly rinsed with de-ionized water before use. An acridine orange (AO) solution was prepared by dissolving 400 mg AO in 1 litre of a citrate phosphate buffer, pH 6.6. The AO solution was stored as 10 ml aliquot volumes. All solutions and the water were filtered (pore size 0.2 μm) and sterilized by autoclaving. A portion of the sample was filtered onto a pre-stained Nuclepore filter at 0.8 MPa and stained for 7 minutes with AO. The number of bacteria was counted using blue light (390-490 nm) in an epi-fluorescence microscope (Olympus BH-2, filter 515 nm). At least 400 bacteria or a minimum of fifteen microscope fields (0.0064 mm^2 each), were counted on each filter. The frequency distribution of the number bacteria counted per microscope field follows a Poisson distribution (Hallbeck & Pedersen, 1990). This means that the precision of the mean of the counted bacteria is only dependent on the number of bacteria counted. One filter then predicts the sample mean with a precision of 5 % if 400 or more bacteria are counted (Niemelä, 1983).

2.4.2 Attached bacteria on surfaces

Surfaces from the biofilm reactors were rinsed with a 1% NaCl solution in a surface rinse described by Pedersen et. al. (1986) to remove unattached bacteria, stained with acridine orange, rinsed with de-ionized water, dried and counted as the Nuclepore filters.

2.4.3 Sampling occasions

Laxemar
The total number of unattached (ml^{-1}) and attached bacteria (cm^{-2}) were counted after the following periods of exposure of the surfaces to flowing groundwater: 830-841 m, 20 September to 9 October 1990, 19 days; 910-921 m, 12 October to 30 October 1990, 18 days; 999-1078 m, 1 November to 20 November 1990, 19 days.

Stripa
The total numbers of unattached bacteria (ml^{-1}) were counted at seven

different occasions, 17 September 1987, 17 April, 1 June and 1 October 1990, 15 April, 29 May and 26 June 1991. The total number of attached bacteria (cm^{-2}) were counted after eight different experimental periods: After 56 days of attachment and growth between 21 February to 17 April 1990; 117 days, 8 June to 1 October 1990; 27 days, 29 May to 26 June; 43 days, 15 April to 29 May; 71 days, 15 April to 26 June; 90 days, 15 January to 15 April; 133 days, 15 January to 29 May; 161 days, 15 January to 26 June.

2.5 ASSIMILATION OF CO_2 AND ORGANIC COMPOUNDS BY UNATTACHED BACTERIA.

2.5.1 Laxemar

Ten ml volumes of groundwater were added to 100 ml sterile serum bottles with aluminium crimp-sealed butyl rubber stoppers under 100% nitrogen atmosphere via hypodermic syringes mounted directly on the tubings from the different sampling depths. A number of different ^{14}C- or ^{3}H-labeled organic compounds and $Na_2{}^{14}CO_3$ (Amersham Sweden AB) were subsequently added in 1 ml oxygen-free portions to assay the nutritional responses of the populations studied. The radioactive concentrations of the ^{14}C-labeled organic compounds were adjusted to 14 MBq ml^{-1} sample. The $Na_2{}^{14}CO_3$ was adjusted to 73 MBq ml^{-1} to compensate for the isotope dilution caused by the carbonate present in the groundwaters. The following final concentrations and specific activities were used: $Na_2{}^{14}CO_3$, 38 μM (1.92 GBq/mmol); ^{14}C-formate, 7 μM (2.05 GBq/mmol); 1,2,3-^{3}H-acetate, 1.3 μM (123.2 GBq/mmol); U-^{14}C-lactate, 2.6 μM (5.7 GBq/mmol); U-^{14}C-glucose, 1.6 μM (9.1 GBq/mmol) and L-4,5-^{3}H-leucine 6 nM (5.63 TBq/mmol). The samples were incubated horizontally on a shake at 20 rotations min^{-1} at 20 °C for 2, 4 and 6 h (only 6 h for 830-841 m), whereafter formalin was added to a final concentration of 2%. Investigation of aerobic assimilation was achieved by replacement of the nitrogen atmosphere with air at sampling and with a subsequent incubation for 6 h. Controls for abiotic adsorption of the labeled compounds were achieved by addition of formalin (2%) together with the labeled compounds at sampling (time 0 h) and processed as the other samples. Control counts were subtracted from sample counts. Samples of 10 ml were filtered through Nuclepore filters (pore size 0.2 μm, 13 mm), rinsed three times with 1 ml portions of filtered Milli-Q water (pore size 0.2 μm) and subsequently placed in 20 ml scintillation cocktail (Redy-Safe, Beckman) and counted in a Beckman LS 3801 scintillation counter.

2.5.2 Stripa

Ten ml volumes of groundwater, sampled 1 October 1990, were added to 100 ml sterile serum bottles with aluminium crimp-sealed butyl rubber stoppers under 100% nitrogen atmosphere via hypodermic syringes mounted directly on the tubings from the different sampling depths. A number of different ^{14}C- or ^{3}H-labeled organic compounds and $Na_2{}^{14}CO_3$ (Amersham Sweden AB) were subsequently added in 1 ml oxygen-free portions to assay the nutritional responses of the populations studied. The radioactive concentrations of the ^{14}C-labeled organic compounds were adjusted to 14 MBq per ml sample. The $Na_2{}^{14}CO_3$ was adjusted to 73 MBq ml^{-1} to compensate for the isotope dilution caused by the carbonate present in the groundwaters. The following final concentrations and specific activities were used: $Na_2{}^{14}CO_3$, 20 (15 April and 26 June 1991), 38 (1 October 1990) or 40 (29 May 1991) μM (1.92 GBq/mmol); ^{14}C-formate, 7 μM (2.05 GBq/mmol); 1,2,3-^{3}H-acetate, 1.3 μM (123.2 GBq/mmol); U-^{14}C-lactate, 1 (26 June 1991) or 2.6 μM (5.7 GBq/mmol); U-^{14}C-glucose, 1.6 μM (9.1 GBq/mmol) and L-4,5-^{3}H-leucine 6 nM (5.63 TBq/mmol). The samples were incubated horizontally on a shake at 20 rotations min^{-1} at 10 °C for 6 h, after which formalin was added to a final concentration of 2%. Controls for abiotic adsorption of the labeled compounds were achieved by addition of formalin (2%) together with the labeled compounds at sampling and processed as the other samples. Control counts were subtracted from sample counts. Subsamples of 2 x 2.5 ml (970-1240 m samples) or 5 ml (799-807 and 812-820 m samples) were filtered through Nuclepore filters (pore size 0.2 μm, 13 mm), rinsed three times with 1 ml portions of Milli-Q filtered water (pore size 0.2 μm) and subsequently placed in 10 ml scintillation cocktail (Ready-Safe, Beckman) and counted in a Beckman LS 3801 scintillation counter.

2.6 ASSIMILATION OF CO_2 AND ORGANIC COMPOUNDS BY ATTACHED BACTERIA.

2.6.1 Laxemar

Groundwater from the sampling depths were filtered in volumes of 20 ml under nitrogen atmosphere through Dynagard hollow fibre syringe filters, mounted directly on the tubings (pore size 0.2 μm), into 50 ml sterile polypropylene centrifuge tubes with lids (Nunc) with a 100% nitrogen atmosphere and labeled compounds as above (2.5.1). Microscope slides from the biofilm reactors were transferred under nitrogen atmosphere to tubes with corresponding filtered groundwater, one slide per tube. The

$Na_2{}^{14}CO_3$ was added after this step. The microscope slides were incubated horizontally on a shake at 20 rotations min^{-1} with the equivalent controls as described previously (2.5.1), rinsed with the surface rinse (Pedersen *et al.*, 1986), cut into 4 pieces with a diamond knife, placed in 20 ml scintillation cocktail (Ready-Safe, Beckman) and counted. There was no difference in the counting efficiency between pieces placed individually in scintillation vials or when all the pieces were counted in a single vial.

2.6.2 Stripa

Groundwater, sampled on 1 October 1990, 15 April, 29 May and 26 June from the sampling depths, was filtered in 20 ml volumes under nitrogen atmosphere through Dynagard hollow fibres syringe filters (pore size 0.2 μm), into sterile 50 ml polypropylene centrifuge tubes with lids (Nunc). Labeled compounds were added as above (2.5.2) 1 October 1990. 15 April, 29 May. Microscope slides from the biofilm reactors were transferred under nitrogen atmosphere to tubes with corresponding filtered groundwater, one slide per tube. The $Na_2{}^{14}CO_3$ was added after this step. The microscope slides were incubated horizontally on a shake at 20 rotations min^{-1} at 10 or 20 °C for 2 to 6 h, rinsed with the surface rinse (Pedersen *et al.*, 1986), cut into 4 pieces with a diamond knife, placed in 20 ml scintillation cocktail (Ready-Safe, Beckman) and counted. Control counts were obtained as described above (2.5.2) and subtracted from the data from 1 October 1990, but not from data achieved 1991 (shown in the figures instead).

2.7 MICROAUTORADIOGRAPHIC STUDIES OF UNATTACHED BACTERIA

MARG studies of unattached bacteria. The MARG procedure followed was the MARG-E method described by Tabor & Neihof (1982). Briefly, 2.5 ml to 10 ml volumes from the sample bottles used for the assimilation studies, including the control samples, were filtered through Nuclepore filters (pore size 0.2 μm) and rinsed three times with 1 ml portions of filtered 1% wv NaCl in Milli-Q water. The filters were transferred to clean microscope slides previously dipped in Kodak NTB-2 autoradiographic emulsion, placed in a water-chilled PVC-container (10 °C), moved to a desiccator after solidifying and left for exposure under vacuum over silica gel for 3 days at 4 °C. A bacterium was scored to be active, assimilating the labeled compound, if at least three silver grains were located at a maximal distance of 1 μm away from the bacteria labeled with ^3H and 3 μm away from the bacteria labeled with ^{14}C.

2.8 MICROAUTORADIOGRAPHIC STUDIES OF ATTACHED BACTERIA

The MARG procedure for attached bacteria followed the one described for unattached bacteria with the following modifications: the microscope slides with bacteria were rinsed and dipped directly into NTB-2 emulsion and allowed to solidify and exposed as above.

2.9 ESTIMATE OF THE LOWER LIMIT OF DETECTION OF THE MARG METHOD.

The facultative anaerobe *Pseudomonas fluorescens* (CCUG-25085) and a SRB, isolated from the 860 m level in a borehole called KAS02 and the 680 m level in KLX01 respectively (Pedersen & Ekendahl, 1990), were used to estimate the lower limit of detection of the MARG method. The bacteria were cultured as described by Pedersen and Ekendahl (1990) with the addition of 0.2 to 20 nM L-4,5-^{3}H-leucine, 2.3 μM U-^{14}C-lactate or 1.4 μM U-^{14}C-glucose incubated for 30 min to 24 h before sampling and processed as for the LSC and MARG studies with unattached bacteria.

2.10 RESPIRATION OF LACTATE BY STRIPA BACTERIAL POPULATIONS

2.10.1 Lactate respiration

Microscope slides from Stripa, 15 April, 29 May and 26 June, were transferred as described above (2.6.2) to tubes with labeled ^{14}C-lactate and rubber stoppers. They were added with 0.8 ml 0.3 M HCl after incubation between 2 to 6 h at 10 or 20°C (970-1240 m) and transported to the laboratory. N_2 was bubbled through the tubes for 15 minutes and the released $^{14}CO_2$ (originated from the lactate) was trapped in 20 ml serum bottles. They contained 10 ml of etanolamine-methanol (60:40 v%, three traps, April) or 1.9 M KOH (three traps in May, two in June). The traps were preceded by an empty safety bottle. Two subsamples were taken from each trap, put into glass or plastic vials with scintillation cocktail and the radioactivity measured. The subsamples were in April: 0.1 and 1 ml to 10 ml Ready Safe (Beckman) and in May and June: 0.1 and 1 ml to 10 ml Hionic Flour (Packard). Background controls were made with the trap solutions in the cocktails. Duplicate controls showed

that the tubes were air tight and that lactate did not release CO_2 chemically as a result of the added HCl.

2.10.2 Sulfate reduction

Microscope slides from Stripa, 26 June, were transferred as described above (2.6.2) to tubes with 2.8 μM labeled $Na_2{}^{35}SO_4$, 10 μM lactate and rubber stoppers. They were added with 5 ml w/v 20% zinc acetate after incubation between 2 to 6 h at 10 or 20°C (970-1240 m), frozen on dry ice and transported to the laboratory. Sulfate reduction were measured as developed $H_2{}^{35}S$ with a set-up the corresponding to that used for lactate respiration with the following changes: The bubbling time was 30 min, acidification was made with 1.5 ml 5 M HCl and the traps contained 10 ml 5% zinc acetate + 0.1% acetic acid (Fossing & Jörgensen, 1989). Subsamples of 3 ml were taken from the traps to 15 ml Hionic Flour.

2.11 THE EFFECT OF TEMPERATURE ON THE ASSIMILATION RATES OF THE STRIPA 970-1240 M BACTERIAL POPULATIONS

Biofilm reactors were connected in a series to the groundwater from 970-1240 m outside and inside a hut in the drift were the groundwater and reactor system were heated from 10°C (outside) to 20°C (inside). CO_2 and lactate assimilation, lactate respiration and sulfate reduction were measured as described above.

2.12 SCANNING ELECTRON MICROSCOPE STUDIES OF ATTACHED BACTERIA ON GLASS SURFACES FROM STRIPA

Microscope slides from 812-820 m 10 °C, 970-1240 m, 10 °C and 20 °C were sampled 26 June, fixed in glutaraldehyde, dehydrated with alcohol-acetone, critical point dried, sputtered with gold-palladium and observed in a Zeiss scanning electron microscope (SEM).

