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Ion chromatography as a tool for optimization and control of fermentation processes

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ABSTRACT

Information concerning the mineral content of fermentation broth and microorganisms is important for the optimization of fermentation processes. Ion chromatographic methods were implemented for measurement of anions and cations in the range 0.01–100 ppm and transition metals in the range 0.01–100 ppb. An IonPac AS5A column and an OmniPac PAX-500 column were compared for anion measurements. Acid-digested samples were used for the determination of transition metals to overcome problems with complex binding by organic compounds. A rapid method for acid digestion with nitric acid in closed vessels and microwave heating is described. These methods were implemented to follow a fermentation process with methanotrophic bacteria. Acetate was found to accumulate when natural gas was used as a substrate. A washout experiment showed magnesium to be growth limiting when the concentration was below 60 ppb.

INTRODUCTION

The cultivation of microorganisms requires a complex mineral medium reflecting the mineral content of the microorganisms. The expense of minerals is generally not considered to be important in laboratory-scale fermentations or when a high-cost product is produced on a large scale on the basis of a complex mixture of substrates. The tolerable concentration of most minerals is high, and normal practice is to prepare media containing minerals in excess. The mineral expenses can be of importance, however, when a cheap product is to be produced on a large scale. Production of single-cell protein (SCP) from natural gas is such an example. Minerals fed to the process must be balanced by minerals utilized for SCP production to reduce mineral costs and to allow reuse of the process water without a risk of build-up of minerals. Reuse of process water is necessary to minimize the requirement for fresh water and to minimize the amount of waste water.

Natural gas is considered to be a cheap carbon and energy source for the production of SCP [1]. It consists mainly of methane, which can be utilized for growth by methanotrophic bacteria. In addition to methane, natural gas also contains higher hydrocarbons, such as ethane and propane. The ethane content is between 2 and 20% and the propane content between 0.4 and 13%, depending on the location of the gas field [2]. Methanotrophic bacteria can oxidize many different hydrocarbons [3], but only methane is utilized for growth. Ethane and propane are mainly oxidized to acetic acid and propionic acid, respectively, which inhibit the growth of methanotrophic bacteria and are therefore important fermentation parameters.

Methanotrophic bacteria have a special requirement for copper for optimum growth [4]. Like other transition metals, copper is poisonous at high concentrations and the range between the optimum and a poisonous copper concentration is very narrow when SCP production from natural gas is considered. This stresses the importance of proper measurement and control of the copper concentration during the growth of methanotrophic bacteria. The determination of copper and other transition metals in fermentation broth can be hindered by complex formation with organic components. These organic components can be metabolites excreted in the fermentation broth by the bacterial cells or material from lysed cells. To remove these interferences it is necessary to decompose the organic materials in the sample before measurement. Degradation of organic materials is also necessary when samples containing microorganisms are to be analysed for minerals. Organic material can be decomposed by ashing at 550–600°C for several hours [5] or by acid digestion in closed vessels heated in a microwave oven [6].

This paper describes methods for ion chromatographic determinations of anions, cations and transition metals in fermentation broth and microorganisms. It compares the Dionex IonPac AS5A separator column with the Dionex OmniPac PAX-500 analytical column for anion determination and describes a rapid method for acid digestion of samples in closed vessels with microwave heating. The methods were used to follow and optimize a fermentation process with methanotrophic bacteria.

EXPERIMENTAL

Chromatographic systems and eluents

Anions. A Dionex (Sunnyvale, CA, USA) system 4500i ion chromatograph equipped with a gradient pump module, a conductivity detector and an anion MicroMembrane suppressor (AMMS) was used for anion measurement. The sample loop was 25 μl. The IonPac AS5A-5μ separator column (250 mm × 4 mm I.D., 5-μm particle size) and the IonPac AG5A guard column (50 mm × 4 mm I.D., 5-μm particle size) or the OmniPac PAX-500 analytical column (250 mm × 4 mm I.D., 8.5-μm bead diameter) and the OmniPac PAX-500 guard column (50 mm × 4 mm I.D., 8.5-μm bead diameter) were used for the separation of anions as stated. An anion trap column (ATC-1) was placed between the gradient pump and the injection valve to remove anionic contaminants from the eluent. The eluent flow-rate was 1 ml/min. The AMMS suppressor was continuously regenerated using 25 m*M* sulphuric acid as regenerant solution. The regenerant flow-rate was 15 ml/min.

