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Summary

Low and intermediate radioactive cellulosic wastes are disposed in cement and steel containers with an initially very high pH, producing cellulosic degradation components such as isosaccharinic acid (ISA) through hydrolysis. It is well known that ISA is a strong complexing ligand and is able to produce complexes with radionuclides thereby increasing the risk for radionuclide transport from the repository. ISA may be degraded by microorganisms, a process that eventually will produce CH_4. The production of CH_4 will reduce the risk for radionuclide migration, but increase the risk for barrier damage due to high gas pressure from the produced CH_4.

To study if microorganisms can degrade ISA and produce CH_4, anaerobic microbial consortia were enriched from low and intermediate radioactive waste from the VLJ experiment in Olkiluoto, Finland and cultured for 89 days after which CH_4 was analyzed. Three cultures with the highest amount of CH_4 were modified by adding ISA as the main carbon source and sub-cultured for 66 days. DOC, CH_4 and H_2 were analyzed at the end of the cultivation period.

There was a consumption of DOC in cultures with added ISA and in the culture with glucose compared to controls, indicating that microorganisms degraded the organic carbon in those cultures, but only a small variation in the concentration of produced CH_4 between the cultures was observed. The results suggest that the growth periods of three months for enrichment, and two months for cultivation with ISA were a too short time to allow the consortia to adapt and degrade the added ISA with concomitant production of CH_4.

Contents

1 Introduction

1.1 Radioactive wastes and isosaccharinic acid

Barrels filled with low and intermediate level radioactive wastes, consisting of biodegradable material with a high proportion of cellulose were deposited in the VLJ repository in Olkiluoto and placed in a tank of cement filled with water. Isosaccharinic acid (ISA) is a product produced from alkaline degradation of cellulose. The alkaline degradation of cellulose generates two isomers, α-ISA and β-ISA. ISA may establish complex with metals and radionuclides because the acid has strong complexing ligands (Johnsson 2006). Methanogenic consortia are assumed to produce methane during degradation of ISA (Glaus et al. 1999, Small et al. 2008). The production of methane from ISA will reduce the risk for radionuclide migration, but increase the risk for barrier damage due to high gas pressure from the produced methane.

1.2 CH_4 production

Organic materials, such as protein, fat and carbohydrates are decomposed by several substantial degradation pathways, eventually producing CH_4. The degradation process, shown in Figure 1-1, consists of four digestion steps, i.e. hydrolysis, fermentation, anaerobic oxidation, and methanogenesis. In the hydrolysis step, microorganisms decompose polymeric substances such as carbohydrates, fat and proteins into smaller molecules, such as sugar, fatty acids, amino acids, and alcohols. These smaller organic molecules are used as substrate in the second step, fermentation, in which the organic molecules are further degraded to various organic acids, such as acetic, propionic, and lactic acid, as well as to CO_2 (carbon dioxide), H_2 (hydrogen), and ammonia; depending on the diversity of microorganisms that take part in the degradation process. The fatty acids produced during hydrolysis are degraded in the third digestion step, anaerobic oxidation, in which fatty acids and organic acids are degraded to CO_2, H_2, and acetate. Methanogenesis, the last step of the anaerobic degradation process, produces CH_4 from H_2 and CO_2 or from acetate (Jarvis and Schnürer 2009). Methane will be the final product in the absence of alternative electron donors such as sulphate, ferric iron or nitrate.

Depending on the diversity of microorganisms that are active in the degradation process, CH_4 is produced via various degradation pathways; as shown in Figure 1-1 and Figure 1-2. The hydrolysis of organic material to smaller organic molecules is often dominated by heterotrophic anaerobes belonging to the genera *Clostridium*, and *Bacteroides*. In the fermentation step microorganisms belonging to the genera *Bacteroides*, *Acetobacterium*, and *Eubacterium*, produce H_2, ammonia, and organic acids. Acetogens, such as *Clostridium aceticum* produces acetic acid while *Acetoanaerobium* produces acetate from H_2 and CO_2. In anaerobic oxidation H_2, CO_2, and acetate are produced from fatty acids degradation by syntrophs; the second fermenting bacteria, such as *Syntrophomonas wolfei*, and *Thermosyntropha lipolytica*. Two kinds of methanogens, hydrogenotrophic and acetotrophic, are involved in methanogenesis, the final degradation step. Hydrogenotrophic methanogens, such as *Methanobacterium* and *Methanococcus* consumes H_2 producing CH_4, in which the acetotrophic methanogens, for example *Methanosaeta* and *Methanosarcina*, produce CH_4 by cleaving acetate (Drake 1994, Jarvis and Schnürer 2009, Liu and Whitman 2008, McInerney et al. 2008, Schink 1997).