3 RESULTS

3.1 THE COMPOSITION OF THE GROUNDWATERS

3.1.1 Laxemar

Table 3-1 shows the major parameters of the groundwater in the Laxemar borehole KLX01. The flow rates were measured as ml min^{-1} and calculated to m sec^{-1} over the microscope slides in the biofilm reactors. The concentrations of $N\text{-}NO_2^-$, $N\text{-}NO_3^-$, $N\text{-}NH_4^+$ and $P\text{-}PO_4^{3-}$ were close to or below the detection limits (10, 1, 1 and 1 μg $litre^{-1}$, respectively) and are not shown. Table 3-3 shows the gas content in the groundwaters. N_2 dominated, followed by $He+H_2$, CO_2, CH_4 and CO. H_2 and He were not separated in the chromatograms and are presented together.

3.1.2 Stripa

Table 3-2 shows the major parameters and Table 3-4 shows the gas content of the groundwaters in the Stripa borehole V2. The sulfate, sulphide, carbonate and conductivity data differed between the sampling depths indicating that the groundwaters were obtained from fracture systems without any close hydraulic connections. The temperatures were measured earlier with a borehole sond during the geological well logging program (Nordstrom *et al.*, 1985). The waters were chilled to 10 °C when flowing from the sampling depths up to the drift and this was the temperature at which the incubations were done unless else is stated. The flow was measured as ml min^{-1} and converted to cm sec^{-1} over the surfaces in the biofilm reactors.

Table 3-1 The major parameters of the groundwaters in the Laxemar borehole KLX01.

sampling depth (m)	pH	Eh (mV)	temp (°C)	SO_4^{2-} (μM)	S^{2-} (μM)	CO_3^{2-} (μM)	conductivity (μS cm^{-1})	flow (ml min^{-1})	flow (m sec^{-1})	drilling water[a] (%)
830-841	8.2	-270	19.5	7000	2.3	104	2600	53	2.1×10^{-3}	0.1
910-921	8.4	...[b]	21.2	8100	11.0	98	3200	98	3.9×10^{-3}	0.9
999-1078	8.5	-220	22.9	7200	5.6	190	3600	100	4.0×10^{-3}	1.8

[a] the amount of water left from the drilling operations
[b] no data

Table 3-2 The major parameters of the Stripa borehole V2.

sampling depth (m)	pH	Eh (mV)	temp[a] (°C)	SO_4^{2-} (μM)	S^{2-} (μM)	CO_3^{2-} (μM)	conductivity (μS cm^{-1})	flow (ml min^{-1})	flow (m sec^{-1})	drilling water[a] (%)
799-807	9.5	+205	18	52	<0.01	158	425	32.6	1.4×10^{-3}	...[b]
812-820	9.4	+199	18	1433	106	50	1640	66.3	2.8×10^{-3}	...
970-1240	10.2	-3	26	520	233	57	1180	12.4	0.5×10^{-3}	...

[a] The water had been chilled to 10 °C when it reached the drift (410 m)
[b] no data

Table 3-3 The major gas content of the groundwaters in the Laxemar borehole KLX01.

sampling depth (m)	H_2+He (μl l^{-1})	N_2 (μl l^{-1})	O_2 (μl l^{-1})	CO (μl l^{-1})	CO_2 (μl l^{-1})	CH_4 (μl l^{-1})	C_2H_6 (μl l^{-1})	$C_2H_{2\text{-}4}$[a] (μl l^{-1})	volume extracted gas (%)
830-841	4600	46500	<1000	0.5	460	26	<0.1	<0.1	5.7
910-921	3500	37000	<1000	0.1	500	27	<0.1	<0.1	4.4
999-1078	2450	18000	<1000	0.7	1600	31	<0.1	<0.1	3.5

[a] the content of C_2H_2 + C_2H_4

Table 3-4 The major gas content of the groundwaters in the Stripa borehole V2.

sampling depth (m)	H_2 (μl l^{-1})	He (μl l^{-1})	N_2 (μl l^{-1})	O_2 (μl l^{-1})	CO (μl l^{-1})	CO_2 (μl l^{-1})	CH_4 (μl l^{-1})	C_2H_6 (μl l^{-1})	$C_2H_{2\text{-}4}$[a] (μl l^{-1})	volume extracted gas (%)
799-807	<10	580	25000	<1000	<1	32	245	0.3	<0.1	2.4
812-820	<10	760	31000	80[b]	<1	11	170	0.6	<0.1	3.4
970-1240	<10	380	24500	0[b]	<1	10	290	2.9	<0.1	2.7

[a] the content of C_2H_2 + C_2H_4
[b] determined with the Winkler titration method

3.2 NUMBERS OF BACTERIA

3.2.1 Laxemar

Table 3-5 shows the numbers of bacteria counted in the groundwaters and on surfaces exposed to flowing groundwater from the sampling depths. There were more bacteria in the groundwater from 999-1078 m than from the other two depths. The attached numbers of bacteria were not different between the sampling depths. The bacteria on the surfaces were distributed in uneven patterns in clusters indicating that some bacteria grew on the surfaces rather than just attached randomly from the passing waters.

Table 3-5 The total number of bacteria in the KLX01 borehole and the random variability of the total number of bacteria in samples from the same sampling interval.

sampling date	830-841 m N[a]	bacteria x 10^{5}[b]	SD %	910-921 m bacteria x 10^{5}[b]	SD %	999-1078 m bacteria x 10^{5}[b]	SD %
Groundwater sampling							
20 September 1990	6	0.15	14	...[c]	...	...	...
30 October 1990	6	...	...	0.21	12	...	...
20 November 1990	6	...	...	...	...	0.68	17
Surfaces exposed to flowing groundwater							
20 September 1990, 19 days	6	0.94	51	...	...	...	...
30 October 1990, 18 days	6	...	...	1.19	23	...	...
20 November 1990, 19 days	6	...	...	...	...	1.04	11

[a] N is the number of independent samples
[b] populations are ml^{-1} or cm^{-2}
[c] no data

3.2.2 Stripa

The numbers of bacteria counted at different occasions in groundwater and on surfaces exposed to flowing groundwater from the sampling depths are shown in Table 3-6. There were 10 to 100-fold more bacteria in the groundwater from 970-1240 m depth than from the other two depths, but this difference was not reproduced on the surfaces exposed to the flowing groundwaters. The random variability of the total number of unattached and attached bacteria in samples from the same depth examined, on one occasion, ranged between 6 and 75 % of the mean. The bacteria on the

surfaces were distributed in uneven patterns in clusters, indicating that they had grown on the surfaces rather than just attached randomly from the passing waters (Figs 3-12, 3-13, 3-16, 3-17, 3-20 and 3-21).

Table 3-6 The total number of unattached bacteria (ml^{-1}) in groundwater, and attached bacteria on surfaces (cm^{-2}) exposed to flowing groundwater from three sampling depths of the Stripa borehole V2, 799-807 m, 812-820 m and 970-1240 m, measured at different occasions.

sampling date		799-807 m bacteria			812-820 m bacteria			970-1240 m bacteria		970-1240 m bacteria	
		N[a]	x 10^{5}[b]	SD %	x 10^{5}[b]	SD %		x 10^{5}[b] 10 °C	SD %	x 10^{5}[b] 20°C	SD %
Groundwater sampling											
17 September	1987	1	0.097	...[c]	0.061	...		2.3	...	...	...
18 April	1990	1	0.036	...	0.016	...		1.6	...	...	...
8 June	1990	1	0.240	...	0.047	...		2.3	...	...	...
1 October	1990	6	0.054	26	0.018	45		1.2	12	...	...
15 April	1991	3	0.225	33	0.129	59		3.4	21	...	...
29 May	1991	2	0.102	10	0.232	14		2.5	11	...	...
26 June	1991	2	0.143	20	0.222	49		1.4	79	...	...
Surfaces exposed to flowing groundwater											
26 June	1991, 27 days	2	...	...	6.4	99		13.8	8	2.4	54
29 May	1991, 43 days	4	...	...	...	...		15.1	17	10.7	27
17 April	1990, 56 days	3	10.0	6	72.0	20		22.0	4	...	...
26 June	1991, 71 days	2	...	...	...	...		50.3	75	18.3	11
15 April	1991, 90 days	4	...	...	46.2	12		42.3	5	...	...
1 October	1990, 117 days	6	12.0	30	71.0	38		59.0	31	...	...
29 May	1991, 133 days	4	...	...	105	20		...	...	...	...
26 June	1991, 161 days	1	...	...	85.8	4		...	...	...	...

[a] N is the number of independent samples
[b] populations are ml^{-1} or cm^{-2}
[c] no data

3.3 ESTIMATE OF THE LOWER LIMIT OF THE MICROAUTORADIOGRAPHIC METHOD.

Fig. 3-1 shows the relation between the percentage of bacteria scored to be active using the MARG method and the mean number of disintegrations per minute (DPM) per bacterium of the samples. The lowest radioactivity that resulted in active bacteria was 10^{-3} DPM per bacterium and 80% of the bacteria were scored active at 10^{-2} DPM per cell. This corresponds to between 0.1 to 1 x 10^{-16} mol ^{14}C per bacterium and 0.21 to 2.1 x 10^{-19} mol ^{3}H per bacterium and are the minimum amounts of the isotopes per

bacterium that can be detected with the MARG method. A comparison of the data in Tables 3-7 and 3-8 shows that several samples demonstrated significant assimilation of the ^{14}C-labeled Na_2CO_3, formate and glucose using LSC, but bacteria to be scored active in the MARG procedure could not be detected. This is explained by the higher sensitivity of the LSC technique compared to the MARG technique on the assumption that the bacteria assimilated too little ^{14}C ($<0.1 \times 10^{-16}$ mol per cell) to be scored active using the MARG technique, but sufficient ^{14}C to give a measurable contribution using the LSC.

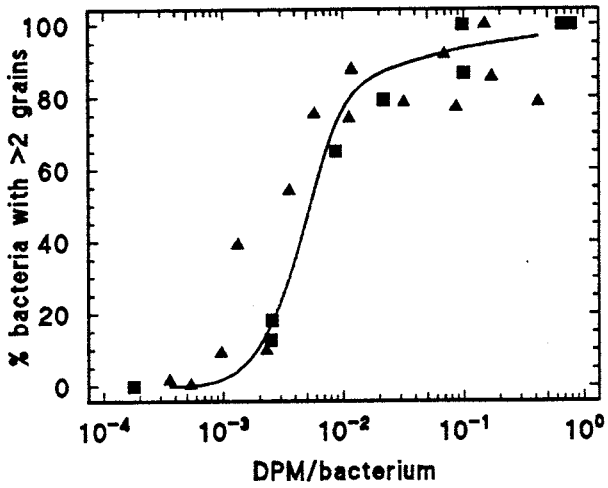

Fig. 3-1 The relation between the percentage of bacteria scored to assimilate the labeled compounds using the microautoradiographic method and the mean number of disintegrations per minute per bacterium in cultures with *Pseudomonas fluorescens* (CCUG-25085) (triangles) and a sulfate reducing bacterium (squares), amended with 0.2-20 nM L-4,5-^{3}H-leucine, 2.3 μM U-^{14}C-lactate or 1.4 μM U-^{14}C-glucose between 30 min and 24 h before sampling.

3.4 ASSIMILATION OF CO_2 AND ORGANIC COMPOUNDS

3.4.1 Laxemar

Table 3-7 shows the assimilation of ^{14}C and ^{3}H from $Na_2{}^{14}CO_3$ labeled compounds by unattached (ml^{-1}) and attached (cm^{-2}) bacterial populations after 6 h, measured with LSC (mole assimilated ^{14}C and ^{3}H) and MARG techniques (% bacteria scored to be active, assimilating the labeled compounds). The LSC results are presented as mole isotope atoms assimilated, because the metabolic pathways of the organic compounds used are unknown for the populations studied. Stoichiometric calculations back to mole organic compounds might therefore be misleading. For

instance, many SRB split lactate into acetate, which is assimilated via the TCA-pathway, and CO_2, which is expelled by the bacterium. The average control counts (the number of samples is 3) for abiotic sorption of the labeled compounds, calculated as mole isotope x 10^{-14} (± % standard deviation) for unattached (ml^{-1}) and attached bacteria (cm^{-2}), were: $Na_2{}^{14}CO_3$, 42 (±80%) and 442 (±99%); ^{14}C-formate, 14 (-) and 31 (-); acetate, 0.16 (±22%) and 0.24 (±93%); lactate, 25 (±38%) and 35 (±50%); glucose, 29 (±63%) and 105 (±118%) and leucine, 0.5 (±57%) and 0.2 (±8%), respectively. A measurement was registered as a detected assimilation of the labeled compound if the sample count exceeded the corresponding control count by 50%.

A comparison of the data in Table 3-7 shows that several populations demonstrated significant assimilations of the CO_2, formate and glucose, but bacteria to be scored active with the MARG procedure could not be detected. This is explained by the higher sensitivity of the LSC technique compared to the MARG technique under the assumption that the bacteria assimilated too little ^{14}C to be scored active with the MARG technique, but enough ^{14}C to give a measurable contribution in the LSC (confer 3-3).

Table 3-7 shows that the amounts of the labeled compounds assimilated were 10 - 500 fold higher cm^{-2} than the amounts assimilated ml^{-1} at the 830-841 and 910-921 m depths while the number of bacteria of the populations that were responsible for the assimilations was approximately 6 times higher on the surfaces than in the water (Table 3-5). There were significant assimilations of CO_2 by all populations, except for the unattached bacteria at 830-841 m depth, indicating *in situ* production of organic carbon from carbonate. Assimilation of formate was detected in two populations, indicating the presence of bacteria able to substitute CO_2 with formate. Acetate, lactate and glucose assimilations demonstrated the presence of heterotrophic bacteria. The assimilation of lactate by the attached bacteria dominated over acetate and glucose at all depths and gave MARG results from 2 up to 83 %. Leucine was assimilated by from 20 up to 98 % of the populations which showed that major portions of the populations studied were viable.

Incubation in air decreased the assimilations of CO_2, formate, lactate and leucine by attached and unattached bacteria at the 910-921 m depth and by the unattached bacteria at the 999-1078 m depth. This effect also appeared with acetate and glucose, with the attached bacteria at the 910-921 m and 999-1078 m (only glucose) depths.