Anions were separated on the IonPac AS5A-5μ separator column by use of a modification of the gradient elution method described by Dionex [7] (see Table I). A modification of the gradient elution method described by Dionex [8] was used for anion separation on the OmniPac PAX-500 analytical column (see Table I).

Monovalent and divalent cations. Alkali and alkaline earth metals were measured with the Dionex DX-100 ion chromatograph. The system was equipped with a conductivity cell placed in a thermal stabilizer, an IonPac CS 10 analytical column (250 mm × 4 mm I.D., 8.5-μm particle size), an IonPac GC guard column (50 mm × 4 mm I.D., 8.5-μm particle size) and a cation MicroMembrane suppressor (CMMS). The sample loop was 25 μl.

The cations were separated isocratically using a 40 m*M* hydrochloric acid–4 m*M* DL-2,3-diaminopropionic acid (DAP–HCl) as eluent [9]. The eluent flow-rate was 1 ml/min. The CMMS was continuously regenerated with 0.1 *M* tetrabutylammonium hydroxide as regenerant solution. The regenerant flow-rate was 10 ml/min. The regenerant solution was continuously recycled using the Dionex AutoRegen accessory.

Transition metals. Transition metals were measured as described [10] on a Dionex Model 4500i ion chromatograph equipped with a variable-wavelenth detector. Isocratic chelation ion chromatography was used. The samples were adjusted to pH 5.3–5.6 by addition of 2 ml of 2.0 *M* ammonium acetate solution. The pH of acid-digested samples was adjusted by addition of 10 ml of 2.0 *M* ammonium acetate to 20 ml of digest. Sample volumes of 20 ml were used for measurements.

Data system. All ion chromatographic systems were connected to an IBM AT compatible computer by a Dionex AI-450 Model II data system.

Preparation of standards and samples

Anion and cation standards were prepared at the 0.01, 0.1, 1, 10 and 100 ppm (mg/l) levels and transition metals standards at the 0.01, 0.05, 0.1, 1, 10 and 100 ppb (μg/l) levels. All standards were prepared from pure reagents (analytical-reagent grade). Anion standards were stored at −20°C to

TABLE I

CONDITIONS FOR THE ANION COLUMNS

Eluent composition: A = pure water; B = 0.75 m*M* NaOH; C = 200 m*M* NaOH; D = 20% methanol. The methanol content of eluent D was reduced to 10% and 2% in experiments with 5% and 1% methanol in the eluent, respectively.

Column	Time (min)	A (%)	B (%)	C (%)	D (%)	Comments
AS5A	0.0	0	100	0	0	Inject sample
	5.0	0	100	0	0	Start gradient ramp
	15.0	0	85	15	0	
	30.0	0	57	43	0	
	30.1	0	0	100	0	Start wash
	35.0	0	0	100	0	
	35.1	0	100	0	0	Equilibrate
	48	0	100	0	0	Ready to load
PAX 500	0.0	50	0	0	50	Inject sample
	0.1	50	0	0	50	Start gradient ramp
	10.0	42	0	8	50	
	25	28	0	22	50	
	30	0	0	50	50	
	37	0	0	50	50	
	37.1	50	0	0	50	Equilibrate
	50.0	50	0	0	50	Ready to load

prevent deterioration of acetate and formate. The other standard solutions were prepared daily from 1000 ppm (cations) or 1 ppm (transition metals) stock solutions. The stock solutions were renewed every month.

Fermentation broth samples, *i.e.*, samples of fermentation liquid centrifuged (3000 *g*, 10 min) to remove bacterial cells (biomass), were filtered (0.45-μm filter, type HV, Millipore) before measurements of anions and cations, and acid digested before measurement of transition metals. Fermentation liquid samples containing bacterial cells were acid digested before measurement of anions, cations and transition metals.

Microwave acid digestion

A CEM (Matthews, NC, USA) MDS-2000 microwave sample preparation system equipped with a pressure-measuring and control device was used for acid digestion.

Sample solutions of 20 ml were placed in 100-ml Teflon PFA vessels and 0.5 ml of 65% nitric acid was added unless stated otherwise. The vessels were closed, placed in the microwave oven and heated according to Table II. Usually two samples were digested at a time but it was possible to digest up to twelve samples in one run by increasing the heating time.

Before the acid digestion of samples for transition metals measurements, it was necessary to clean the Teflon containers carefully to reduce the background. The Teflon vessels were thoroughly washed and filled with 5 ml Suprapur nitric acid. The vessels were heated according to Table II and the digest was discarded. The procedure was repeated and the vessels were used for sample digestion.