This study aimed to investigate if microorganisms produce CH_4 degrading organic wastes withholding α-ISA from the VLJ repository. Consortia needed to be established, producing CH_4, before α-ISA could be added to cultures. This acid is not commercially available. α-ISA was kindly made available by from Mark Foreman at Chalmers University of Technology.

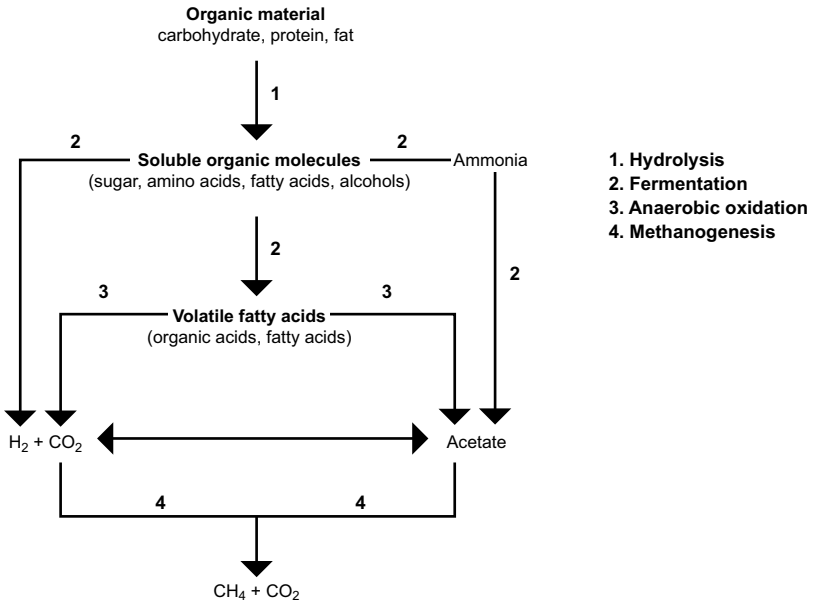

Figure 1-1. *The four degradations steps in CH_4 production, involving hydrolysis, fermentation, anaerobic oxidation, and methanogenesis. Image adapted from Jarvis and Schnürer (2009).*

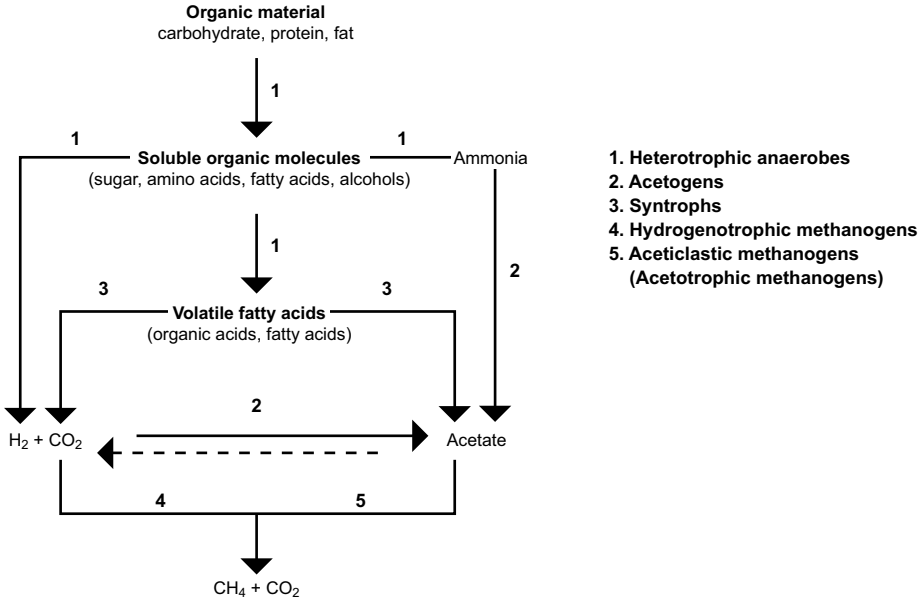

Figure 1- 2. *Microorganisms involved in CH_4 production process, involving heterotrophic anaerobes, acetogens, syntrophs, hydrogenotrophic-, and acetotrophic methanogens. Image from Jarvis and Schnürer (2009).*

