



Sludge preparation for agricultural use: results from the ROUTES project

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Sludge separation

Large WWTPs > 100,000 inhabitants

With primary sedimentation - With/Without nutrient removal - Low/High organic load - Medium pollution level

Problem: High sludge production at medium pollution level

Solution: Separation of primary and secondary sludge treatments.



TT process: Thermal pretreatment + Thermophilic digestion



Test #1: OLR=1.0 kgVS m⁻³d⁻¹ Test #2: OLR=1.7 kgVS m⁻³d⁻¹ Test #3: OLR=3.7 kgVS m⁻³d⁻¹

Parallel tests were carried out simultaneously, feeding untreated and pretreated sludge, at different loading rates.

UMT process: Ultrasonic pretreatment + Two-stage digestion (1st mesophilic and 2nd thermophilic)



Test #1: OLR=1.7 kgVS m⁻³d⁻¹ Test #2: OLR=3.1 kgVS m⁻³d⁻¹ Parallel tests were carried out simultaneously, feeding untreated and pretreated sludge, at different loading rates. 5 of 43

Enhanced Stabilization Processes

Effect of pretreatments:

Untreated WAS floc



Sonication N = 20 kHz; t = 2 min DD_{COD} = 3–4 %





Thermal pretreatment

T = 134°C; P = 3.2 bar; t = 20 min DD_{COD} = 13%

Specific energy ~ 0.5 kWh/kg TS



Small aggregates, dispersed cells

Specific energy ~ 5 kWh/kg TS

Floc destructuration, no small aggregates 6 of 43

Results: VS removal and biogas production at low and high loading rate

Low loading rate



Supernatant characteristics and filterability



OLR=1.0 – 1.7 kgVS/m3d CST (sec L /gTS) OLR=3.1 – 3.7 kgVS/m3d 40 35 30 25 20 15 10 5 0 Conventional Untreated Pretreated Untreated Pretreated MAD UMT UMT TT TT



Enhanced processes caused an increase in soluble COD and ammonia in anaerobic supernatants, with respect to conventional MAD.

Enhanced processes caused also worse dewaterability of digested sludge.

Sequential anaerobic-aerobic digestion



Basic motivation: to improve stabilization performance with different reaction environments anaerobic and aerobic suitable for a more efficient biodegradation of the different VS sludge fractions. **Additional achievements:** nitrogen removal by intermittent

aeration in the aerobic stage (nitrification - denitrification process)

Experimental apparatus

Reactors: 7.4 L cylindrical vessels operated in semi-continuous mode and fed daily



Aerobic reactor

- T= 20±0.5°C in the 1st and 2nd period, 37±0.5°C in the 3rd period
- V= 4.5 L
- DO ≈ 3-5 mg/L
- SRT= 12 d
- Intermittent aeration (40 min on - 20 min off)

Two-phase digested sludge

Anaerobically digested sludge

Performances

Mixed sludge					
SGP (Specific Biogas production) [Nm ³ /(l	g VS 0.82 ± 0.15				
destroyed × d)]					
CH ₄	67%				
Nitrification efficiency	97 ± 1% (mixed sludge at 2	20°C)			
Denitrification efficiency	70 ± 7% (mixed sludge at 2	20°C)			
Secondar	y sludge				
SGP (Specific Biogas production)	0.78 ± 0.24 1 st series				
[Nm ³ /(kg VS destroyed × d)]	0,81 ± 0.25 2 nd series				
CH ₄	65-68%				
Nitrification efficiency	90 ± 6% (20°C 1 st series); 86 ± 6%				
	(20°C 2 nd series);				
	65 ± 10% (37°C 3 rd series)				
Denitrification efficiency	62 ± 11% (20°C 1 st series), 66 ± 12%				
	(20°C 2 nd series);				
	75 ± 8% (37°C 3 rd series)				



0

1

Secondary sludge: 1st series (cumulative 40+25%)





Secondary sludge: 2nd series (cumulative 43+20%) Secondary sludge: 3rd series (37°C) (cumulative 40+33%)

3

week

5

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4

2

Pollutants fate during anaerobic digestion

Pollutant load (feed)	ANAEROBIC DIGESTION ANAEROBIC DIGESTION Anaerobic process	xpected concentration in the digested sample: ormalized feed concentration (NF) with respect to the original mass Theoretical accumulation of pollutant
Organic micropollutant (mg/kg dm)	Feed sludge concentr (mg/kg dm)	ation Literature range (mg/kg dm)
EOX	4.7 – 12	
Non-ionic surfactants	1 -4	22-650
Anionic surfactants	115 – 630	400-700
PAHs	1.7 – 3.6	1-3
PCBs	0.011 - 0.022	0.003-0-7
Phthalates	25 – 86	0.2-150

Evaluation of pollutants removal

- ⇒ The normalized feed concentration (NF) represents the theoretical pollutant concentration in the digested sample if no degradation and volatilization of the pollutant occurs. It is the concentration of the feed sludge normalized at the digested solid concentration.
- ⇒ The pollutant concentration in the digested sample (D) represents the concentration after the treatment.