3.4.2 Stripa

The assimilation of ^{14}C and ^{3}H from labeled compounds by unattached

(ml^{-1}) and attached (cm^{-2}) bacterial populations after 6 h (from 1 October 1990), measured with LSC (mol ^{14}C and ^{3}H ml^{-1} or cm^{-2}) and MARG (% bacteria scored to be active) techniques is shown in Table 3-8. A measurement was registered as a detected assimilation of the labeled compound if the sample count exceeded the corresponding control count by 50%

There was significant assimilation of CO_2 in all samples, except for the unattached bacteria at 799-807 m depth, indicating the *in situ* production of organic carbon from CO_2. Assimilation of formate followed that of CO_2 except for the unattached bacteria at 970-1240 m depth and indicated the presence of bacteria able to substitute formate for CO_2. The acetate, lactate and glucose assimilation demonstrated the presence of heterotrophic bacteria. The assimilation of lactate by the attached bacteria dominated over glucose at all depths and gave MARG responses up to 74%. Leucine was assimilated by up to 99 % of the populations which showed that major fractions of the populations studied were viable.

3.5 GROWTH RATES OF ATTACHED BACTERIA IN STRIPA

Fig. 3-3 shows the attachment and growth of bacteria per cm^2 on slides after up to 161 days of exposure to the flowing groundwaters from V2. The number of bacteria increased more rapidly during the first month after which what seems to be a slow growth phase continues. If the first month is considered to be dominated by attachment of bacteria from the water, the following period would be growth of these attached bacteria (confer Escher & Characklis, 1990). Linear regressions from the 27:th day onward result in a generation time of 43 days for the 970-1240 m population at 10°C, ($r=0.80$) 16 days for the 970-1240 m population at 20°C ($r=0.91$) and 37 days for the 812-820 m population ($r=0.90$).

3.6 THE RATE OF ASSIMILATION OF CO_2

The assimilation of CO_2 without and with lactate is shown in Fig. 3-4, 3-5, 3-6. The slope of each line represents the initial rates of assimilation, shown in table 3-9, of different population sizes. The rates are generally higher when there are more bacteria present. Fig. 3-7 compares the rates for the different population studied and Table 3-10 shows that the assimilation of CO_2 increases with depth and temperature. There was not

Table 3-7 The total assimilation of CO_2 + $^{14}CO_2$ and of ^{14}C and ^{3}H from $Na_2{}^{14}CO_3$ and labeled organic compounds by unattached bacteria in groundwater and attached bacteria on surfaces exposed to flowing groundwater from three sampling depths of the Laxemar borehole KLX01, and the percentage of the populations scored to be active with the microautoradiographic method, assimilating the labeled compounds.

		unattached bacteria			attached bacteria		
labeled compound	Depth (m)	in N_2	in air	percent bacteria active in N_2	in N_2	in air	percent bacteria active in N_2
		mol isotope x 10^{-14} ml^{-1}			mol isotope x 10^{-14} cm^{-2}		
CO_2[a] (^{14}C)							
	830-841	-[b]	.[c]	-	2200	.	25
	910-921	17	-	-	2700	-	3
	999-1078	100	-	3	3600	290	2
formate (^{14}C)							
	830-841	-	...	-	-	...	-
	910-921	-	-	-	110	38	-
	999-1078	51	36	-	-	-	-
acetate (^{3}H)							
	830-841	0.2	...	29	91	...	17
	910-921	0.6	0.7	27	200	65	62
	999-1078	0.7	0.4	21	2	3	13
lactate (^{14}C)							
	830-841	140	...	21	6500	...	8
	910-921	420	105	16	15000	1320	60
	999-1078	1900	160	83	360	310	2
glucose (^{14}C)							
	830-841	43	...	2	1400	...	10
	910-921	10	26	-	600	120	12
	999-1078	29	390	-	320	-	2
leucine (^{3}H)							
	830-841	3	...	56	53	...	20
	910-921	14	3	87	150	48	87
	999-1078	66	4	98	16	20	73

[a] the data has been corrected for isotope dilution caused by the measured $CO_3{}^{2-}$ and CO_2 contents of the groundwaters (Tables 3-3 and 3-4)
[b] not detected
[c] no data

Table 3-8 The assimilation of ^{14}C and ^{3}H from CO_2 and labeled organic compounds by unattached bacteria in groundwater and attached bacteria on surfaces exposed to flowing groundwater from three sampling depths of the Stripa borehole V2 and the percentage of the population scored to be active using the microautoradiographic method, assimilating the labeled compounds.

		unattached bacteria		attached bacteria	
labeled compound	depth (m)	mol isotope x 10^{-14} ml^{-1}	% active bacteria	mol isotope x 10^{-14} cm^{-2}	% active bacteria
CO_2[a] (^{14}C)					
	799-807	312	5	-[b]	-
	812-820	66	5	1200	-
	970-1240	88	-	4380	-
formate (^{14}C)					
	799-807	11	4	-	-
	812-820	3	6	29	8
	970-1240	-	-	81	-
acetate (^{3}H)					
	799-807	0.05	46	39	35
	812-820	0.15	16	72	27
	970-1240	0.2	3	238	21
lactate (^{14}C)					
	799-807	26	16	5100	11
	812-820	10	34	13700	24
	970-1240	68	6	92400	74
glucose (^{14}C)					
	799-807	19	5	123	-
	812-820	19	8	4800	5
	970-1240	66	-	2600	-
leucine (^{3}H)					
	799-807	1	55	160	77
	812-820	5	23	290	99
	970-1240	5	9	280	38

[a] the data has been corrected for isotope dilution caused by the measured CO_3^{2-} and CO_2 contents of the groundwaters (Tables 3-3 and 3-4)
[b] not detected

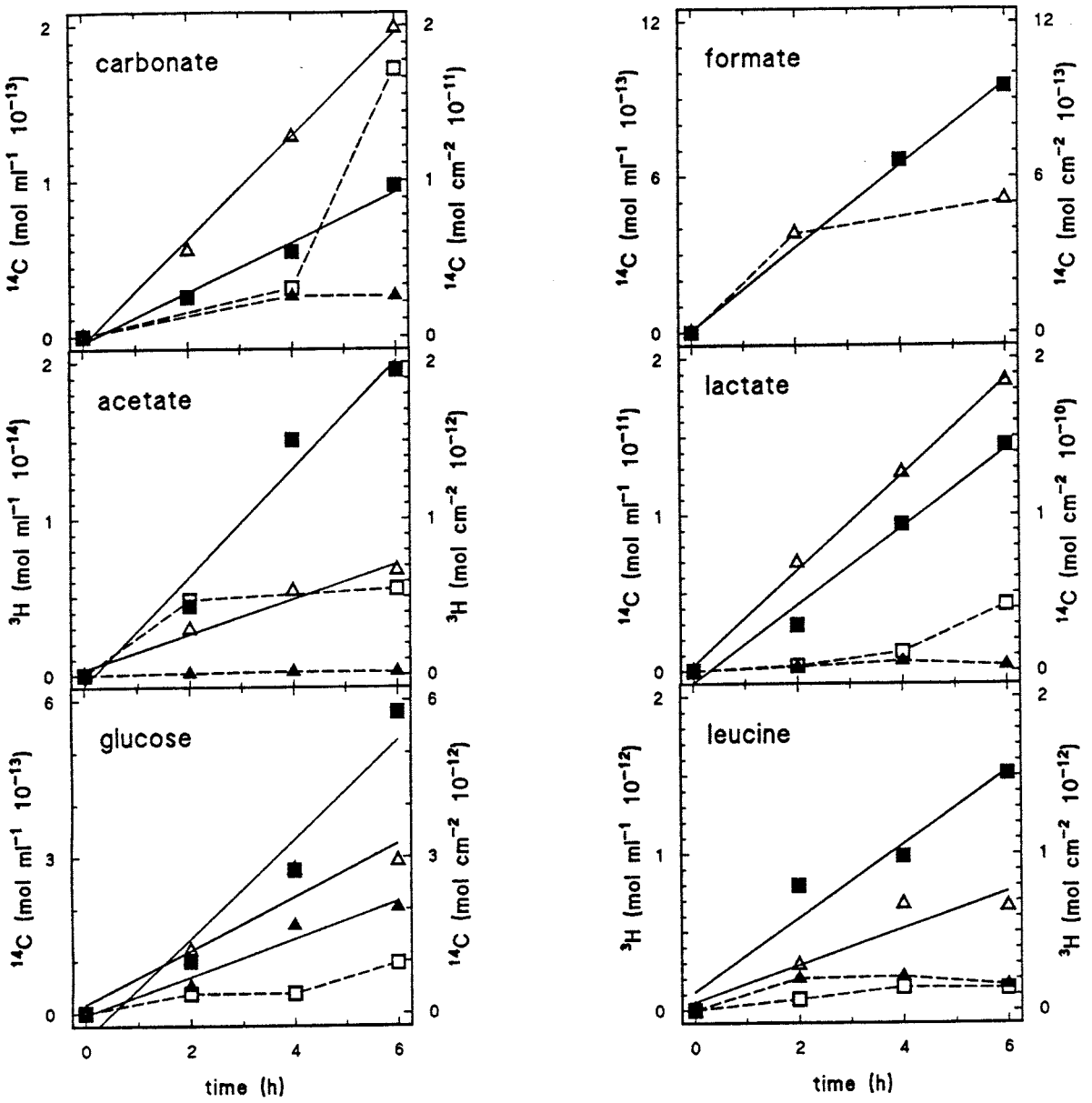

Fig. 3-2 The assimilation of ^{14}C and ^{3}H from $Na_2{}^{14}CO_3$ and radiolabeled organic compounds by unattached bacteria (open symbols) and attached bacteria (solid symbols) from two sampling depths at Laxemar in a 100% nitrogen atmosphere. Control counts were subtracted (confer 3.4.1). Symbols: square, 910-921 m; triangle, 999-1078 m; Solid lines denote data that in a linear regression had a correlation coefficient, *r*, larger than 0.95 and dashed lines denote data that had a *r* smaller than 0.95.

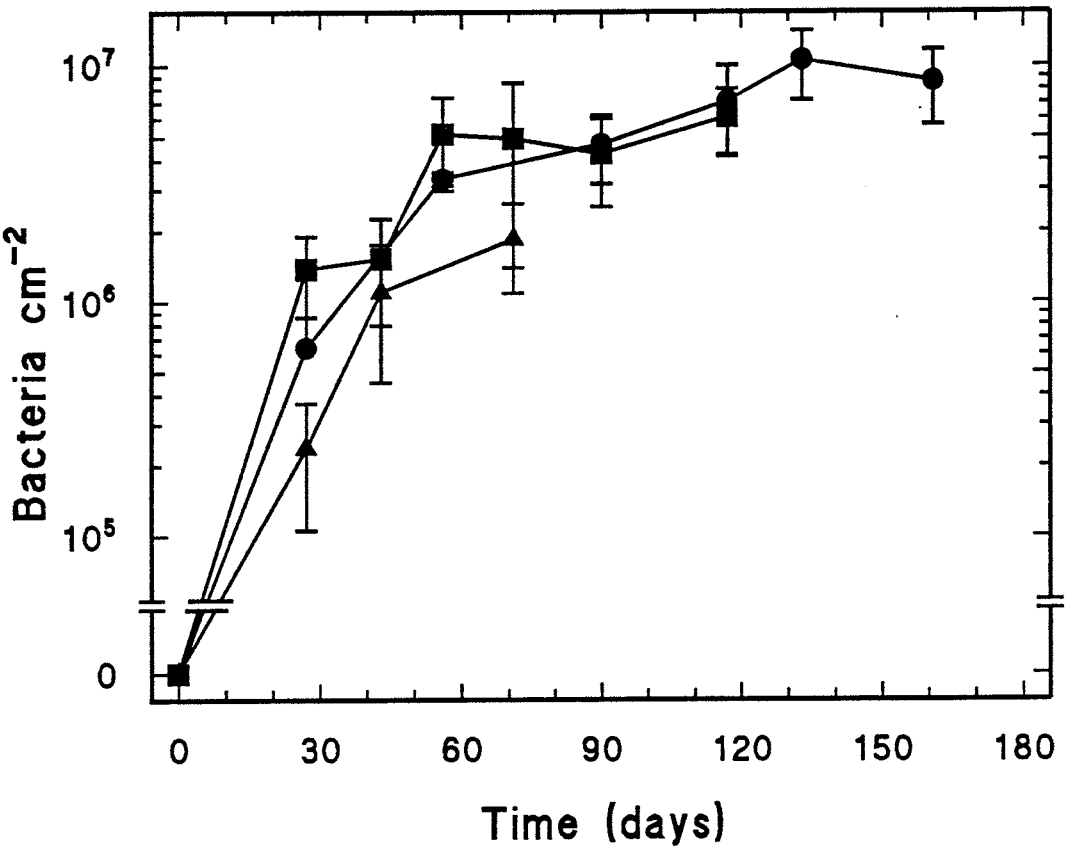

Fig. 3-3 The total number of attached bacteria on surfaces exposed to flowing groundwater from the Stripa borehole V2 for different times (confer Table 3-6). Symbols: circle, 812-820 m 10°C; square, 970-1240 m, 10°C; triangle, 970-1240 m, 20°C. Bars indicate SD.