2 Material and methods

2.1 Cultivation medium

2.1.1 Basal culture medium for methanogenic consortia

Basal medium was made up of (g L^{-1}): NaCl, 7.0; $CaCl_2 \times 2H_2O$, 1.0; KCl, 0.67; NH_4Cl, 1.0; KH_2PO_4, 0.15; and $MgCl_2 \times 6H_2O$, 0.5. After sterilization, the medium was made anaerobic by purging with a mix of nitrogen (80%) and carbon dioxide (20%). Anaerobic solutions consisting of essential trace elements, vitamins, and organic acids were added to the basal medium. The total concentration of DOC in this medium was 15 mM. Approximately 50 mL medium was added to 120 mL sterile anaerobic butyl rubber-stopped bottles using syringes (Hallbeck and Pedersen 2008).

2.1.2 Media combinations

The basal culture medium was modified by adding organic carbon sources resulting in six media combinations. The α – ISA, collected from Mark Foreman at Chalmers University, was dissolved in water and a drop of concentrated hydrogen chloride was added before the ISA was made anoxic. Medium 1 contains of (mM) sodium acetate, 20; formate, 30; and methanol, 37. Medium 2 contained (mM) valeric acid, 10; propionic acid, 10; butyric acid, 10; and approximately 15 mg tissue paper. Medium 3 contained (mM) glucose, 20; and 32.5 mg filter paper. Medium 4 contained of (mM) valeric acid, 1; propionic acid; 1; byturic acid, 1; and microcrystalline cellulose, 10. Medium 5 and 6 contained (mM) ISA, 3 and 6 respectively.

2.2 Sampling of VLJ

Materials were collected from five positions in the VLJ repository with various degrees of biodegradable materials and inoculated into media combinations number 1–4, as shown in Table 2-1 and Table 2-2. The pH of the media was adjusted to 7.5–8.0. The cultures were incubated at room temperature (18–20°C) for 89 days until gas analysis.

Table 2-1. The five sample positions collected on the VLJ repository.

Sample position	Position	Position number	Sampling explanation
104	Positions inside a drum containing 4.9% biodegradable material	2 of 3	Sampling of liquid inside a drum
110	Positions inside a drum containing 38.7% biodegradable material	2 of 3	Sampling of liquid inside a drum
115	Positions inside a drum containing 94.7% biodegradable material	1 of 3	Sampling of liquid inside a drum
116	Positions inside a drum containing 94.7% biodegradable material	2 of 3	Sampling of liquid inside a drum
121	Tubes between the drum	Lowest (of middle and top)	Sampling of liquid between drums

Table 2-2. Consortia inoculated from the five sample positions to four media.

Sample position	Medium number	Sample name
104	1	Sodium acetate (20 mM), formate (30 mM) and methanol (37 mM)
104	2	Organic acids (30 mM) and 15 mg tissue paper
104	3	Glucose (20 mM) and 32.5 mg filter paper
104	4	Organic acids (3 mM) and cellulose (10 mM)
110	1	Sodium acetate (20 mM), formate (30 mM) and methanol (37 mM)
110	2	Organic acids (30 mM) and 15 mg tissue paper
110	3	Glucose (20 mM) and 32.5 mg filter paper
110	4	Organic acids (3 mM) and cellulose (10 mM)
115	1	Sodium acetate (20 mM), formate (30 mM) and methanol (37 mM)
115	2	Organic acids (30 mM) and 15 mg tissue paper
115	3	Glucose (20 mM) and 32.5 mg filter paper
115	4	Organic acids (3 mM) and cellulose (10 mM)
116	1	Sodium acetate (20 mM), formate (30 mM) and methanol (37 mM)
116	2	Organic acids (30 mM) and 15 mg tissue paper
116	3	Glucose (20 mM) and 32.5 mg filter paper
116	4	Organic acids (3 mM) and cellulose (10 mM)
121	1	Sodium acetate (20 mM), formate (30 mM) and methanol (37 mM)
121	2	Organic acids (30 mM) and 15 mg tissue paper
121	3	Glucose (20 mM) and 32.5 mg filter paper
121	4	Organic acids (3 mM) and cellulose (10 mM)