D = NF	NO removal
D < NF	removal
D > NF	desorption

LAS, PCB, PAH, DEHP, NP/NPE removal have been investigated for the enhanced AD

Polycyclic aromatic hydrocarbons



Polychlorinated biphenyls



Phthalates



Concentration (µg/kg)

200.000

180.000



Non-ionic surfactants



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Anionic surfactants

Linear Alkylbenzene Sulphonates



Microbiological quality

	Secondary sludge Average (Range) (sample number)	Mixed sludge (Literature data)
E. coli (CFU/g dm)	5.7 10 ⁵ (3.0 10 ³ -2.9 10 ⁶) (18)	4.4 10 ⁵ – 1.1 10 ⁶ MPN /g DW (Pourcher et al. 2005) 4.6 10 ³ -1.6 10 ⁶ MPN /g DW (Carballa et al. 2009) 6.1-6.5 log CFU/g DW (Astals et al. 2012) 6.51-6.63 log MPN /g DW (Chen et al. 2012)
Cl. perfringens spores (CFU /g dm)	3.6 10 ⁵ (9.6 10 ³ -1.9 10 ⁶) (18)	4.5 10 ⁶ –1.9 10 ⁷ MPN /g DW (Pourcher et al. 2005) 8.4 10 ⁴ –8.1 10 ⁶ CFU/g DW (Carballa et al. 2009)
Somatic coliphages (PFU/g dm)	3.7 10 ⁶ (7.1 10 ⁵ -9.7 10 ⁶) (14)	2.8-3.9 10 ⁸ PFU /10 g DW (Guzman et al. 2007) 6.5-8.4 log PFU/g DW (Astals et al. 2012)
Salmonella (MPN /g dm)	1.2 10 ² (2 10-2 10 ²) (4)	1.2–3.2 MPN/g DW (Pourcher et al. 2005) 6.3 10 ² MPN/g DW (Dahab and Surampalli 2002) ^a 23.7 MPN/4g DW (Forster-Carneiro et al. 2010) 1884±3286 MPN /4g DW (Pepper et al. 2010) 5.58-6.55 log MPN /g DW (Chen et al. 2012)
Enteroviruses (PFU/g dm)	<0.1-2.03 (4)	15-18/ g DW (Pourcher et al. 2005) 4.8 10 ² -2 10 ³ / 10 g DW (Guzman et al. 2007) 19.02±31.6 /4g DW (Pepper et al. 2010)

Reduction of microbial indicators in the enhanced stabilization processes

		TH	SON	TAD	MAD	AA	UMT	UMT son
	Log removal	3.2 - 5.3	NR	2.9 - 5.3	1.2	2.4	3.5 - 5.3	3.5 - 5.3
E. coli	(positive treated samples/total samples)	(1/9)	(4/4)	(0/8)	(7/7)	(4/7)	(0/4)	(0/4)
	Log removal	3.9 - 5.2	NR	2.2	0.9	2.0	2.3	2.4
SOMCPH	(positive treated samples/total samples)	(2/9)	(4/4)	(4/5)	(6/6)	(6/6)	(4/4)	(4/4)
	Log removal Average±dev.st	2.5 - 5.1	NR ^c	NR	NR	NR	NR	NR
SPORES	(positive treated samples/total samples)	(0/9)	(4/4)	(8/8)	(7/7)	(7/7)	(4/4)	(4/4)
Salmonalla	Log removal	0.9 - 2.3	NR	0.9 - 1.3	0.9 - 2.0	0.9 - 2.1	0.8 - 2.1	0.8 - 2.1
Samonena	(positive treated samples/total samples)	(0/2)	(1/1)	(0/1)	(0/1)	(0/1)	(0/1)	(0/1)

Compliance to the proposed microbial indicators limits and removal requirements

	E. Coli	E. Coli E. Coli		SOMCPH	
	2 log units removal ^a	<500 CFU/g dm ^a	<1/50 g ww ^a	<10 ⁴ PFU/g dm ^a	
Th	100% (9)	89% (9)	100% (4)	100% (9)	
Son	0% (4)	0% (4)	0% (4)	0% (4)	
MAD	0% (7)	0% (7)	100% (3)	0% (9)	
AA	100% (7)	43% (7)	100% (3)	33% (6)	
TAD	100 % (8)	100 (8)	100% (3)	20% (5)	
UMT ^b	100% (8)	100% (8)	100% (2)	25% (8)	

a: percentage of samples (total samples); b: UMT and UMT-son are reported together

Bacterial regrowth: Comparison between thermophilic digested sludge and compost with sludge