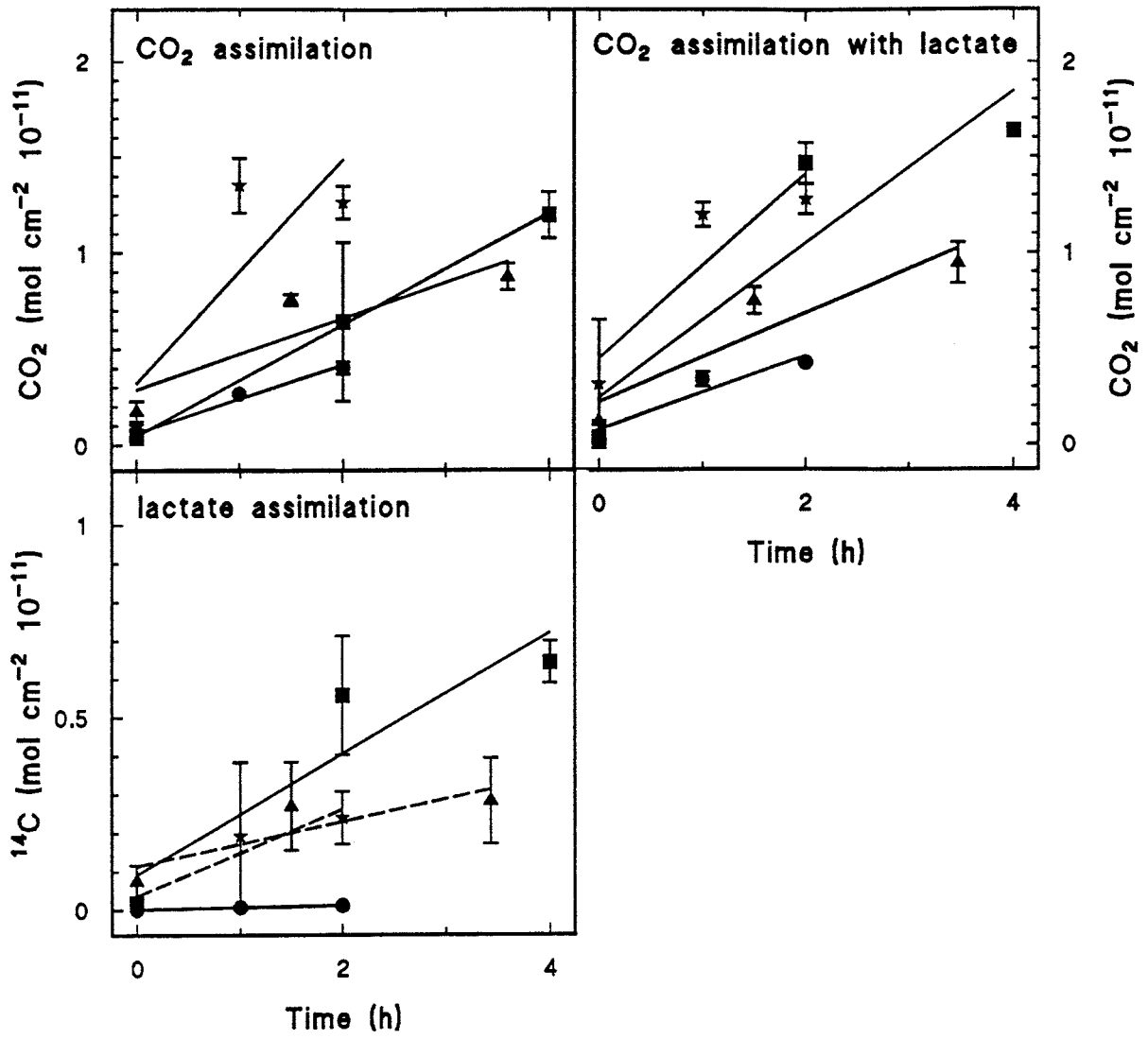

Fig. 3-4 The *in situ* assimilation of CO_2 without and with the addition of 10 μM unlabeled lactate and the assimilation of ^{14}C from U-^{14}C-labeled lactate by attached bacteria in groundwater from the Stripa borehole V2, 812-820 m, 10°C. Control counts were not subtracted from the data. Symbols: circle, 0.6×10^6 bacteria cm^{-2}; square, 4.6×10^6 bacteria cm^{-2}; star, 8.6×10^6 bacteria cm^{-2}; triangle, 10.5×10^6 bacteria cm^{-2}. Solid line denote sample that in a linear regression had a correlation coefficient, r, larger than 0.8 and a probability, p, that the sample was taken from a population in which there was no correlation, smaller than 0.05. Dashed line denote data that had a r smaller than 0.8 and a p larger than 0.05 (confer table 3-9).

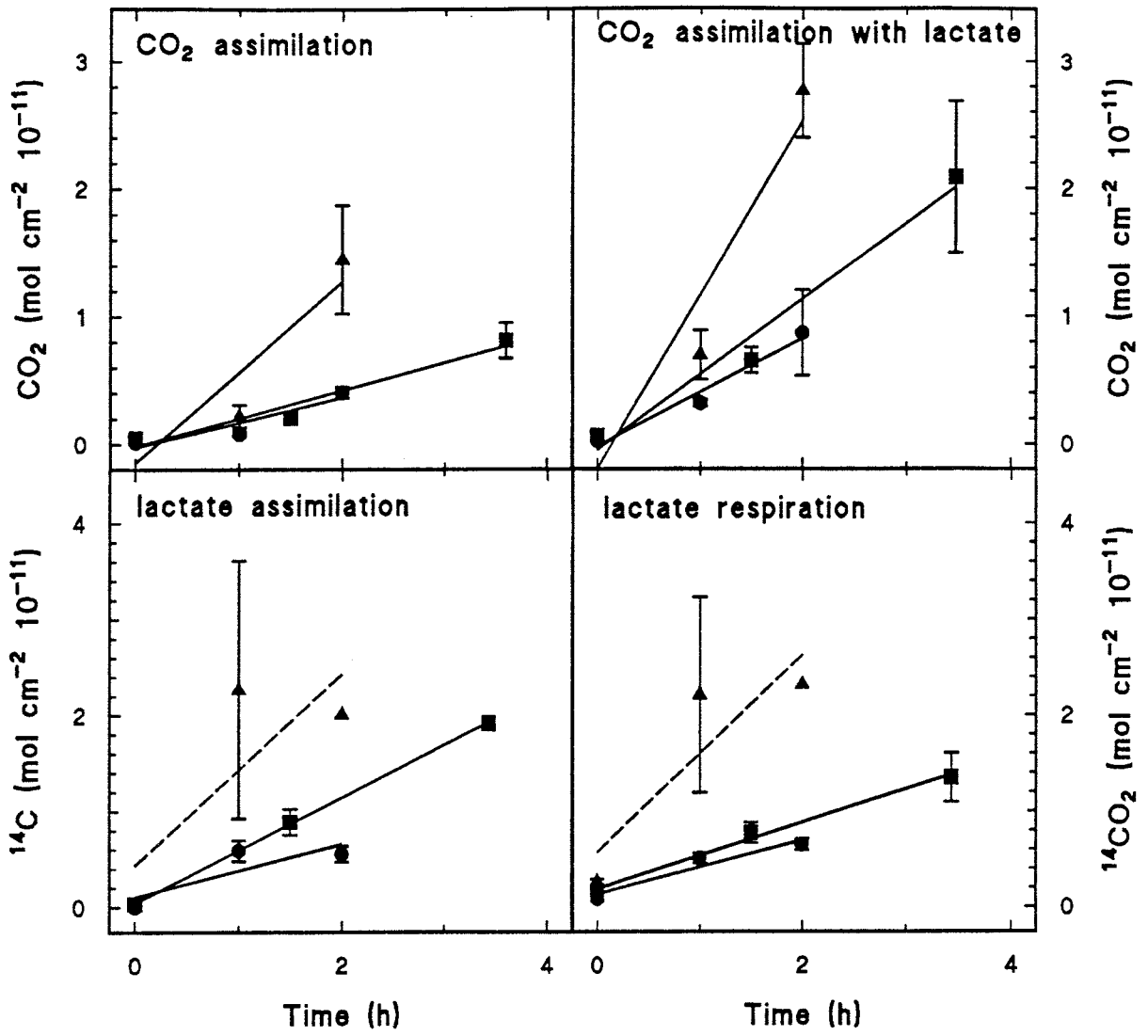

Fig. 3-5 The *in situ* assimilation of CO_2 without and with the addition of 10 μM unlabeled lactate, the assimilation of ^{14}C from U-^{14}C-labeled lactate and the respiration of assimilated ^{14}C-lactate, measured as $^{14}CO_2$ evolution, by attached bacteria in groundwater from the Stripa borehole V2, 970-1240 m, 10°C. Control counts were not subtracted from the data. Symbols: circle, 1.4×10^6 bacteria cm^{-2}; square, 1.5×10^6 bacteria cm^{-2}; triangle, 5.0×10^6 bacteria cm^{-2}. Solid line denote sample that in a linear regression had a correlation coefficient, r, larger than 0.8 and a probability, p, that the sample was taken from a population in which there was no correlation, smaller than 0.05. Dashed line denote data that had a r smaller than 0.8 and a p larger than 0.05 (confer table 3-9).

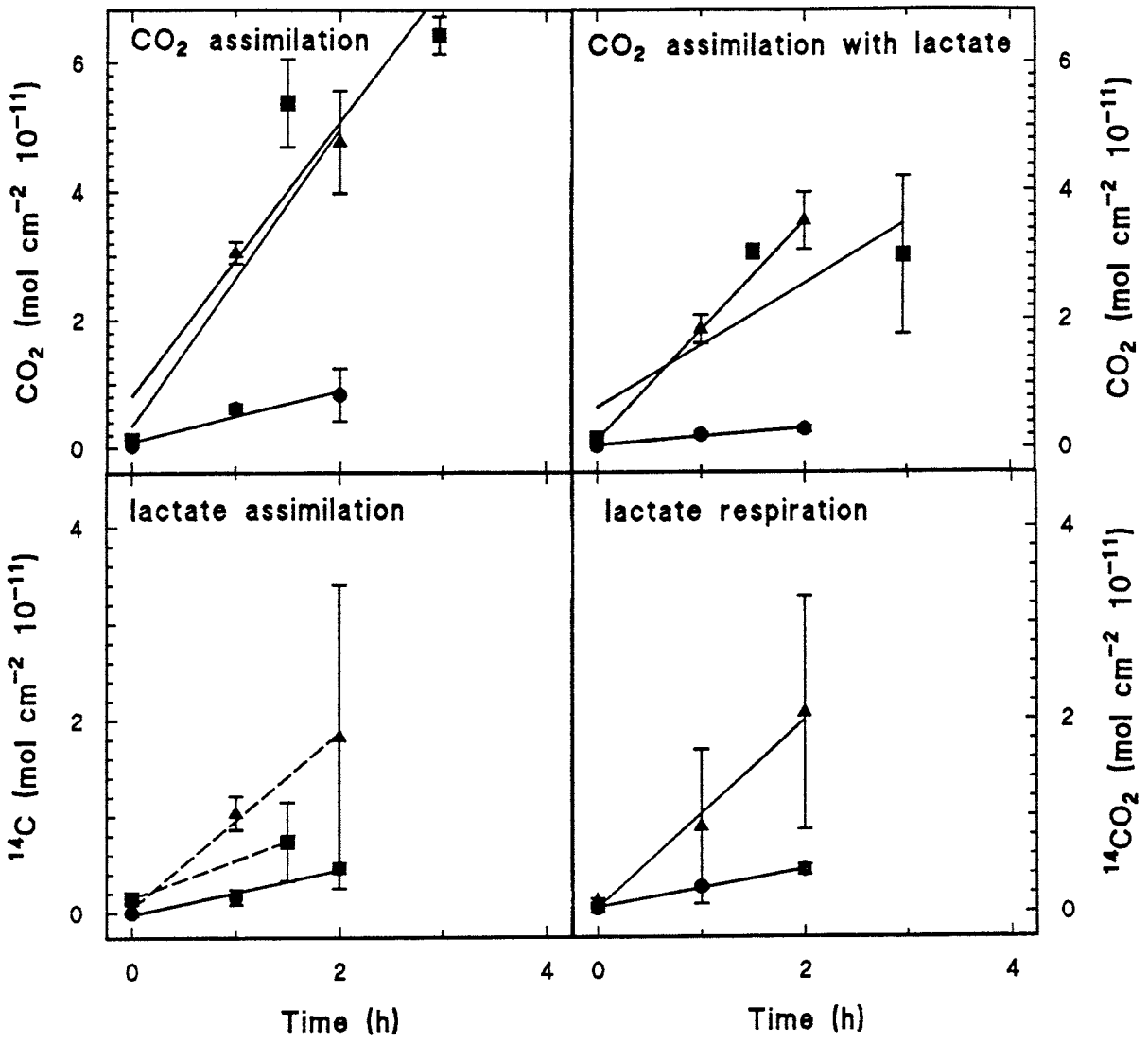

Fig. 3-6 The *in situ* assimilation of CO_2 without and with the addition of 10 μM unlabeled lactate, the assimilation of ^{14}C from U-^{14}C-labeled lactate and the respiration of assimilated ^{14}C-lactate, measured as $^{14}CO_2$ evolution, by attached bacteria in groundwater from the Stripa borehole V2, 970-1240 m at 20°C. Control counts were not subtracted from the data. Symbols: circle, 0.2×10^6 bacteria cm^{-2}; square, 1.1×10^6 bacteria cm^{-2}; triangle, 1.8×10^6 bacteria cm^{-2}. Solid line denote sample that in a linear regression had a correlation coefficient, r, larger than 0.8 and a probability, p, that the sample was taken from a population in which there was no correlation, smaller than 0.05. Dashed line denote data that had a r smaller than 0.8 and a p larger than 0.05 (confer table 3-9).