Figure 2-1. *The inoculation process in VLJ repository to anaerobic butyl rubber-stopped bottles using syringes.*

Figure 2-2. *Sample position number 115 consisting of 94.7% biodegradable material inoculated to media number 1–4.*

2.3 Cultivation with ISA

Three consortia cultures with the highest amount of produced CH_4 were sub-cultured and modified. The cultures were:

- Culture originating from position number 116 and inoculated in medium number 2 (consortium X).
- Culture originating from position number 121 and inoculated in medium number 3 (consortium Y).
- Culture originating from position number 121 and inoculated in medium number 4 (consortium Z).

α-ISA was added, as shown in Table 2-3, to final concentrations of 3 mM and 6 mM with inoculums from above cultures as the main carbon and energy source. A mix of nitrogen (80%) and carbon dioxide (20%) was added to the cultures to a total pressure of 3 bars. Cultures were adjusted to pH 7.7–8.0 and incubated in room temperature for 66 days when analysis commenced.

2.4 Gas analysis

Methane (CH_4) in concortia was analysed on a Varian Star 3400CX gas chromatograph using a Flame Ionization Detector (FID) with an oven temperature of 65°C and a detector temperature of 200°C. The gas was separated using a Porapak-Q column (2 m × 1/8 inch diameter) and analysed on the FID with N_2 as the carrier gas. The gas samples were diluted with nitrogen to reach needed analysis range, i.e < 20 ppm; this method was used to detect which cultures had produced CH_4.

Table 2-3. Cultures sub-cultured from consortium X, Y and Z. In specific cultures ISA were added to final concentrations of 3 or 6 mM, in medium number 5 and 6 respectively.

Consortium	Culture number	Medium number	Medium composition
X	Culture 1	2	Organic acids (30 mM) and 15 mg tissue paper
X	Culture 2	2	Organic acids (30 mM) and 15 mg tissue paper
X	Control	2	Organic acids (30 mM) and 15 mg tissue paper
X	Culture1	5	α – ISA (3mM)
X	Culture 2	5	α – ISA (3mM)
X	Control	5	α – ISA (3mM)
X	Culture 1	6	α – ISA (6mM)
X	Culture 2	6	α – ISA (6mM)
X	Control	6	α – ISA (6mM)
Y	Culture 1	3	Glucose (20 mM) and 32.5 mg filter paper
Y	Culture 2	3	Glucose (20 mM) and 32.5 mg filter paper
Y	Control	3	Glucose (20 mM) and 32.5 mg filter paper
Y	Culture1	5	α – ISA (3mM)
Y	Culture 2	5	α – ISA (3mM)
Y	Control	5	α – ISA (3mM)
Y	Culture 1	6	α – ISA (6mM)
Y	Culture 2	6	α – ISA (6mM)
Y	Control	6	α – ISA (6mM)
Z	Culture 1	4	Organic acids (3 mM) and cellulose (10 mM)
Z	Culture 2	4	Organic acids (3 mM) and cellulose (10 mM)
Z	Control	4	Organic acids (3 mM) and cellulose (10 mM)
Z	Culture1	5	α – ISA (3mM)
Z	Culture 2	5	α – ISA (3mM)
Z	Control	5	α – ISA (3mM)
Z	Culture 1	6	α – ISA (6mM)
Z	Culture 2	6	α – ISA (6mM)
Z	Control	6	α – ISA (6mM)

The concentration of CH_4 in ISA cultures were analyzed using Varian Star 3400CX as above and H_2 was analysed on a Bruker 450 gas chromatograph equipped with a CP7355 PoraBOND Q (50 m × 0.53 mm, ID) and a CP7536 MOLSIEVE 5A PLOT (25 m × 0.32 mm, ID) and a Pulsed Discharge Helium Ionization Detector (PDHID) (Bruker Daltonics Scandinavia AB, Vallgatan 5, SE-17067 Solna, Sweden). The analyzed amounts of CH_4 and H_2 in moles per gas phase are shown in Table A-1.