	Sample		data	E. coli		SF	RC	Som	ncph
Name	Time (days)	Code	uale	cfu/g ww	cfu/g ww	cfu/gww	cfu/g ww	pfu/g ww	pfu/g ww
1	0	1 t0 A	26/09/2012	1,18E+02	+02 1.025.02	2,65E+04	2 025104	4,40E+01	E 00E+01
1	0	1 t0 B	26/09/2012	8,64E+01	1,020+02	1,40E+04	2,030+04	5,60E+01	5,00E+01
1		1 t5 A	01/10/2012	5,71E+01		1,70E+04	1.075+04	1,05E+02	0.055.01
1	5	1 t5 B	01/10/2012	5,29E+01	5,50E+01	2,23E+04	1,970+04	7,60E+01	9,050+01
1	20	1 t20 A	16/10/2012	1,80E+01	1 505,01	9,53E+03	1.075+04	7,00E+01	C 255101
1	20	1 t20 B	16/10/2012	1,20E+01	1,502+01	1,19E+04	1,070+04	5,50E+01	0,250+01
1	10	1 t40 A	06/11/2012	5,00E+00		1,10E+04	0.025.02	2,10E+01	2 155,01
1	40	1 t40 B	06/11/2012	8,00E+00	6,50E+00	6,66E+03	8,83E+03	4,20E+01	3,15E+01
1	60	1 t60 A	28/11/2012	9,00E+00	1,20E+01	1,45E+04	2,05E+04	3,10E+01	3,35E+01
Sample			E. coli		SRC		Somcph		
	Sample			E. (coli	SE	RC	Som	ncph
Name	Sample	Code	date	E.	coli cfu/a ww	SF cfu/aww	RC cfu/g	Son	ncph pfu/g
Name	Sample Time (days)	Code	date	E. cfu/g ww	coli cfu/g ww	SF cfu/gww	RC cfu/g ww	Som pfu/g ww	ncph pfu/g ww
Name 3	Sample Time (days)	Code 3 t0 A	date 02/10/2012	<i>cfu/g ww</i> 6,36E+02	coli cfu/g ww 6.18E+02	SF <i>cfu/gww</i> 8,00E+04	<u>cfu/g</u> ww 5.23E+04	Som <i>pfu/g ww</i> 6,55E+04	ncph pfu/g ww 6.41F+04
Name 3	Sample Time (days) 0	Code 3 t0 A 3 t0 B	date 02/10/2012 02/10/2012	<i>cfu/g ww</i> 6,36E+02 6,00E+02	coli cfu/g ww 6,18E+02	SF cfu/gww 8,00E+04 2,45E+04	<u>cfu/g</u> ww 5,23E+04	Son <i>pfu/g ww</i> 6,55E+04 6,27E+04	ncph pfu/g ww 6,41E+04
Name 3	Sample Time (days)	Code 3 t0 A 3 t0 B 3 t5 A	date 02/10/2012 02/10/2012 08/10/2012	<i>cfu/g ww</i> 6,36E+02 6,00E+02 5,77E+02	coli cfu/g ww 6,18E+02	SF cfu/gww 8,00E+04 2,45E+04 3,00E+04	<u>cfu/g</u> ww 5,23E+04	Son <i>pfu/g ww</i> 6,55E+04 6,27E+04 2,71E+04	ncph pfu/g ww 6,41E+04 2 76E+04
Name 3 3	Sample Time (days) 0 5	Code 3 t0 A 3 t0 B 3 t5 A 3 t5 B	date 02/10/2012 02/10/2012 08/10/2012 08/10/2012	<i>cfu/g ww</i> 6,36E+02 6,00E+02 5,77E+02 5,05E+02	coli cfu/g ww 6,18E+02 5,41E+02	SI cfu/gww 8,00E+04 2,45E+04 3,00E+04 1,58E+04	cfu/g ww 5,23E+04 2,29E+04	Som pfu/g ww 6,55E+04 6,27E+04 2,71E+04 2,80E+04	ncph pfu/g ww 6,41E+04 2,76E+04
Name 3 3	Sample Time (days) 0 5	Code 3 t0 A 3 t0 B 3 t5 A 3 t5 B 3 t20 A	date 02/10/2012 02/10/2012 08/10/2012 08/10/2012 23/10/2012	<i>E.</i> <i>cfu/g ww</i> 6,36E+02 6,00E+02 5,77E+02 5,05E+02 7,55E+02	coli cfu/g ww 6,18E+02 5,41E+02	SI cfu/gww 8,00E+04 2,45E+04 3,00E+04 1,58E+04 2,05E+04	C cfu/g ww 5,23E+04 2,29E+04	Som pfu/g ww 6,55E+04 6,27E+04 2,71E+04 2,80E+04 4,00E+04	ncph pfu/g ww 6,41E+04 2,76E+04 2,28E+04