Table 3-9 The assimilation and respiration rates of different populations of attached bacteria grown in groundwaters from the Stripa borehole V2. The rates were calculated from linear correlations of the data presented in Fig. 3-4 to 3-6. r is the regression coefficient and p is the probability, p, that the sample was taken from a population in which there was no correlation. The number of pairs in the samples, n, were 6.

depth (m)	temp (°C)	days	bacteria cm^{-2}	rate mole h^{-1}	r	p	figure
CO_2-assimilation							
812-820	10	27	$0.6x10^6$	$1.8x10^{-12}$	0.98	0.0004	fig. 3-3
812-820	10	90	$4.6x10^6$	$2.9x10^{-12}$	0.94	0.006	fig. 3-3
812-820	10	133	$10.5x10^6$	$1.9x10^{-12}$	0.89	0.02	fig. 3-3
812-820	10	161	$8.6x10^6$	$5.8x10^{-12}$	0.83	0.04	fig. 3-3
970-1240	10	27	$1.4x10^6$	$2.0x10^{-12}$	0.94	0.007	fig. 3-4
970-1240	10	43	$1.5x10^6$	$2.2x10^{-12}$	0.96	0.002	fig. 3-4
970-1240	10	71	$5.0x10^6$	$7.1x10^{-12}$	0.89	0.02	fig. 3-4
970-1240	20	27	$0.2x10^6$	$3.9x10^{-12}$	0.86	0.03	fig. 3-5
970-1240	20	43	$1.1x10^6$	$21.3x10^{-12}$	0.93	0.007	fig. 3-5
970-1240	20	71	$1.8x10^6$	$23.2x10^{-12}$	0.97	0.001	fig. 3-5
CO_2-assimilation with 10 μM lactate							
812-820	10	27	$0.6x10^6$	$1.9x10^{-12}$	0.95	0.004	fig. 3-3
812-820	10	90	$4.6x10^6$	$4.0x10^{-12}$	0.91	0.01	fig. 3-3
812-820	10	133	$10.5x10^6$	$2.3x10^{-12}$	0.92	0.009	fig. 3-3
812-820	10	161	$8.6x10^6$	$4.8x10^{-12}$	0.86	0.03	fig. 3-3
970-1240	10	27	$1.4x10^6$	$4.2x10^{-12}$	0.92	0.01	fig. 3-4
970-1240	10	43	$1.5x10^6$	$5.9x10^{-12}$	0.95	0.004	fig. 3-4
970-1240	10	71	$5.0x10^6$	$13.6x10^{-12}$	0.95	0.004	fig. 3-4
970-1240	20	27	$0.2x10^6$	$1.3x10^{-12}$	0.97	0.002	fig. 3-5
970-1240	20	43	$1.1x10^6$	$9.7x10^{-12}$	0.81	0.05	fig. 3-5
970-1240	20	71	$1.8x10^6$	$17.1x10^{-12}$	0.99	0.0002	fig. 3-5
Lactate assimilation							
812-820	10	27	$0.6x10^6$	$0.1x10^{-12}$	0.89	0.02	fig. 3-3
812-820	10	90	$4.6x10^6$	$1.6x10^{-12}$	0.90	0.02	fig. 3-3
812-820	10	133	$10.5x10^6$	$0.6x10^{-12}$	0.70	0.1	fig. 3-3
812-820	10	161	$8.6x10^6$	$1.1x10^{-12}$	0.72	0.1	fig. 3-3
970-1240	10	27	$1.4x10^6$	$2.7x10^{-12}$	0.82	0.05	fig. 3-4
970-1240	10	43	$1.5x10^6$	$5.5x10^{-12}$	1.00	0.00002	fig. 3-4
970-1240	10	71	$5.0x10^6$	$9.9x10^{-12}$	0.71[a]	0.2[a]	fig. 3-4
970-1240	20	27	$0.2x10^6$	$2.3x10^{-12}$	0.97	0.002	fig. 3-5
970-1240	20	43	$1.1x10^6$	$3.9x10^{-12}$	0.82[b]	0.2[b]	fig. 3-5
970-1240	20	71	$1.8x10^6$	$9.1x10^{-12}$	0.75	0.08	fig. 3-5
Lactate respiration							
970-1240	10	27	$1.4x10^6$	$2.8x10^{-12}$	0.95	0.003	fig. 3-4
970-1240	10	43	$1.5x10^6$	$3.5x10^{-12}$	0.97	0.001	fig. 3-4
970-1240	10	71	$5.0x10^6$	$10.3x10^{-12}$	0.81[a]	0.1[a]	fig. 3-4
970-1240	20	27	$0.2x10^6$	$2.0x10^{-12}$	0.98	0.0005	fig. 3-5
970-1240	20	71	$1.8x10^6$	$9.8x10^{-12}$	0.80	0.06	fig. 3-5

[a] n=5, [b] n=4

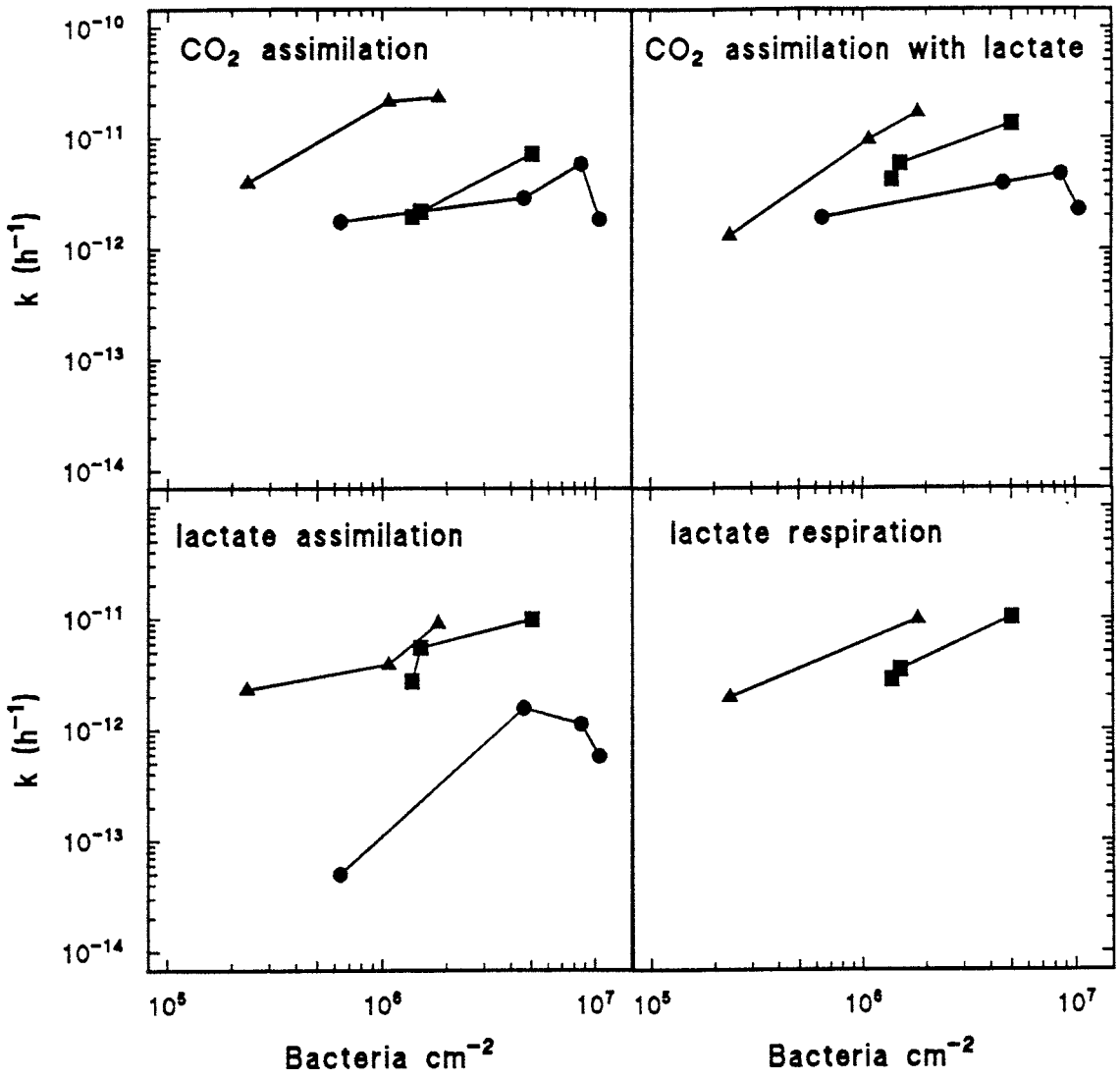

Fig. 3-7 The *in situ* assimilation rates of CO_2 without and with the addition of 10 μM unlabeled lactate, the assimilation rates of ^{14}C from U-^{14}C-labeled lactate and the respiration rates of assimilated ^{14}C-lactate, measured as $^{14}CO_2$ evolution, by attached bacteria in groundwater from the Stripa borehole V2. Symbols: circle, 812-820 m; square, 970-1240 m, 10°C; triangle, 970-1240 m, 20°C.

Table 3-10 The *in situ* assimilation rates of CO_2 without and with the addition of 10 μM unlabeled lactate, the assimilation rates of ^{14}C from U-^{14}C-labeled lactate and the respiration rates of assimilated ^{14}C-lactate, measured as $^{14}CO_2$ evolution, by an average attached bacterium in groundwater from the Stripa borehole V2 at a density of $1.8x10^6$ bacteria cm^{-2} (confer Fig. 3-7).

depth (m)	temp (°C)	rate (mole h^{-1} $bacterium^{-1}$)
assimilation of CO_2		
812-820	10	$1.2x10^{-18}$
970-1240	10	$1.6x10^{-18}$
970-1240	20	$12.9x10^{-18}$
assimilation of CO_2 with 10 μM lactate		
812-820	10	$1.6x10^{-18}$
970-1240	10	$3.6x10^{-18}$
970-1240	20	$9.5x10^{-18}$
lactate assimilation		
812-820	10	$0.2x10^{-18}$
970-1240	10	$6.0x10^{-18}$
970-1240	20	$9.1x10^{-18}$
lactate respiration		
970-1240	10	$2.2x10^{-18}$
970-1240	20	$5.4x10^{-18}$

any consistent effect from the addition of lactate on the CO_2 assimilation rates (Table 3-10).

3.7 THE RATE OF ASSIMILATION OF LACTATE

The assimilation of lactate is shown in Fig. 3-4, 3-5, 3-6. The slope of each line represents the initial rates of assimilation, shown in table 3-9, of different population sizes. The rates are higher when there are more bacteria present at 970-1240 m but decreases at the highest bacterial numbers in the 812-820 m populations. Fig. 3-7 compares the rates for the different population studied and Table 3-10 shows that the assimilation of lactate increases with depth and temperature.

3.8 RESPIRATION OF LACTATE BY STRIPA BACTERIAL POPULATIONS

The respiration of lactate is shown in Fig. 3-5 and 3-6. The slope of each line represents the initial rates of assimilation, shown in table 3-9, of different population sizes. The rates increases with increasing bacterial density (Table 3-9) and increasing temperature (Fig. 3-7 and Table 3-10).

3.9 AODC AND SEM STUDIES OF ATTACHED BACTERIA ON GLASS SURFACES FROM STRIPA

Fig. 3-10 demonstrates a thin film that cover the microscope slide exposed to flowing groundwater (2.8×10^3 m sec^{-1}) for 161 days from the Stripa borehole V2, 812-820 m. It has dried during the preparation for SEM. Fig. 3-9, 3-11, 3-12 and 3-13 show that the bacteria were short rods, < 1 μm and grew in colonies beneath the film.

Fig. 3-14 to 3-17 show microscope slides exposed to flowing groundwater (0.5×10^3 m sec^{-1}) for 71 days from the Stripa borehole V2, 970-1240 m, 10 °C. The bacteria were 1-2 μm long rods appearing single or in clusters like small colonies.

Fig. 3-18 to 3-21 show microscope slides exposed to flowing groundwater (0.5×10^3 m sec^{-1}) for 71 days from the Stripa borehole V2, 970-1240 m, 20 °C. The bacteria were 1-2 μm long rods or filaments (Fig. 3-18 and 3-20) and appearing in clusters like small colonies (3-19 and 3-21). They excreted thin, 100 nm, threads that seem to act as holdfasts and eventually for cell-cell interactions (Fig. 3-19). Occasionally, stalked bacteria with a morphology like *Caulobacter* could be observed (not shown).

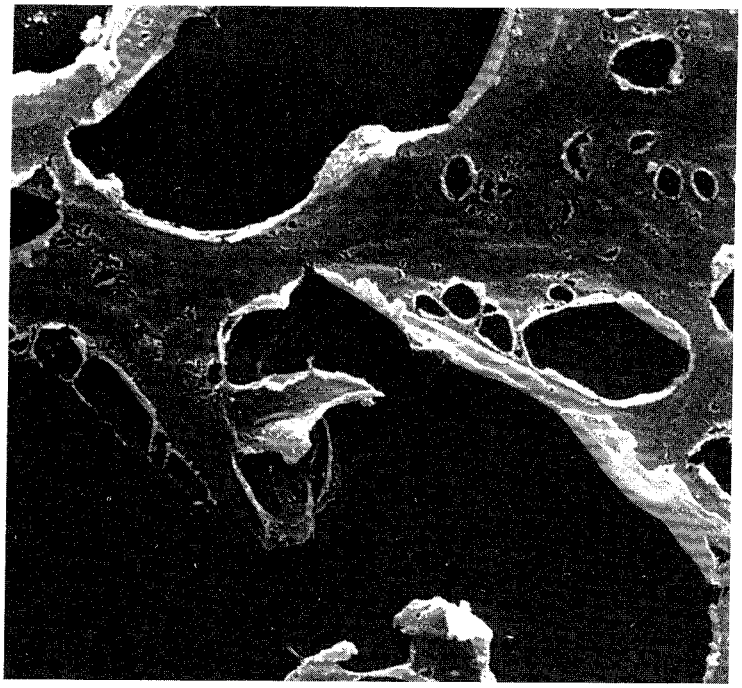

Fig. 3-8 SEM image of a microscope slide exposed for 161 days to flowing groundwater (2.8 x 10^3 m sec^{-1}) from the Stripa borehole V2, 812-820 m. 1 cm = 2 μm; magnification: x 5000.

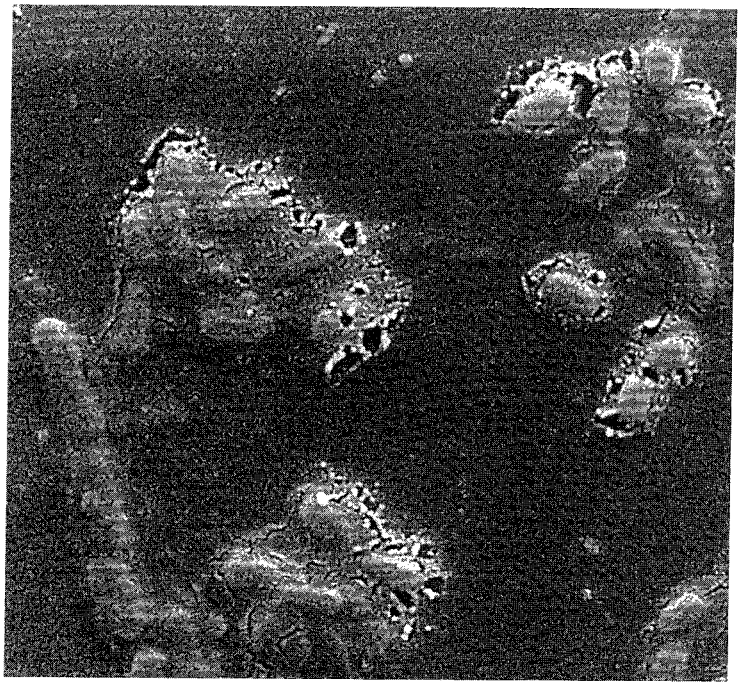

Fig. 3-9 SEM image of a microscope slide exposed for 161 days to flowing groundwater (2.8 x 10^3 m sec^{-1}) from the Stripa borehole V2, 812-820 m. 1 cm = 1 μm; magnification: x1000.