Both chromatographs were calibrated using certified gas mixes that mimic the gas composition of the analysed samples

2.5 Dissolved organic carbon analysis

Dissolved organic carbon (DOC) was analyzed by ALS Scandinavia, Täby, Sweden using their accredited method for total organic carbon. Because the samples were filtered (0.2 μm) the results show DOC. Samples were diluted before shipment to reach the analytical range of the method.

3 Results

3.1 Consortia

The investigation of the potential for microbial degradation of ISA in low and intermediate level radioactive wastes required establishment of methanogenic consortia. Cultures were inoculated from five sample positions to four media, and CH_4 was analyzed after 89 days and the results are shown in Table 3-1.

The inoculated cultures from sample position number 115 produced a CH_4 concentration that were over range for the GC FID detector, as did the culture with medium number 2 that originated from position number 104, and the culture with medium number 1 inoculated from position 116. Active methanogens fluoresce green-blue due to the coenzyme F_{420}. Cultures were, therefore, studied under UV-light in a fluorescence microscope to verify what cultures should be sub-cultured. Three cultures that produced high concentrations of CH_4 and that showed strong fluorescence under UV-light were further sub-cultured.

3.2 Cultivation with ISA

Three cultures with high concentrations of CH_4 and a large presence of methanogens as indicated by UV-light detection were inoculated. α-ISA was added to some cultures as the main carbon and energy source, to final concentrations of 3 or 6 mM. The cultures were incubated in room temperature until analysis at day 66. DOC was analyzed by ALS Scandinavia to investigate if added organic carbon been consumed. Subtracting the DOC level of sterile controls from cultures mean values indicated if organic carbon was consumed by the microbiological consortia (Table 3-2).

Table 3-1. Consortia CH_4 concentrations, measured at day 89. O.R = over range for the detector.

Sample position	Medium number	Culture name	CH_4 (µL/L gas phase)
104	1	Sodium acetate (20 mM), formate (30 mM) and methanol (37 mM)	639
104	2	Organic acids (30 mM) and 15 mg tissue paper	O.R.
104	3	Glucose (20 mM) and 32.5 mg filter paper	315
104	4	Organic acids (3 mM) and cellulose (10 mM)	2,072
110	1	Sodium acetate (20 mM), formate (30 mM) and methanol (37 mM)	94
110	2	Organic acids (30 mM) and 15 mg tissue paper	95
110	3	Glucose (20 mM) and 32.5 mg filter paper	401
110	4	Organic acids (3 mM) and cellulose (10 mM)	228
115	1	Sodium acetate (20 mM), formate (30 mM) and methanol (37 mM)	O.R.
115	2	Organic acids (30 mM) and 15 mg tissue paper	O.R.
115	3	Glucose (20 mM) and 32.5 mg filter paper	O.R.
115	4	Organic acids (3 mM) and cellulose (10 mM)	O.R.
116	1	Sodium acetate (20 mM), formate (30 mM) and methanol (37 mM)	O.R.
116	2	Organic acids (30 mM) and 15 mg tissue paper	3,995
116	3	Glucose (20 mM) and 32.5 mg filter paper	2,286
116	4	Organic acids (3 mM) and cellulose (10 mM)	1,050
121	1	Sodium acetate (20 mM), formate (30 mM) and methanol (37 mM)	7,454
121	2	Organic acids (30 mM) and 15 mg tissue paper	1,053
121	3	Glucose (20 mM) and 32.5 mg filter paper	4,156
121	4	Organic acids (3 mM) and cellulose (10 mM)	1,439

Table 3-2. Culture CH_4, H_2, and DOC concentrations.