TABLE II

POWER AND HEATING TIME FOR MICROWAVE DIGESTION OF SAMPLES

Parameter	Initial heating	Heating	Cooling
Power (W)	650	325	0
Time (min)	5	5	15
Maximum pressure (p.s.i.)	100	150	–

Chemicals

Hydrochloric acid, acetic acid, 2-dimethylaminoethanol (all from Merck), and sodium hydrogencarbonate (Riedel-de Haën) were of analytical-reagent grade. Ammonia solution (25%) and nitric acid (65%) were of Suprapur grade (Merck). D,L-Diaminopropionic acid monohydrochloride (DAP) was obtained from Sigma and pyridine-2,6 dicarboxylic acid (PDCA) from Aldrich. Ammonium acetate (2.0 *M*) and ammonium nitrate (0.1 *M*) were both Dionex class 100 reagents and monosodium 4-(2-pyridylazo)recorcinol monohydrate (Dionex) was >95% pure. All other chemicals were of analytical-reagent grade. Ultra-pure water (18.2 MΩ/cm resistivity at 25°C), obtained by use of a Milli-Q water purification system (Millipore), was used throughout.

RESULTS AND DISCUSSION

Determination of anions, cations and transition metals

The gradient method for measurement of anions with the IonPac AS5A column described by Dionex [7] was initially used. At first we had many problems in applying the method. The chromatograms showed a peak with a retention time (t_R) of 18 min and irreproducible gradient ramps with a high (≫4 μS) change in the background conductance. Eventually we found that the problems were caused by carbonate contamination. Atmospheric carbon dioxide trapped by the basic eluents caused a build-up of carbonate in the system. The problems were solved by installation of an anion trap column before the injection valve, by replacing the plastic eluent containers with glass containers (atmospheric carbon dioxide can diffuse through the walls of a plastic container), and by washing with 0.2 *M* NaOH for 5 min after each run. These modifications gave reproducible gradient ramps with about a 4 μS change in background conductance. This change is slightly higher than the 1–3 μS mentioned by Dionex [7].

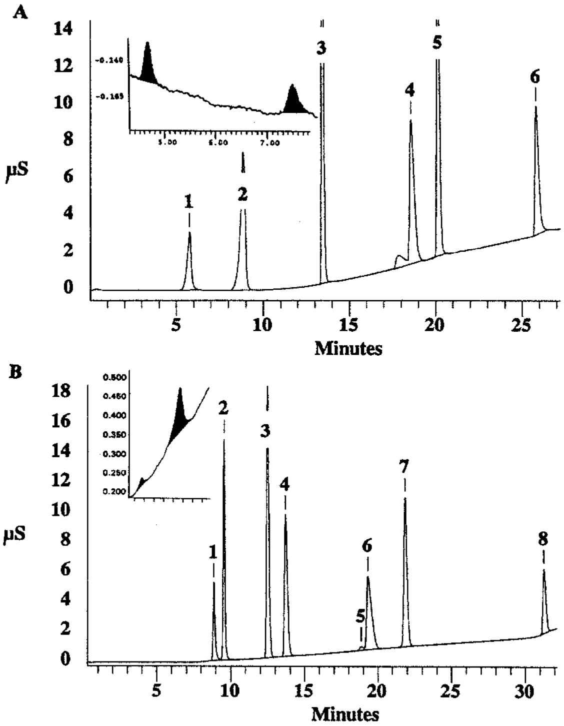

Fig. 1. (A) Chromatogram obtained on the IonPac AS5A column for a 10 ppm standard solution. Inset: chromatogram of 10 ppb standard solution of acetate and formate. Peaks: 1 = acetate; 2 = formate; 3 = chloride; 4 = nitrate; 5 = sulphate; 6 = phosphate. (B) Chromatogram obtained on the OmniPac PAX-500 column of a 10 ppm standard solution. Inset: chromatogram of a 10 ppb standard solution of acetate and formate. Peaks: 1 = acetate; 2 = formate; 3 = chloride; 4 = nitrite; 5 = carbonate; 6 = nitrate; 7 = sulphate; 8 = phosphate.