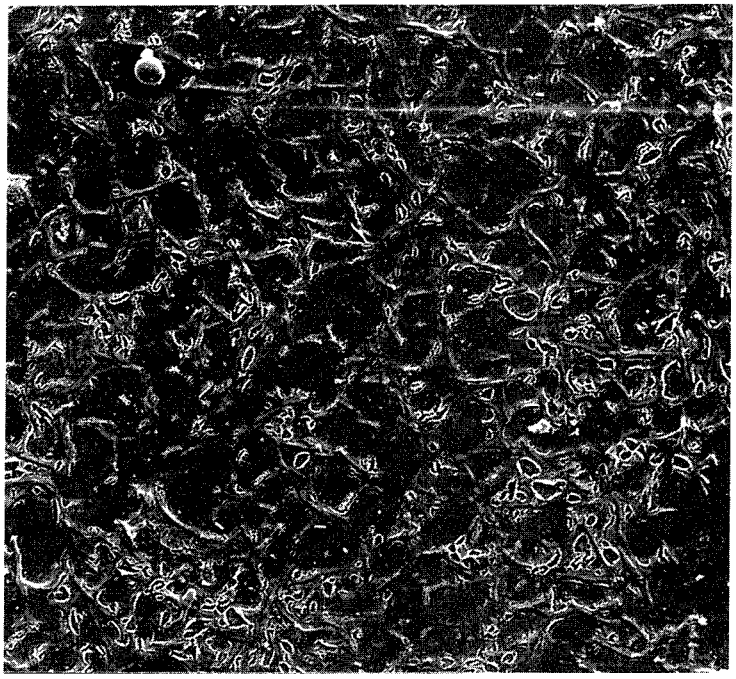

Fig. 3-10 SEM image of a microscope slide exposed for 161 days to flowing groundwater (2.8 x 10^3 m sec^{-1}) from the Stripa borehole V2, 812-820 m. 1 cm = 10 μm; magnification: x1000.

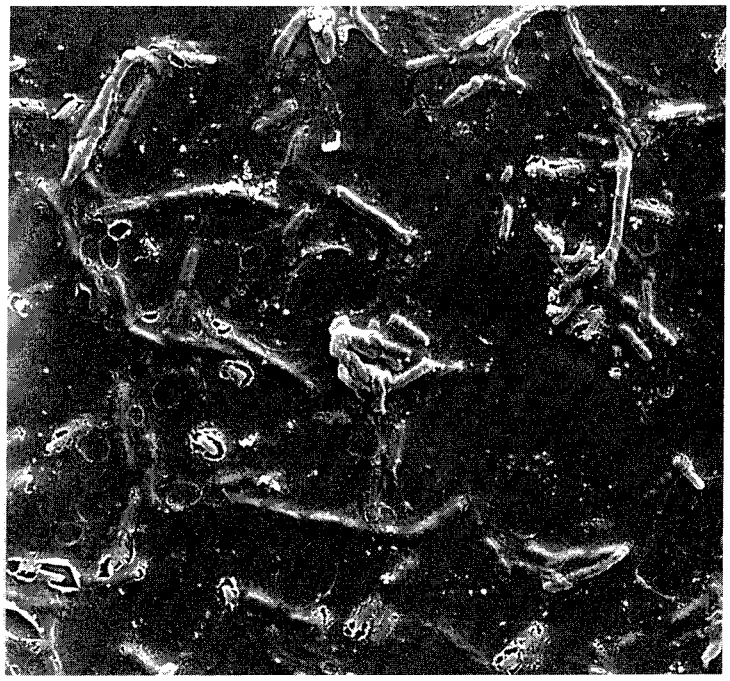

Fig. 3-11 SEM image of a microscope slide exposed for 161 days to flowing groundwater (2.8 x 10^3 m sec^{-1}) from the Stripa borehole V2, 812-820 m. 1.5 cm = 5 μm; magnification: x3000.

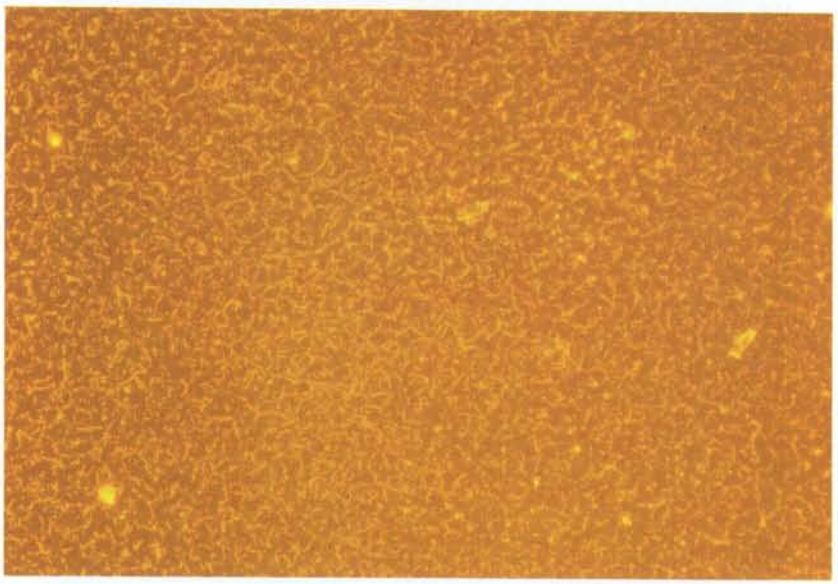

Fig. 3-12 AODC image of a microscope slide exposed for 161 days to flowing groundwater (2.8×10^3 m sec^{-1}) from the Stripa borehole V2, 812-820 m. 1 cm = 40 μm; magnification: x250.

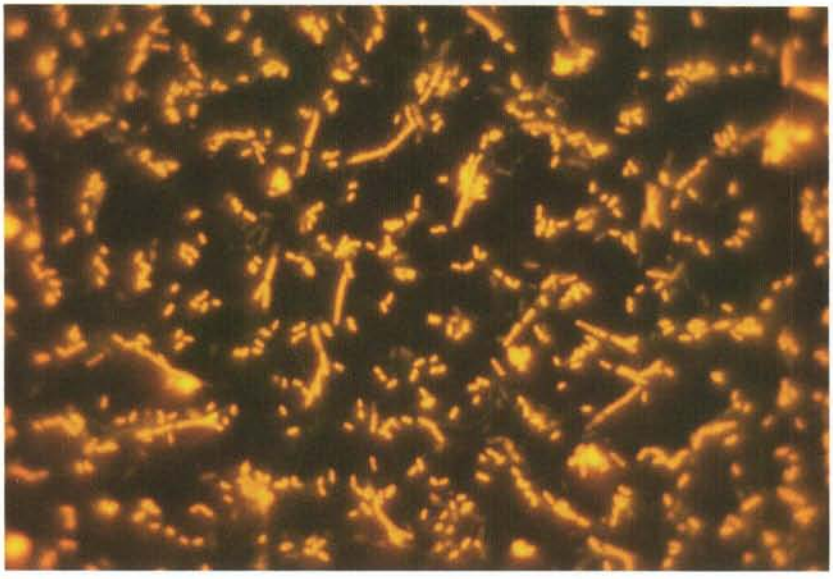

Fig. 3-13 AODC image of a microscope slide exposed for 161 days to flowing groundwater (2.8×10^3 m sec^{-1}) from the Stripa borehole V2, 812-820 m. 1 cm = 8 μm; magnification: x1250.

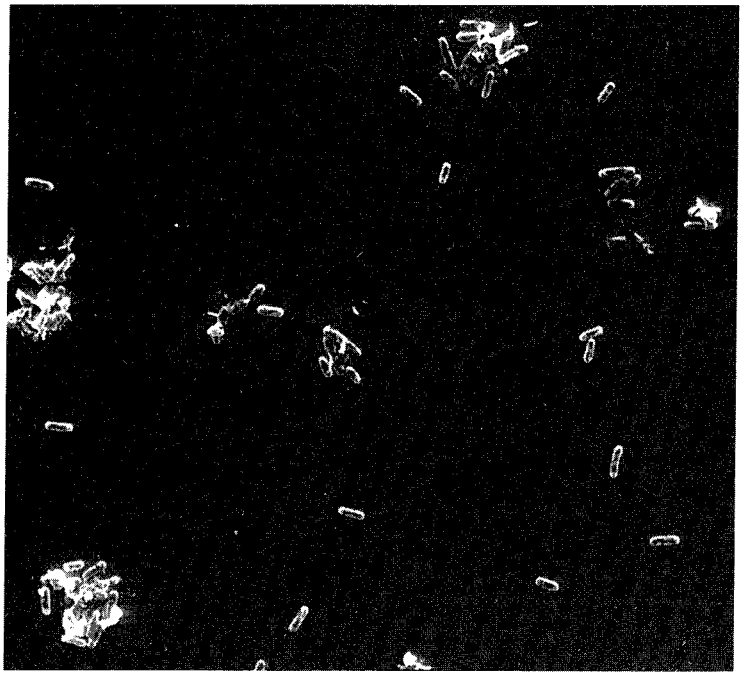

Fig. 3-14 SEM image of a microscope slide exposed for 71 days to flowing groundwater (0.5 x 10^3 m sec^{-1}) from the Stripa borehole V2, 970-1240 m, 10 °C. 1 cm = 5 μm; magnification: x2000.

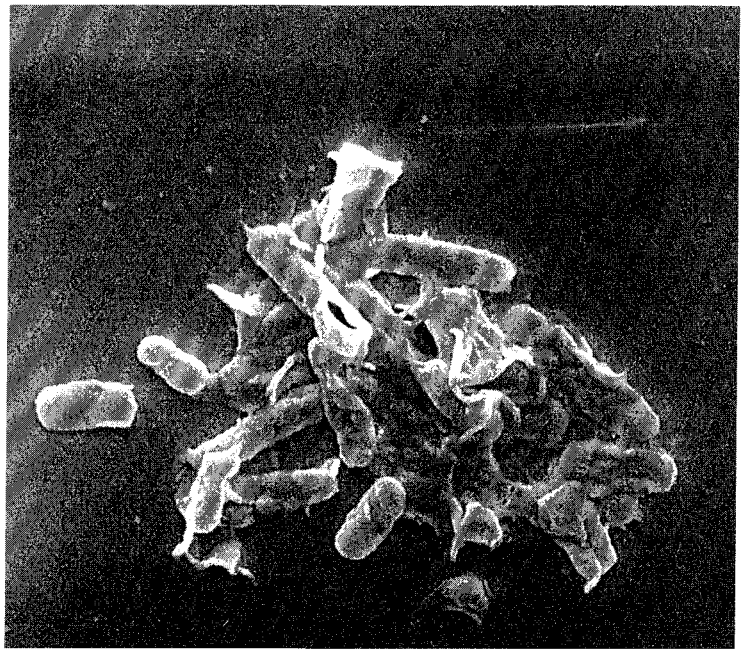

Fig. 3-15 SEM image of a microscope slide exposed for 71 days to flowing groundwater (0.5 x 10^3 m sec^{-1}) from the Stripa borehole V2, 970-1240 m, 10 °C. 1 cm = 1 μm; magnification: x10000.

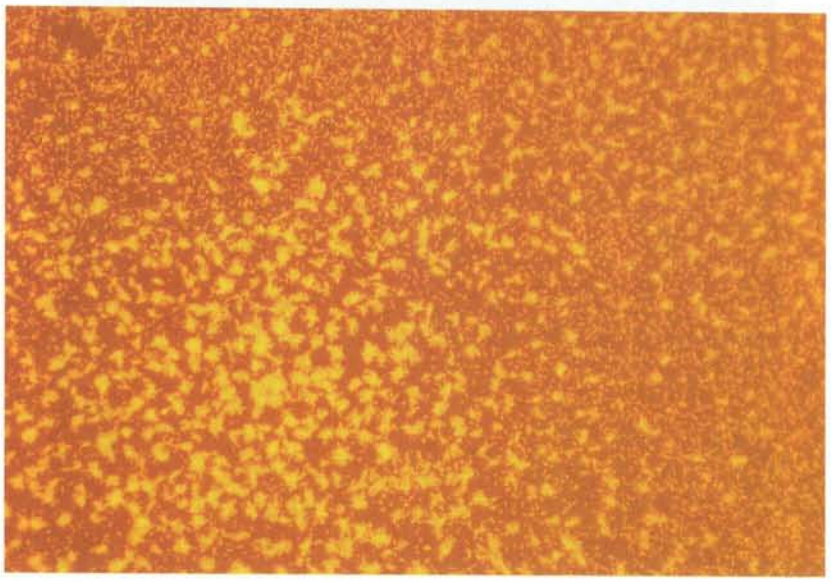

Fig. 3-16 AODC image of a microscope slide exposed for 71 days to flowing groundwater (0.5 x 10^3 m sec^{-1}) from the Stripa borehole V2, 970-1240 m, 10 °C. 1 cm = 40 μm; magnification: x250.