Consortium	Culture name	Culture number	Medium number	Medium composition	CH_4 (µM)	H_2 (µM)	DOC (mM)
X	A1	Culture 1	2	Organic acids (30 mM) and 15 mg tissue paper	230	0.6	120
X	A1	Culture 2	2	Organic acids (30 mM) and 15 mg tissue paper	341	0.8	119
X	A2	Control	2	Organic acids (30 mM) and 15 mg tissue paper	1.0	7.5	113
X	B1	Culture 1	5	α – ISA (3mM)	224	0.6	26.9
X	B1	Culture 2	5	α – ISA (3mM)	322	0.8	37.0
X	B2	Control	5	α – ISA (3mM)	1.4	2.3	32.0
X	C1	Culture 1	6	α – ISA (6mM)	230	0.8	56.0
X	C1	Culture 2	6	α – ISA (6mM)	294	0.5	52.0
X	C2	Control	6	α – ISA (6mM)	0.8	2.1	56.9
Y	D1	Culture 1	3	Glucose (20 mM) and 32.5 mg filter paper	249	3,979	88.3
Y	D1	Culture 2	3	Glucose (20 mM) and 32.5 mg filter paper	126	5,681	110
Y	D2	Control	3	Glucose (20 mM) and 32.5 mg filter paper	0.8	5.2	183
Y	E1	Culture 1	5	α – ISA (3mM)	112	0.6	44.7
Y	E1	Culture 2	5	α – ISA (3mM)	166	0.6	37.6
Y	E2	Control	5	α – ISA (3mM)	1.0	4.0	43.0
Y	F1	Culture 1	6	α – ISA (6mM)	161	0.6	58.7
Y	F1	Culture 2	6	α – ISA (6mM)	143	0.5	63.3
Y	F2	Control	6	α – ISA (6mM)	0.4	4.1	69.3
Z	G1	Culture 1	4	Organic acids (3 mM) and cellulose (10 mM)	299	2,004	55.7
Z	G1	Culture 2	4	Organic acids (3 mM) and cellulose (10 mM)	214	2,400	61.4
Z	G2	Control	4	Organic acids (3 mM) and cellulose (10 mM)	1.4	4.0	34.0
Z	H1	Culture 1	5	α – ISA (3mM)	236	0.8	37.8
Z	H1	Culture 2	5	α – ISA (3mM)	212	0.7	37.3
Z	H2	Control	5	α – ISA (3mM)	1.5	2.4	38.3
Z	I1	Culture 1	6	α – ISA (6mM)	246	0.5	53.2
Z	I1	Culture 2	6	α – ISA (6mM)	51	0.6	51.4
Z	I2	Control	6	α – ISA (6mM)	1.4	3.9	60.0

A somewhat lower CH_4 concentration, 51 µM, was observed in I1 of culture 1 with α-ISA (6 mM) from consortia Z, than what was observed for the other cultures, added with ISA originating from consortia Z, in which CH_4 concentration were between 212 to 246 µM.

The H_2 concentration was high in G1, cultures 1 and 2, just like culture 1 and 2 of D1, in which H_2 concentrations were 2004, 2400, 3979, and 5681 µM respectively. This was much higher than what was observed in the other cultures in which the H_2 concentration was between 0.5 to 7.5 µM.

An increase by 6.25 mM in DOC was observed in the A1 cultures DOC compared to the control; while samples with added α-ISA, 3 and 6 mM, from consortia X, had consumed 0.04 and 2.92 mM DOC, respectively (Figure 3-1). The DOC concentration in the D1 cultures, decreased by 84.17 mM, while cultures with 3 and 6 mM ISA, from consortia Y, decreased 1.88 and 8.33 mM respectively.

Decreases in DOC concentration of 0.83 and 7.71 mM were found in the H1 and I1 cultures, containing 3 and 6 mM α-ISA from consortia Z; while the DOC in G1 cultures increased by 24.54 mM (Table 3-2).