The IonPac AS5A and the OmniPac PAX-500 columns gave nearly identical results when acetate, formate, chloride, nitrate, sulphate and phosphate were measured (see Fig. 1 and Table III). The calibration graphs were linear in the tested range of 10 ppb–100 ppm, except for sulphate and phosphate, which showed deviations from linearity at low concentrations owing to the high background level of these anions. The correlation coefficients were above 0.998 in all instances. The linearity of the phosphate and sulphate calibration graphs was extended to 10 ppb when their signals were background corrected. The detection limit for all the measured anions was below 10 ppb. The AS5A column had a lower detection limit for acetate than the PAX-500 column (insert in Fig. 1 and Table III). Five repeated injections of a 10 ppm standard solution showed relative standard deviations (R.S.D.s) below 3% for both columns. The AS5A column performed slightly better than the PAX-500 column. Analysis of fermentation broth showed more

TABLE III

LINEAR RANGE OF CONCENTRATIONS TESTED, LINEAR CORRELATION COEFFICIENTS, SIGNAL-TO-NOISE RATIOS, RETENTION TIMES AND RELEVANT R.S.D.

Species	Linear range (ppm)	r[a]	S/N[b]	R.S.D.[c] (%)	R.S.D.[d] (%)	t_R[e] (min)	R.S.D.[f] (%)
Anions measured with the IonPac AS5A-5μ column							
Acetate	0.01–100	0.999	10	0.7	8.3	4.98	6.1
Formate	0.01–100	1.000	8	0.9	3.9	7.79	6.0
Chloride	0.01–100	1.000	40	1.0	3.1	13.4	0.55
Nitrate	0.01–100	1.000	–[g]	0.8	4.0	19.3	0.31
Sulphate	0.01–100	1.000	–	2.8	5.9	20.0	0.51
Phosphate	0.01–100	1.000	6	1.4	6.0	25.6	0.54
Anions measured with the OmniPac PAX-500 column							
Acetate	0.01–100	0.998	4	1.86	–	8.43	3.1
Formate	0.01–100	0.999	25	2.15	–	9.17	2.3
Chloride	0.01–100	1.000	8	2.21	–	11.9	2.5
Nitrate	0.01–100	1.000	4	2.57	–	19.3	1.6
Sulphate	0.01–100	1.000	6	2.21	–	21.4	2.0
Phosphate	0.01–100	1.000	3	2.76	–	30.9	1.6
Cations							
Sodium	0.01–100	1.000	8	4.4	16.3	2.16	3.9
Ammonium	0.01–100	0.997	2	1.2	10.1	2.38	0.9
Potassium	0.01–100	1.000	2	4.5	18.0	2.84	3.5
Manganese	0.01–100	0.999	4	4.6	22.4	9.1	6.6
Calcium	0.01–100	1.000	4	4.1	20.3	17.3	7.2
Transition metals	(ppb)						
Fe^{3+}	0.05–100	0.995	–	3.70	–	5.5	5.0
Cu^{2+}	0.01–100	1.000	–	0.63	–	9.3	6.4
Ni^{2+}	0.10–100	0.997	3	1.60	–	10.4	6.3
Zn^{2+}	0.50–100	0.998	–	2.13	–	11.2	6.3
Co^{2+}	0.05–100	1.000	3	0.57	–	12.7	6.2
Mn^{2+}	0.10–100	0.999	5	2.09	–	15.3	5.8

[a] Linear correlation coefficient. The peak area of phosphate and sulphate was background corrected before calculation of the correlation coefficient.

[b] Signal-to-noise ratio of 10 ppb (anions and cations), 0.05 ppb (Co^{2+}) or 0.01 ppb (Ni^{2+} and Mn^{2+}) standard solutions.

[c] Relative standard deviation of five replicates of 10 ppm (anions and cations) or 10 ppb (transition metals) standard solutions.

[d] Relative standard deviation of nine measurements of 10 ppm standard solutions (anions) or 28 measurements of 100 ppm standard solutions (cations) on different days.

[e] Mean retention time for a period of 9 days (anions on the IonPac AS5A column), 5 days (anions on the OmniPac PAX-500 column), 28 days (cations) or 5 days (transitions metals).

[f] Relative standard deviation of the retention times.

[g] – = Not determined.

peaks on the OmniPac PAX-500 column than on the IonPac AS5A column. One of these peaks was found to be citrate, the others were not identified. The OmniPac PAX-500 column was tested with 1, 5 and 10% of methanol in the eluent. Neither the gradient ramp nor the elution time of the anions tested were affected by the methanol level. Propionate and butyrate were found in separate experiments to elute with t_R 8.7 and 9.0 min, respectively. An improved separation of the short-chain organic acids on the OmniPac PAX-500 column was obtained by isocratic elution with 0.5 mM NaOH during the first 18 min. The t_R values were then 10.5, 11.3, 12.8 and 16.7 min for acetate, propionate, butyrate and formate, respectively. The total elution time was in that case extended to 1 h.