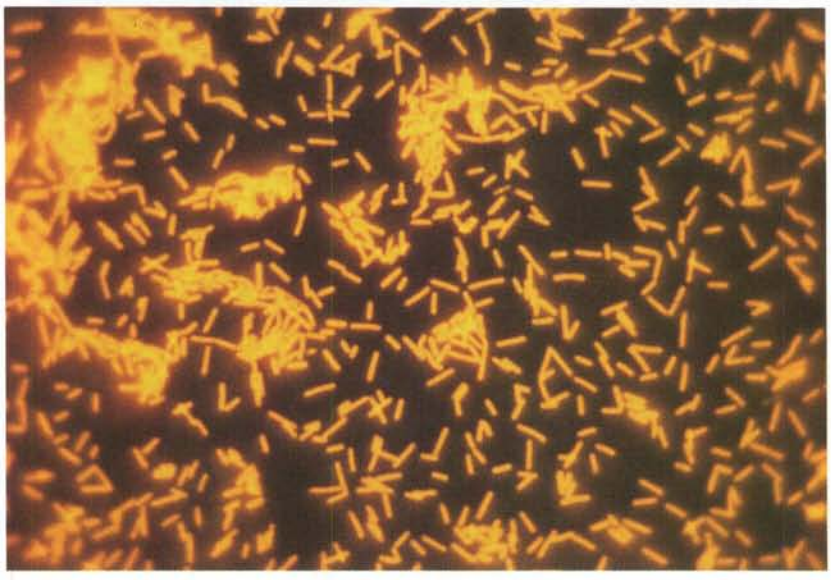

Fig. 3-17 AODC image of a microscope slide exposed to flowing groundwater (0.5 x 10^3 m sec^{-1}) for 71 days from the Stripa borehole V2, 970-1240 m, 10 °C. 1 cm = 8 μm; magnification: x1250.

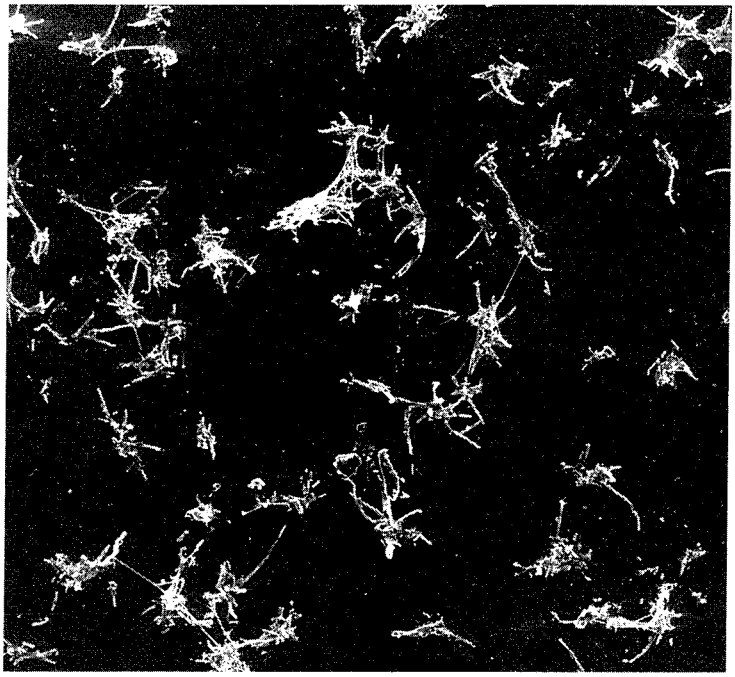

Fig. 3-18 SEM image of a microscope slide exposed for 71 days to flowing groundwater (0.5 x 10^3 m sec^{-1}) from the Stripa borehole V2, 970-1240 m, 20 °C. 1 cm = 20 μm; magnification: x500.

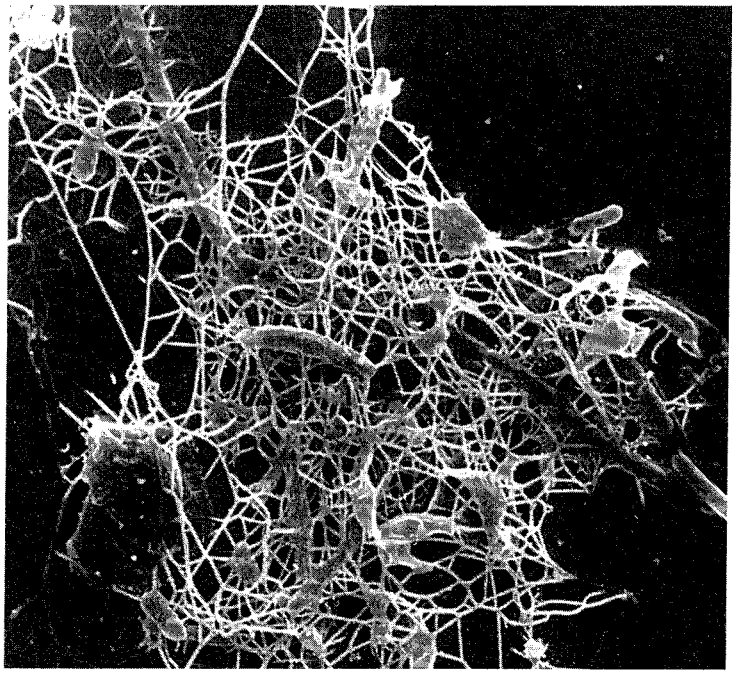

Fig. 3-19 SEM image of a microscope slide exposed for 71 days to flowing groundwater (0.5 x 10^3 m sec^{-1}) from the Stripa borehole V2, 970-1240 m, 20 °C. 1 cm = 2 μm; magnification: x5000.

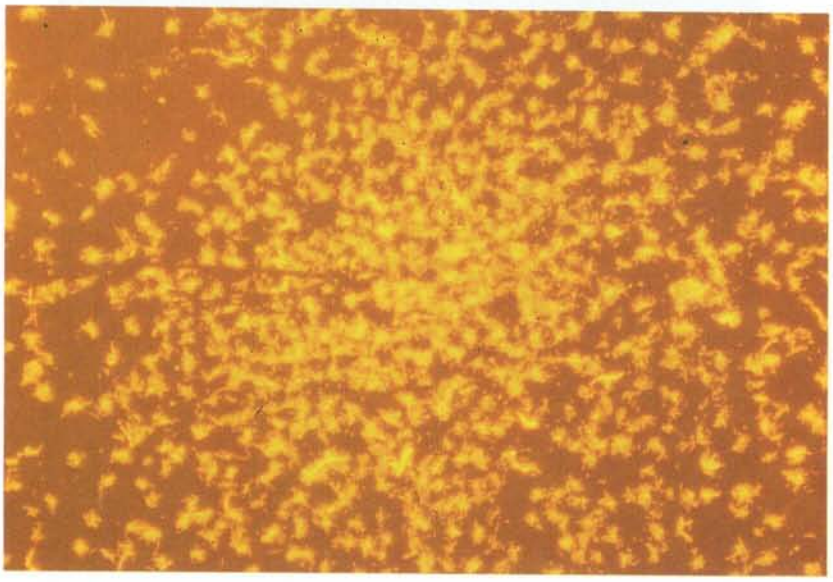

Fig. 3-20 AODC image of a microscope slide exposed for 71 days to flowing groundwater (0.5×10^3 m sec^{-1}) from the Stripa borehole V2, 970-1240 m, 20 °C. 1 cm = 40 μm; magnification: x250.

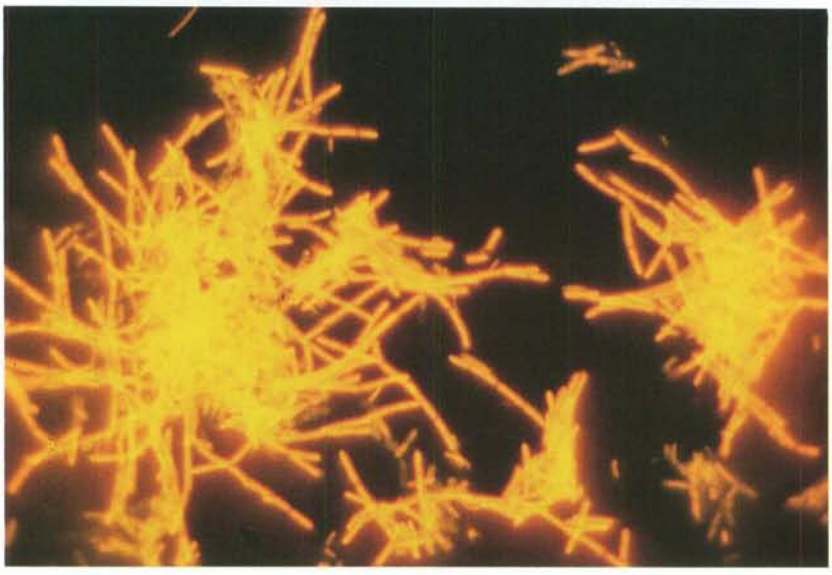

Fig. 3-21 AODC image of a microscope slide exposed for 71 days to flowing groundwater (0.5×10^3 m sec^{-1}) from the Stripa borehole V2, 970-1240 m, 20 °C. 1 cm = 8 μm; magnification: x1250.

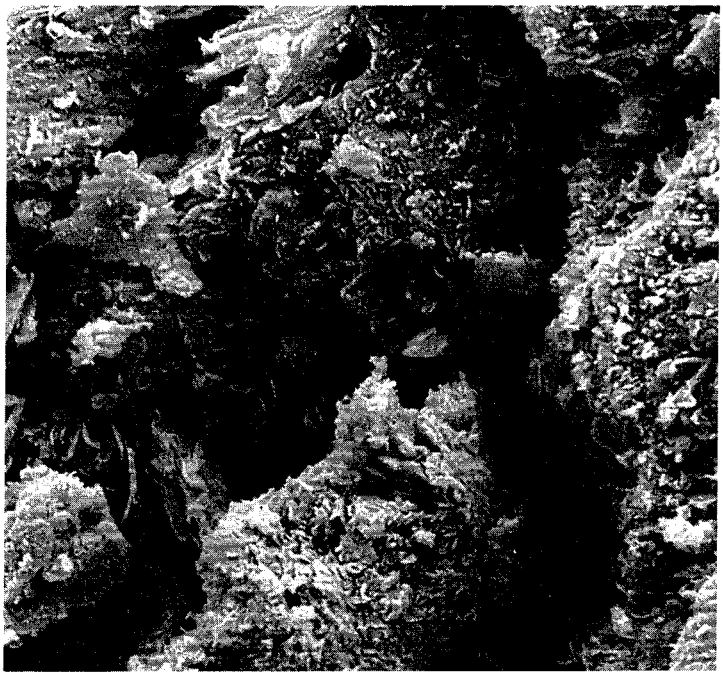

Fig. 3-22 SEM image of the surface of a water conducting fracture, collected from 160 m depth in the Äspö tunnel the hours after explosion.
1 cm = 2 μm; magnification: x5000.

Fig. 3-23 SEM image of the surface of a water conducting fracture, collected from 160 m depth in the Äspö tunnel the hours after explosion.
1 cm = 0.5 μm; magnification: x20000.

4 DISCUSSION

4.1 THE DEEP GROUNDWATER ENVIRONMENT

The study by Gustafsson et al. (1989) shows that the salinity profile of the borehole KLX01 at Laxemar is homogeneous, which is typical for groundwater in crystalline rock that has been intruded by saline waters such as sea-water. This probably occurred during the era when the Baltic Sea was called the Litorina Sea and had a salinity three times higher than the present level. The conductivity increases with depth (Table 3-1) and reflects this homogeneity.

The study by Nordstrom *et al.*, (1985) showed that the salinity profile of the borehole V2 at Stripa is heterogeneous, which is typical for groundwaters in crystalline bedrock that have not been intruded by saline waters such as that from the sea. The different conductivities, carbonate, sulfate and sulphide concentrations of the sampling depths (Table 3-2) reflect this heterogeneity and indicate that the groundwaters came from fracture systems without close hydraulic connections. The two lower sampling depths had a considerably higher salinity than the upper depth indicating that these waters are old (in excess of 20000 years using conventional ^{14}C measurements, confer. Nordstrom *et al.*, 1985). The 799-807 m groundwater is probably mixed with surface water via the mine, which may have diluted the salinity above 810 m (Nordstrom *et al.*, 1985).

The negative redox potentials and the sulphide contents of the borehole KLX01 (Table 3-1) and the sulphide content of the two lowest depths of

the borehole V2 indicate that these habitats were anaerobic, and that facultative or obligate anaerobic bacteria should be expected.

Incubation in air decreased the assimilations of CO_2, formate, lactate and leucine by attached and unattached bacteria at the 910-921 m depth in the borehole KLX01 (Table 3-7) and by the unattached bacteria at the 999-1078 m depth. This effect also appeared with acetate and glucose, with the attached bacteria at the 910-921 m and 999-1078 m (only glucose) depths. This indicates that portions of the studied populations were obligate anaerobes as their ability to assimilate the added compounds was sensitive to oxygen and to changes in the chemical environment associated with exposure of the anoxic groundwater to air, e.g. changes of pH, Eh and solubilities of metals, nutrients etc. in the groundwater. Facultative anaerobic bacteria might have been responsible for the remaining assimilations in air.

There was a significant content of methane in the groundwaters (Table 3-3 and 3-4), which may have two different origins. 1) Methane bacteria can use CO_2 and formate as carbon sources, and use CO_2 as terminal electron acceptor in their energy metabolism producing methane (Fuchs, 1990; Ormeland, 1988). Enrichment cultures made earlier have indicated the presence of anaerobic bacteria in the KLX borehole, presumably methanogenic bacteria, capable of growth on C-1 compounds with hydrogen Pedersen & Ekendahl (1990). The small but significant assimilation of CO_2 and formate support the assumed presence of methane bacteria. 2) Another methane source might be gas migrating from natural deposits of nonbiological chemical origin, thermocatalytic methane, (Barker & Fritz, 1982) of the type proposed for the Siljan deep gas project area, approximately one hundred kilometres north of Stripa. Large gas and oil deposits, formed as a result of an ancient collision of a meteorite with the earth, are postulated to lie several kilometres below ground.