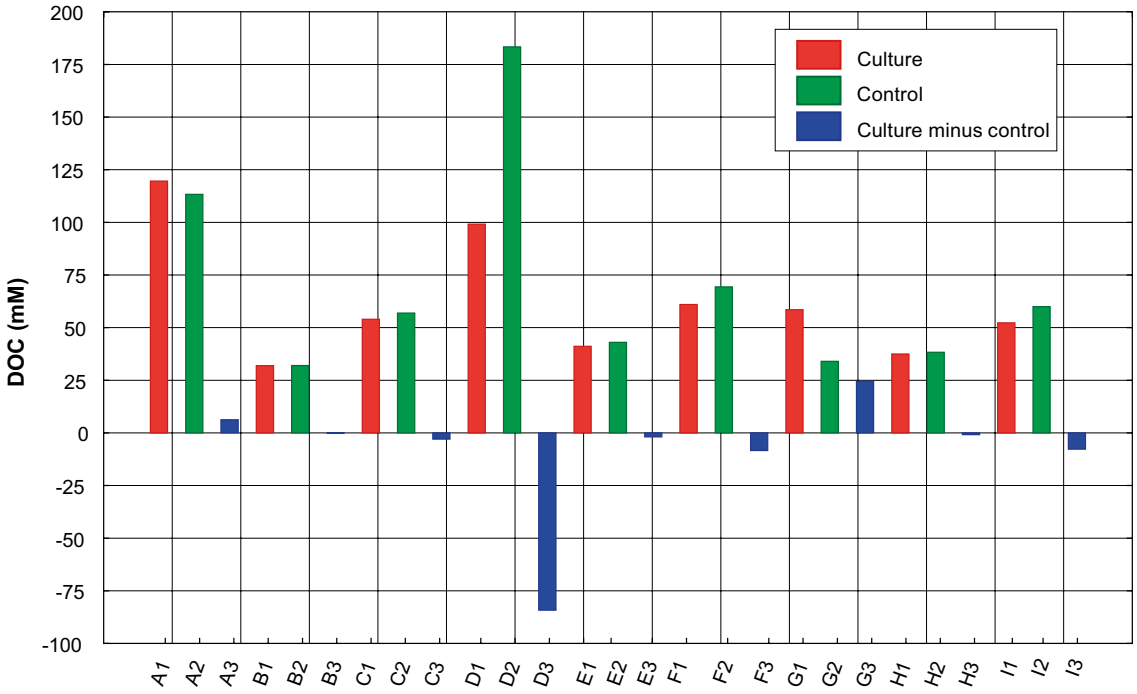

Figure 3-1. *Culture DOC values: A-C, consortia X on medium number 2, 5 and 6; D-F, consortia Y on medium number 3, 5 and 6; G-I, consortia Z on medium 4, 5 and 6.*

4 Discussion

The purpose of the work presented in this report was to study if microorganisms enriched from the VLJ experiment in Olkiluoto, Finland can degrade ISA and produce CH_4. Because the pH of the VLJ experiment had rapidly decreased from above 11 to approximately 8 when the samples were collected (Small et al. 2008), it was not possible to enrich for high pH consortia. Such enrichment has, however, been found successful elsewhere (Charles C J, Rout S P, Humphreys P N, 2014. *The generation and degradation of isosaccharinic acid within a hyperalkaline environment*. Poster presented at IGD-TP Geodisposal conference, Manchester, 24–26 June 2014). That study used cellulose cotton samples from boreholes as inoculums to cultures with ISA at pH 11. The established consortia produced CH_4, CO_2, and H_2S as end products. A second study using the organic waste at neutral pH showed that ISA was fermented to smaller chain acids such as acetic acid, which was further degraded by acetotrophic and hydrogenotrophic methanogens to CH_4 (Rout S P, Charles C J, Doulgeris C, McCarthy A J, Rooks D J, Loughnane P, Laws A P, Humphreys P N, 2014. *Effect of pH on the microbial degradation of anaerobic alkaline cellulose degradation products relevant to geological disposal*. Poster presented at IGD-TP Geodisposal conference, Manchester, 24–26 June 2014). After the initial study at neutral pH, the scientists increased pH while significant amounts of ISA remained in the samples. The CH_4 production rate decreased by the increased pH, and methanogens could be found up to pH 10.

The DOC concentration in culture with added α-ISA decreased, indicating that microorganisms consumed DOC in these cultures. However, the decrease was at most 8 mM which is much less that the total available DOC in the cultures (Table 3-2). It is, therefore, at the present stage not possible to conclude if the decrease corresponds to degradation of ISA or the organic compounds of the medium, or both. Such conclusions need analysis of ISA and that specific analytical protocol was not available in the laboratory during the experiments due to budget restrictions. A large proportion of the DOC was consumed in the culture with glucose (20 mM) and 32.5 mg filter paper which is expected because glucose is a very easily degradable sugar. The organic acid (30 mM) and 15 mg tissue paper and organic acid (3 mM) and cellulose (10mM) cultures demonstrated increases in the DOC concentration. This was likely due to microbial release of DOC from the added tissue paper and crystalline cellulose during degradation.