The linearity of the calibration graphs and the low R.S.D.s found for all the anions indicate that quantification by peak area instead of by peak height is sufficiently accurate and reproducible even for compounds with resolution below 1.

The stabilities of the anion standard solutions were initially tested. The levels of formate and acetate in the standards decreased from day to day when the standards were stored at room temperature in glass containers, even when the pH was increased to 10 by addition of NaOH. However, the levels of formate and acetate were found to be constant for several months when the standards were stored at −20°C.

Sodium, ammonium, potassium, magnesium and calcium were separated on the IonPac CS10 column with detection limits of about 10 ppb set by the pumping noise of the DX-100 system (Table III). The R.S.D. of the peak areas was below 5% for five repeated injections. The day-to-day R.S.D. in peak area was 10–20% and stressed the need for a daily calibration. The retention times were fairly constant with a day-to-day R.S.D. of 1–7%.

Transition metals were measured by the isocratic chelation ion chromatographic method with a detection limit of 0.01–0.05 ppb (Table III). The signals for manganese, cobalt, iron, nickel and copper were linear in the range 0.1–100 ppb and for zinc in the range 1–100 ppb. The R.S.D. for five 10 ppb samples was below 2.5% except for iron, which had an R.S.D. of 3.7% owing to a high background level.

Microwave acid digestion

Acid digestion was initially tested on samples of pure water and samples of fermentation liquid containing 10 g dry weight of biomass per litre. The medium used for the fermentation contained 75 ppm of Mg^{2+} and 41 ppm of Ca^{2+}. At first, 5 ml of 65% nitric acid was used for the digestion of 20 ml of sample. However, cation chromatograms of the digest showed noisy baselines with a negative peak which had the same retention time as calcium and the anion chromatograms of the digest were totally dominated by the nitrate peak. Therefore, the release of magnesium and calcium was tested using different amounts of nitric acid for the digestion. Cation chromatograms of the digest showed the same amount of magnesium and calcium when 0.125–2 ml of nitric acid were used (Table IV). The measured concentrations were close to the levels of magnesium and calcium in the fermentation medium, showing a 100% recovery of magnesium and calcium even with the very small addition of nitric acid.

TABLE IV

RELEASE OF MAGNESIUM AND CALCIUM BY NITRIC ACID DIGESTION OF MICROORGANISMS

20-ml fermentation liquid samples containing 10 g dry weight per litre were digested with the indicated amount of nitric acid. Magnesium and calcium were measured using ion chromatography.

HNO_3 (ml)	Mg^{2+} (ppm)	Ca^{2+} (ppm)
0.125	74.8	40.7
0.25	76.0	40.8
0.5	70.4	39.3
0.5	83.9	42.1
1.0	79.9	41.6
2.0	84.8	46.2
2.0	75.4	39.2
Mean	77.9	41.4
R.S.D. (%)	6.7	5.7

Anion chromatograms of acid-digested biomass with 0.5 ml of nitric acid could be obtained after a 1:10 dilution of the digest. The dilution was necessary owing to the high content of nitrate from the nitric acid added for the digestion. Fig. 2 shows an

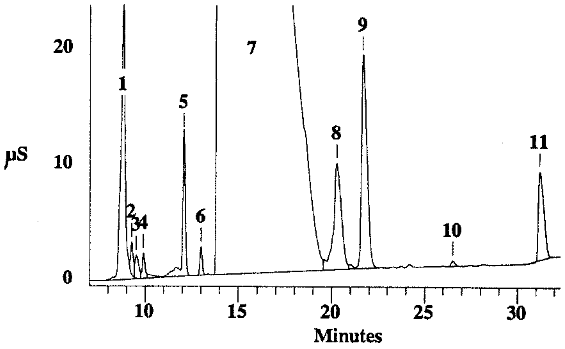

Fig. 2. Anion chromatogram of an acid-digested fermentation liquid sample containing about 10 g dry weight of biomass per litre. The anions were separated on the OmniPac PAX-500 column. Peaks: 1 = acetate; 2 = propionate; 3 = butyrate; 4 = formate; 5 = cloride; 6 = nitrite; 7 = nitrate; 8 = unknown; 9 = sulphate; 10 = unknown; 11 = phosphate.