The contents of total organic carbon (TOC) in the KLX01 groundwaters were previously determined to be 1.4 mg litre^{-1} (Pedersen & Ekendahl, 1990) and to 1.1 mg litre^{-1} in the groundwaters of the borehole V2 (0.4 to 4 mg l^{-1}, 8 measurements) (Nordstrom *et al.*, 1985). This is in agreement with the range of what has been found at depths below 600 m in the KAS boreholes in the Laxemar-Äspö area (Pedersen & Ekendahl, 1990) and in 22 other deep groundwaters from crystalline bedrock (average 2 mg litre^{-1}) (Lakksoharju, 1990). A significant part of this DOC consists of fulvic acids (Pettersson *et al.*, 1990). The amounts of organic compounds used were large, 0.32 (formate), 0.07 (acetate), 0.23 (lactate) and 0.29 (glucose) mg litre^{-1}, ranging from 5 up to 23 % of the TOC. The presence of *in situ* concentrations of unlabeled corresponding organic compounds giving significant misleading data due to isotope dilution is then unlikely.

4.2 ORIGIN OF THE OBSERVED POPULATIONS

The physical and chemical parameters and the flows measured from the borehole V2 at Stripa have not differed significantly since the borehole was drilled in 1978 (Nordstrom *et al.*, 1985). In addition, each sampling depth exhibited an amount of bacteria per volume of water that differed little between sampling occasions from 1987 to 1990 (Table 3-6). This implies that the bacterial populations studied in Stripa and probably also in Laxemar were in steady states with their environments and that the fractures of the bedrocks constitute stable habitats for bacteria.

The possibility of contamination of the groundwater during drilling can not be excluded at this point, but it can also be argued that bacteria probably inhabited this environment far before the boreholes were drilled. A population of bacteria, slowly migrating vertically at a rate of 0.1 to 1 m a year in the present chemical and physical gradients and with the groundwater movements, would need 1000 to 10000 years to reach a depth of 1000 m. This is rapid in relation to the geological age of many million years of the Laxemar and Stripa bedrocks.

In addition, it must be true, that building a repository will introduce new bacteria, more or less well adapted to this environment, and the repository will also influence eventually indigenous populations. It should then be judged if the activities around the building of a repository will have an effect, remaining long enough to allow the development of bacterial populations relevant for radionuclide migration.

Bacteria, irrespective of wether they are migrating, contaminates or indigenous populations, need carbon and energy sources to survive. The assimilation of CO_2 (Table 3-7 and 3-8) reflects an *in situ* production of organic carbon from the CO_2, which might be sufficient to sustain autotrophic growth of parts of the populations. The energy source for autotrophy could be hydrogen migrating from the earth crust. Hydrogen has been detected in KLX01 (Table 3-3) and in the Stripa groundwaters in a borehole called V1 (Carlsson *et al.*, 1983) but not in borehole V2 (Table 3-4). This is probably as a consequence of the utilization of the hydrogen by resident borehole V2 bacterial populations. Heterotrophic, fermentative or respiratory utilization of geological deposits of organic material is another possible explanation to the observed deep groundwater populations.

4.3 VIABILITY OF THE POPULATIONS

Leucine assimilation is virtually specific for bacteria provided low (nM) concentrations are used and is used by many bacteria during growth as a leucine source in their protein synthesis Kirchman *et al.*, 1985). It can also be used as a carbon and energy source and can be fermented by proteolytic clostridia via Sticklands reaction. High percentages of the populations, up to 98% of the unattached bacteria on the KLX01 borehole at the 999-1078 m depth and 99% of the attached bacteria at the V2 812-820 m depth, assimilated leucine. This assimilation showed that major portions of the studied populations were viable.

4.4 AUTOTROPHIC ACTIVITY OF THE POPULATIONS

4.4.1 Assimilation of CO_2

Our results indicate the presence of metabolically active, growing microbial populations that assimilate CO_2 in deep groundwater. Assuming a dry weight of 2.8 x 10^{-13} g bacterium^{-1} and a carbon content of 50% (Neidhardt *et al.*, 1990) leads to 1.12 x 10^{-14} mole carbon per bacterium. The generation times for the Stripa biofilms, 812-820 m 10°C, 970-1240 m 10°C and 970-1240 m 20 °C were 37, 43 and 16 days (3.5) and these bacteria assimilated CO_2 at rates 1.2, 1.6 and 12.9 x 10^{-18} mole CO_2 h^{-1} bacterium^{-1} respectively (Table 3-10). The assimilations would then theoretically be enough for autothropic growth of 9.4%, 14,5% and 42.7% of respective population. Consequently, there must be an additional organic carbon source to explain the observed growth of bacteria. Such carbon might origin from abstraction processes from geological deposits of organic material to the flowing groundwater. The build-up of films, probably of organic origin, like the one in 812-820 m (Fig. 3-8) support this theory. Heterotrophic or mixotrophic assimilation of CO_2 during fermentative or respiratory utilization of such geological deposits of organic material is another possible explanation for the recorded uptake of CO_2.

There were 50 μM CO_3^{2-} in the groundwater from 812-820 m and 57 μM CO_3^{2-} in the groundwater from 970-1240 m in Stripa. It would take approximately 1 day for the Stripa populations to consume this CO_3^{2-} in a situation with 10^7 bacteria cm^{-2} in a 0.1 mm fracture with totally stagnant groundwater. Respiration of assimilated carbon back to CO_2 will prolong the time, and it must be expected that a flow dependent steady state will

be entered. The rates measured here are based on experiments with flows that probably are several orders of magnitude larger than in a fracture and this will have implications as discussed below (4.7). Nevertheless, the results indicate that deep groundwater bacteria seem to have a CO_2 assimilating potential, that may have a profound influence on the groundwater chemistry through its action on the carbonate system.

4.5 HETEROTROPHIC ACTIVITY OF THE POPULATIONS

4.5.1 Assimilation and respiration of lactate

Assimilation of lactate by the attached bacteria dominated over acetate and glucose at all depths and gave substantial MARG results with all populations (Table 3-7 and 3-8). The deep groundwaters of the Laxemar and Stripa site is anoxic and depleted of nitrate and nitrite but contained 0.05-8 mM SO_4 (Table 3-1 and 3.2) and such an environment will be selective for fermenting bacteria and SRB (Widdel, 1988). Propionate-producing bacteria are among the few bacteria known to ferment lactate without involving a respiratory chain, but they thrive in nutrient rich habitats like cheese, on skin and in mud and not in oligotrophic environments like the Laxemar and Stripa deep groundwaters. Consequently, as the only available electron acceptor for respiration of lactate was sulfate, it is likely that the bacteria that utilized lactate in the anaerobic incubations were SRB. The most probable number of SRB in the KLX01 borehole at 680 m level was earlier determined to be 5.6×10^4 bacteria ml^{-1} (Pedersen & Ekendahl, 1990).

Sulfate reduction by SRB's will increase the $\delta^{34}S$ isotopic content of a groundwater due to the preference for ^{32}S by sulfate reducers (Widdel, 1988) and also result in increasing amounts of sulphide. Fontes *et al.*, (1989) found $\delta^{34}S$ values that were high in borehole V2 and they postulated the presence of viable populations of sulfate reducing bacteria. The 970-1240 m depth revealed the highest $\delta^{34}S$ value achieved during their investigations; this depth had the highest lactate assimilation (9.2×10^{-10} mol cm^{-2}), with 74% of the bacteria actively assimilating lactate and the highest sulphide content in our study (233 μM) (Table 3-1 and 3-2). Our data confirm the hypothesis proposed by Fontes *et al.*, (1989).

The presence of culturable SRB in KLX01 and the isotopic composition of the Stripa groundwaters, together with the observations made in this work, suggests that the SRB constitute a substantial part of the bacterial populations in the fractures of the Laxemar and Stripa crystalline

bedrocks, as have been reported for other deep geological formations (Olson *et al.*, 1981). Demonstration of sulfate reduction with stoichiometric loss of lactate and CO_2 production is however needed to confirm this assumption.

The results indeed show that lactate was respired to CO_2 by the Stripa 812-820 and 970-1240 m populations. However, we could not detect any sulfate reduction with the method used although it worked well with a pure culture of SRB *in vitro*. One reason for the negative result might be that we were to close to the detection limit of what can be measured *in situ*. We have isolated DNA from the Stripa populations and will sequence the 16S-RNA from them in an attempt to describe dominant species in the populations and to test the hypothesis that SRB are present in the Stripa groundwaters.

4.5.2 Glucose and acetate

Glucose and acetate utilization is common among mixotrophic bacteria (Kuenen & Bos, 1989) and most fermenting as well as respiring heterotrophic bacteria. There was assimilation of glucose, which was below the detection limit for active bacteria ($<0.1 \times 10^{-16}$ mol cell^{-1}) in several samples analysed with the MARG method. Since only a few SRB are known to utilize glucose, the registered uptake probably indicates the presence of heterotrophic bacteria other than SRB. Although the amounts of acetate assimilated were low, the use of tritiated acetate allows low detection limits with the LSC and MARG methods and we determined that up to 62 % of the populations were metabolically active, assimilating this compound.

4.6 ASSIMILATION RATES

4.6.1 Laxemar

The assimilations of the introduced carbon sources in Laxemar increased linearly with time for the attached bacterial population from the 910-921 m depth and the unattached bacterial population from 999-1078 m depth except with formate. These populations exhibited generally the highest amounts of assimilated compounds (Table 3-7), enough to give data well separated from the control measurements of abiotic sorption of the compounds. They were also very viable, 98% and 87 % assimilated leucine, respectively. The lack of lag-phases suggests that these populations were metabolically active (Hall *et al.*, 1990) as they

immediately responded to a sudden increase in the concentration of carbon and energy sources. The unattached bacterial populations from the 910-921 m depth and the attached bacterial population from 999-1078 m depth, except for glucose, gave data that were less consistent.

There was a 52 fold higher assimilation of lactate than of glucose by the unattached population at the 999-1078 m depth as measured with the LSC method. The MARG method resulted in 83 % of bacteria active in assimilation of lactate while glucose assimilation could not be detected. This example shows that the determinations of heterotrophic activity in the Laxemar groundwaters were strongly dependent on the choice of introduced compound. The use of several different compounds reduces the risk for false conclusions about the viability and metabolic activity of the studied populations.

The measurement of heterotrophic activity of bacteria as it was done here gives measures relative to the true growth rate and metabolic activity (Hobbie, 1990; Ladd & Costerton, 1990). The amounts of the labeled compounds assimilated were 10 - 500 fold higher cm^{-2} than the amounts assimilated ml^{-1} at the 830-841 and 910-921 depths while the number of bacteria of the populations that were responsible for the assimilations was approximately 6 times higher on the surfaces (cm^{-2}) than in the water (ml^{-1}) (Tables 3-5 and 3-7). This implies that the attached populations were in more metabolically active states than the unattached bacteria. The presence of metabolically active attached bacteria producing complexing agents and other metabolites that affect speciation of trace elements may increase the mobility of radionuclides from a radioactive waste disposal.

4.6.2 Stripa

The assimilation of CO_2 and lactate increased with depth and temperature (Table 3-10). The temperature effect must be considered when discussing the impact of microbes with a repository, since there will be a considerable increase in temperature, up to 80 °C, around the vault. There was not any significant effect from the addition of lactate on the CO_2-assimilation which implies that CO_2 was assimilated by bacteria else than those that assimilated and respired lactate.

4.7 THE DEVELOPMENT OF BIOFILMS ON FRACTURE SURFACES

A fracture in crystalline bedrock is made of two surfaces which are wavy and rough. They are in contact with each other at some points but are at a

distance from each other at others. The openings in the fractures are potential channels for groundwater. Recently several model studies have been made on flow and transport in fractures with variable apertures (Moreno *et al.*, 1988; Tsang *et al.*, 1988). The results indicated that considerable channelling is to be expected in such fractures and that there is a tendency for some pathways to carry much more water than others. In a limited mass of rock one or a few channels will dominate flow, radionuclide transport and transport of nutrients for bacteria. Assuming a mean channel width of 0.1 mm (Moreno *et al.*, 1985), our results imply that there would be from 4 x 10^3 up to 8 x 10^5 more attached than unattached bacteria in a channel after 4 months of contact with Stripa borehole V2 groundwater flowing at 0.5 to 2.8 x 10^{-3} m sec^{-1} (Table 3-2). Fig. 3-22 and 3-23 show that there can be a considerable larger surface available for attachment and growth than calculated from models with simple flat walls. The average hydraulic conductivity, K, has been determined to be 10^{-6} m sec^{-1} or less in fractured rock of Stripa (Carlsson *et al.*, 1983) but it will be considerably higher in individual channels (Neretnieks, 1990). K is a function of the injection flow rate, the injection excess head, the length of the injection interval and the radius of the borehole, (Andersson *et al.*, 1989). The flows used here were probably even higher than in a channel with a high conductivity; instead the experiment time was very short in relation to the time a channel will be open for flowing groundwater and bacteria.

The availability of energy and nutrients over time for a biofilm is flow dependant and will determine whether a biofilm will develop *in situ* and how many bacteria can be maintained. The slower the flow, the slower the development rate of a biofilm down to a limit where the bacteria can no longer grow or maintain a non-growth metabolism. This limit is probably very low for bacteria in an oligotrophic environment like deep groundwater, and selects for bacteria with advanced morphological and physiological mechanisms to survive a very limited availability of nutrients (Kjelleberg *et al.*, 1987). Assessing the influence of groundwater microbiology on the long term safety of nuclear waste disposal involve time scales ranging from hundreds to many millions of years, thus there is practically no time limit for even the slowest developing biofilm to reach a steady state. The presence of attached bacteria might retard transport of radionuclides from a nuclear waste repository unless they produce complexing agents and other metabolites that affect speciation and thus mobility of radionuclides in a contradictory way. The possibility of such *in situ* production by bacteria in fractured bedrock will be an important task for future research, aimed to assess the influence of groundwater microbiology on the long term safety of nuclear waste disposal.

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Description of transport pathways in a KBS-3 type repository
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Concentrations of particulate matter and humic substances in deep groundwaters and estimated effects on the adsorption and transport of radionuclides
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TR 91-51
Gideå study site. Scope of activities and main results
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Rune Nordqvist[2], Christer Ljunggren[2], Sven Tirén[2],
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Fjällveden study site. Scope of activities and main results
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Rune Nordqvist[2], Christer Ljunggren[2], Sven Tirén[2],
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