Relatively low CH_4 concentrations from the ISA cultures (Table 3-2) compared to the enrichment cultures (Table 3-1), together with DOC consumption for several cultures suggest that the methanogens had not got enough time to adapt and grow in the ISA cultures. Syntrophy i.e. cooperation between microorganisms, is essential in the digestion process degrading the organic material, a fact that was recently discussed in detail during the International Conference on Biogas Microbiology 2 (ICBM2), 2014; Biogas, Uppsala, Sweden. Microorganisms and particular methanogenic consortia need time to adapt to new environments before they can be active and produce CH_4. The degradation pathway towards methane is extremely sensitive to disturbances and slight changes of the environmental conditions may interrupt the degradation. For instance, the composition of the organic material influences the CH_4 producing process significantly. Excess of fat may generate accumulation of fatty acids creating long-chain fatty acids, which lower pH below optimum for methanogenesis. Proteins are degraded to amino acids and further to ammonia which inhibits methanogenic activity. A large amount of carbohydrates, especially glucose, destabilizes the degradation process, causing the process to proceed slowly (Carlsson and Uldal 2009, Jarvis and Schnürer 2009).

The H_2 level was significantly higher in cultures with glucose and filter paper, and organic acids (3 mM) and cellulose. This may cause a thermodynamic problem for synthropic methanogenesis because the H_2 level needs to be kept low for the hydrolysis and degradation steps to proceed. This is optimally controlled by the methanogens that consumes H_2, producing CH_4 during autotrophic methanogenesis. If the H_2 concentration increases, it will result in the production of fatty acids that lower pH and inhibit methanogenesis. The H_2 threshold level varies from time to time and is dependent on what kinds of microorganisms that are involved in each specific system (Jarvis and Schnürer 2009).

4.1 Conclusions

- Cultures with α-ISA and glucose and filter paper showed degradation of DOC but it was not possible to distinguish what types of organic compounds were degraded. Analytical tools specific for ISA must be set up in the laboratory.
- Low CH_4 production in combination with DOC consumption indicated that the microorganisms and especially the methanogens had not been fully adapted to the environment in the culture bottles. Future experiments must be kept running for longer times than the 2–3 months applied here.

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Appendix

The CH_4 and H_2 concentrations in moles per gas phase are shown in Table A-1.

Table A-1. Consortia CH_4 and H_2 concentrations calculated in moles per gas phase.

Consortia	Medium number	Medium number	Culture number	Medium composition	CH_4 (µmole in gas phase)	H_2 (µmole in gas phase)
X	2	2	Culture 1	Organic acids (10 mM) and tissue paper	9.4	0.03
X	2	2	Culture 2	Organic acids (10 mM) and tissue paper	14.0	0.03
X	2	2	Control	Organic acids (10 mM) and tissue paper	0.04	0.33
X	5	2	Culture 1	α – ISA (3mM)	10.3	0.03
X	5	2	Culture 2	α – ISA (3mM)	14.8	0.04
X	5	2	Control	α – ISA (3mM)	0.07	0.11
X	6	2	Culture 1	α – ISA (6mM)	10.4	0.04
X	6	2	Culture 2	α – ISA (6mM)	13.3	0.02
X	6	2	Control	α – ISA (6mM)	0.04	0.10
Y	3	3	Culture 1	Glucose (20 mM) and filter paper	10.7	171
Y	3	3	Culture 2	Glucose (20 mM) and filter paper	5.4	244
Y	3	3	Control	Glucose (20 mM) and filter paper	0.03	0.24
Y	5	3	Culture 1	α – ISA (3mM)	5.2	0.03
Y	5	3	Culture 2	α – ISA (3mM)	7.6	0.03
Y	5	3	Control	α – ISA (3mM)	0.05	0.20
Y	6	3	Culture 1	α – ISA (6mM)	7.3	0.03
Y	6	3	Culture 2	α – ISA (6mM)	6.5	0.02
Y	6	3	Control	α – ISA (6mM)	0.02	0.20
Z	4	1	Culture 1	Organic acids (1 mM) and cellulose (10 mM)	13.3	87.2
Z	4	1	Culture 2	Organic acids (1 mM) and cellulose (10 mM)	9.3	104
Z	4	1	Control	Organic acids (1 mM) and cellulose (10 mM)	0.07	0.18
Z	5	1	Culture 1	α – ISA (3mM)	10.9	0.03
Z	5	1	Culture 2	α – ISA (3mM)	9.8	0.03
Z	5	1	Control	α – ISA (3mM)	0.07	0.12
Z	6	1	Culture 1	α – ISA (6mM)	11.1	0.02
Z	6	1	Sample 2	α – ISA (6mM)	2.3	0.03
Z	6	1	Control	α – ISA (6mM)	0.07	0.19