anion chromatogram of a digested sample of biomass. The peaks were identified according to their retention times. The existence of formate, acetate and propionate was confirmed by gas chromatographic–mass spectrometric studies. The organic acids revealed that the bacterial cells were only partly digested. The digest was turbid and formed a light grey precipitate within a few hours. The turbidity of the digest was unaffected by an increase in the digestion time from 5 to 15 min at 150 p.s.i. A clear digest without organic acids was obtained when 2 ml of nitric acid were used for the digestion. However, this also raised the detection limit as it had to be followed by an extra dilution to reduce the nitrate peak and 0.5 ml of nitric acid was used for digestion in the further studies.

Cation and anion spike recovery studies were performed with a fermentation liquid sample of unknown ion composition (Table V). The recovery was 87–115%. The deviation form 100% can be explained by the R.S.D. of the determination of anions and cations combined with the dilution factor. These experiments confirmed that the use of closed Teflon vessels and microwave heating for nitric acid digestion prevents any loss of analyte due to volatilization or adsorption on the container walls, as previously shown by Patterson *et al.* [6].

Transition metals in the digest could be measured after neutralization with 2.0 *M* ammonium acetate solution. The necessary amount of ammonium acetate was found to be 10 ml for a 20-ml sample. Initially we found a high and variable background of transition metals in the digest of pure water. To reduce this background we found it necessary to apply a special cleaning procedure to the Teflon vessels before each acid digestion (see Experimental). The ion chromatographic determination of copper in acid-digested samples was compared with results obtained by potentiometric stripping analysis (PSA). The PSA method is applicable to measurements of copper in biomass and fermentation liquid without prior degradation of organic materials and has been described elsewhere [11]. The same level of copper was found by the two methods. This indicated that the acid-digestion procedure was suitable for the determination of transition metals. Fig. 3 shows a chromatogram of an acid-digested fermentation broth sample. The concentrations of copper, zinc, cobalt and manganese were 7.8, 16.5, 1.7 and 4.0 ppb, respectively. Iron was observed as Fe^{2+} and Fe^{3+} (t_R = 5 min) but was not quantified.

Determination of cations in the fermentation broth

Fermentation broth often contains metabolic byproducts and complex organic components that might interfere with the chromatographic determination of cations [5]. We found the same level of sodium, potassium, magnesium and calcium in un-

TABLE V

RECOVERY OF SODIUM, POTASSIUM, MAGNESIUM, CALCIUM, CHLORIDE, SULPHATE AND PHOSPHATE FROM SAMPLES DIGESTED WITH 0.5 ml OF NITRIC ACID

Species	Standard[a] (ppm)	Sample[b] (ppm)	Spiked sample[c] (ppm)	Recovery[d] (ppm)
Sodium	47.2, 50.2	77,6, 71.7	123, 135	54.3
Potassium	37.5, 41.1	292, 274.5	318, 346	48.9
Magnesium	50.7, 51.2	55.8, 52.7	99.8, 104	47.7
Calcium	52.6, 50.9	41.0, 40.1	88.4, 88.6	48.0
Chloride	–[e]	59.9, 63.6	113.4, 125	57,5
Sulphate	–	264, 266	307, 310	43.5
Phosphate	–	251, 251	292, 297	43.5

[a] 10 ml of 100 ppm standard solution + 10 ml of water.

[b] 10 ml of fermentation liquid sample of unknown ion concentrations + 10 ml of water.

[c] 10 ml of fermentation liquid sample + 10 ml of 100 ppm standard solution.

[d] Mean of the measured concentrations of spiked samples minus measured concentrations of samples.

[e] – = Not determined.

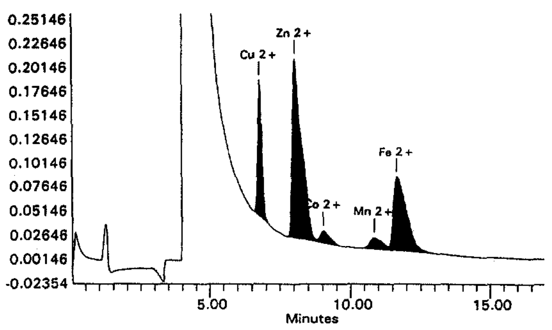

Fig. 3. Chromatogram of an acid-digested fermentation broth sample.

treated as in acid-digested samples of fermentation broth. Hence, it is not necessary to digest fermentation broth before the determination of these cations. In contrast, the level of transition metals measured was clearly influenced by the method of sample treatment prior to chromatographic analysis.

Transition metals are known to form stable complexes with several inorganic and organic compounds. We tested the influence of EDTA, oxalate, cyanide, formate and citrate on the chelation ion chromatographic detection of iron, copper, nickel, zinc, cobalt and manganese. Table VI shows the measured concentrations of the different transition metals when the sample contained one of the complex-forming components. EDTA showed a very strong interference with the chelation ion chromatographic technique. The negative values for iron, copper, zinc and manganese are caused by the binding of background contaminants by EDTA. Oxalate, cyanide and citrate showed a moderate interference with the chelation ion chromatographic technique. Oxalate and cyanide reduced the iron and nickel peaks, but increased the zinc peak. The increased zinc peak is probably due to zinc contamination of the oxalate and cyanide solutions. Citrate showed a different pattern, with a strong reduction of the copper peak and a weaker reduction in the amounts of iron, zinc, cobalt and manganese detected. The results clearly demonstrated that many complex-forming compounds interfere with the chelation ion chromatographic technique and emphasize the need for removal of these interferences.

Fermentation experiments

Fig. 4 shows chromatograms of two fermentation broth samples, one taken before and the other taken 4 h after a switch of substrate from methane (99.95% methane) to natural gas (about 91% methane and 4.7% ethane). Fig. 5 gives the concentrations of formate, acetate and citrate during the experiment. The acetate concentration was very low before the switch from methane to natural gas (*ca.* 2 ppm) but showed a steady increase after the switch. This indicated that the bacteria could oxidize ethane to acetate and that they were unable to utilize acetate. The level of citrate was not affected by the substrate switch. The oscillating behaviour of formate was unexpected and needs further investigation.

Acetate has been found to inhibit the growth of methanotrophic bacteria [12]. To optimize the growth on natural gas it is necessary to prevent ace-

TABLE VI

INFLUENCE OF DIFFERENT COMPONENTS ON THE ION CHROMATOGRAPHIC DETECTION OF TRANSITION METALS

The samples contained 12.5 ppb of each transition metal and 5 m*M* of one of the complex-forming components. Values are in ppb.

Complex-forming component	Fe^{3+}	Cu^{2+}	Ni^{2+}	Zn^{2+}	Co^{2+}	Mn^{2+}
Phosphate	12.5	12.5	12.5	12.5	12.5	12.5
EDTA	−0.9	−0.7	0.0	−3.1	0.0	−0.4
Oxalate	5.0	14.3	8.6	27.7	11.7	13.1
Cyanide	6.8	11.6	6.9	40.0	12.0	12.5
Formate	12.2	12.2	12.3	18.9	12.0	11.6
Citrate	8.6	0.8	11.6	4.9	7.7	9.3

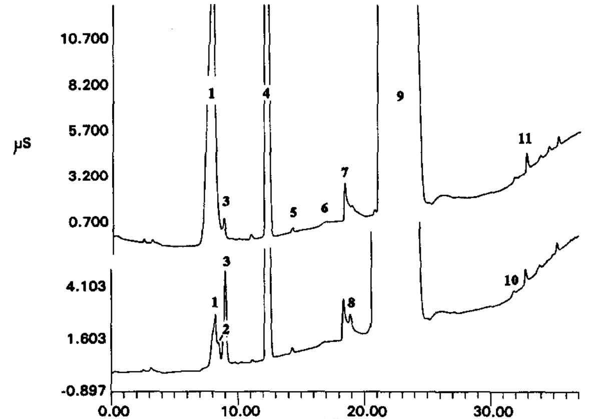

Fig. 4. Anion profile for fermentation of methane and natural gas. The lower curve is a chromatogram of a fermentation broth sample taken from a steady-state methane fermentation. The upper curve is from the same fermentation but 4 h after a shift from methane to natural gas. The OmniPac PAX-500 column and gradient elution with 10% methanol were used for the separation of anions. Peaks: 1 = acetate; 2 = propionate; 3 = formate; 4 = chloride; 5 = nitrite; 6 = carbonate; 7 = unknown; 8 = nitrate; 9 = sulphate; 10 = phosphate; 11 = citrate.

tate accumulation. One possibility is to use a culture consisting of acetate-utilizing bacteria in addition to the methanotrophic bacteria. This was tested by adding *Comamonas acidovorans*, an acetate-utilizing bacterial strain, to the fermenter after 22 hours. This reduced the acetate concentration to about 6 ppm within 12 h (results not shown), confirming the concept.

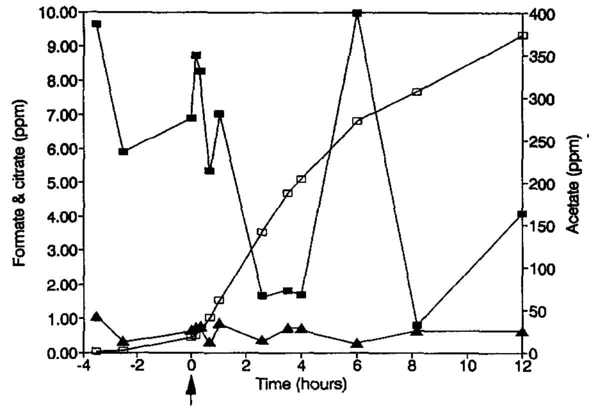

Fig. 5. (■) Formate, (□) acetate and (▲) citrate concentrations in the fermentation broth of a culture of methanotrophic bacteria. The arrow at time zero indicates a change of substrate from methane to natural gas.

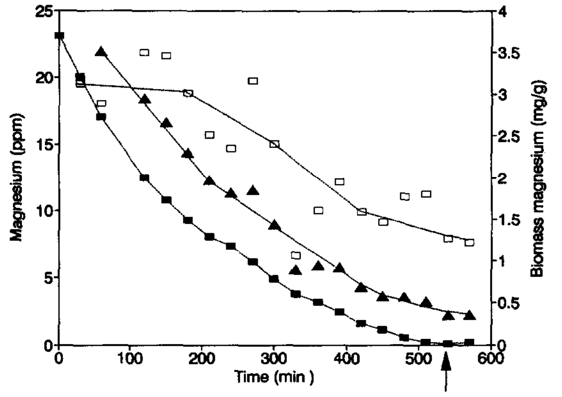

Fig. 6. Magnesium concentration in fermentation broth and fermentation liquid samples with 2 g dry weight of biomass per litre during a washout experiment. At time zero the medium flow was changed to a medium flow without added magnesium. The steady-state growth rate was 0.2 h^{-1}. The arrow indicates a sudden stop in growth rate. ■ = Magnesium in fermentation broth; ▲ = magnesium in acid-digested fermentation samples; □ = calculated magnesium content of the microorganisms (difference between fermentation sample and fermentation broth divided by the dry weight).

Fig. 6 shows a magnesium washout experiment. At time zero the feed was changed from a medium with magnesium to a medium without magnesium. The magnesium concentration in the fermenter was followed by ion chromatographic measurements of fermentation broth samples and of acid-digested fermentation liquid samples. The growth rate was constant and about 0.2 h^{-1} during the first 9 h. The arrow indicates a sudden stop in the growth of the bacteria due to magnesium limitation. The limiting concentration of magnesium was 0.06 ppm. The magnesium content of the biomass was reduced from about 3 to about 1.2 mg/g biomass during the washout. This showed a relationship between fermentation broth magnesium concentration and biomass magnesium content.

This experiment showed that the concentration of magnesium in the fermentation broth has to be above 0.06 ppm to maintain optimum growth conditions and that the magnesium content of the final product to some extent can be controlled by the magnesium content of the fermentation broth. Low-magnesium SCP can be produced by keeping the magnesium content of the fermentation broth close to 0.06 ppm whereas high-magnesium SCP can be produced by keeping the magnesium concentration of the fermentation broth above 10 ppm.

CONCLUSIONS

Anions, alkali metals and alkaline earth metals in the fermentation broth could be determined by ion chromatography after simple removal of the bacterial cells. Determination of transition metals required that complexing compounds were removed. Acid digestion in a microwave oven was a rapid method for removal of complexing compounds and was also useful when preparing samples containing biomass for the chromatographic determination of inorganic anions, cations and transition metals. A total mineral determination of a sample could be obtained in less than 100 min with the combination of a microwave acid digestion and ion chromatography. The implementation of these methods in a fermentation process with methanotrophic bacteria showed that acetate accumulated when natural gas was used as a substrate and that magnesium became the growth-limiting substrate when the magnesium concentration of the fermentation broth was below 60 ppb. These examples show ion chromatography to be a useful tool for the determination of minerals and organic acids in fermentation broth and for the determination of minerals in fermentation liquid and in biomass.

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