Name 3 3 3	Sample Time (days) 0 5 20	Code 3 t0 A 3 t0 B 3 t5 A 3 t5 B 3 t20 A 3 t20 B	date 02/10/2012 02/10/2012 08/10/2012 08/10/2012 23/10/2012 23/10/2012	<i>E.</i> <i>cfu/g ww</i> 6,36E+02 6,00E+02 5,77E+02 5,05E+02 7,55E+02 2,73E+02	coli cfu/g ww 6,18E+02 5,41E+02 5,14E+02	SI cfu/gww 8,00E+04 2,45E+04 3,00E+04 1,58E+04 2,05E+04 2,27E+04	cfu/g ww 5,23E+04 2,29E+04 2,16E+04	Som pfu/g ww 6,55E+04 6,27E+04 2,71E+04 2,80E+04 4,00E+04 2,55E+04	ncph pfu/g ww 6,41E+04 2,76E+04 3,28E+04
Name 3 3 3 3	Sample Time (days) 0 5 20	Code 3 t0 A 3 t0 B 3 t5 A 3 t5 B 3 t20 A 3 t20 B 3 t40 A	date 02/10/2012 02/10/2012 08/10/2012 08/10/2012 23/10/2012 23/10/2012 12/11/2012	<i>E.</i> <i>cfu/g ww</i> 6,36E+02 6,00E+02 5,77E+02 5,05E+02 7,55E+02 2,73E+02 4,91E+02	<i>cfu/g ww</i> 6,18E+02 5,41E+02 5,14E+02	SI cfu/gww 8,00E+04 2,45E+04 3,00E+04 1,58E+04 2,05E+04 2,27E+04 5,00E+04	cfu/g ww 5,23E+04 2,29E+04 2,16E+04	Son <i>pfu/g ww</i> 6,55E+04 6,27E+04 2,71E+04 2,80E+04 4,00E+04 2,55E+04 8,60E+03	ncph pfu/g ww 6,41E+04 2,76E+04 3,28E+04 8 EEE+02
Name 3 3 3 3 3	Sample Time (days) 0 5 20 40	Code 3 t0 A 3 t0 B 3 t5 A 3 t5 B 3 t20 A 3 t20 B 3 t40 A 3 t40 B	date 02/10/2012 02/10/2012 08/10/2012 08/10/2012 23/10/2012 23/10/2012 12/11/2012 12/11/2012	<i>E.</i> <i>cfu/g ww</i> 6,36E+02 6,00E+02 5,77E+02 5,05E+02 7,55E+02 2,73E+02 2,73E+02 4,91E+02 9,42E+02	coli cfu/g ww 6,18E+02 5,41E+02 5,14E+02 7,17E+02	SI cfu/gww 8,00E+04 2,45E+04 3,00E+04 1,58E+04 2,05E+04 2,27E+04 5,00E+04 2,18E+04	cfu/g ww 5,23E+04 2,29E+04 2,16E+04 3,59E+04	Som <i>pfu/g ww</i> 6,55E+04 6,27E+04 2,71E+04 2,80E+04 4,00E+04 2,55E+04 8,60E+03 8,51E+03	ncph pfu/g ww 6,41E+04 2,76E+04 3,28E+04 8,55E+03
Name 3 3 3 3 3 3 3 3	Sample Time (days) 0 5 20 40 60	Code 3 t0 A 3 t0 B 3 t5 A 3 t5 B 3 t20 A 3 t20 A 3 t20 B 3 t40 A 3 t40 B 3 t40 A	date 02/10/2012 02/10/2012 08/10/2012 08/10/2012 23/10/2012 23/10/2012 12/11/2012 12/11/2012 04/12/2012	<i>E.</i> <i>cfu/g ww</i> 6,36E+02 6,00E+02 5,77E+02 5,05E+02 7,55E+02 2,73E+02 4,91E+02 9,42E+02 2,50E+02	coli cfu/g ww 6,18E+02 5,41E+02 5,14E+02 7,17E+02 2,48E+02	SI cfu/gww 8,00E+04 2,45E+04 3,00E+04 1,58E+04 2,05E+04 2,27E+04 5,00E+04 2,18E+04 2,75E+04	RC cfu/g ww 5,23E+04 2,29E+04 2,16E+04 3,59E+04 2,47E+04	Som pfu/g ww 6,55E+04 6,27E+04 2,71E+04 2,80E+04 4,00E+04 2,55E+04 8,60E+03 8,51E+03 6,57E+03	ncph pfu/g ww 6,41E+04 2,76E+04 3,28E+04 8,55E+03 6,43E+03

4	Sampl	e		Е. со	E. coli SRC		Son	ncph	
Name	Time (days)	Code	date	cfu/g ww	cfu/g ww	cfu/gww	cfu/gww	pfu/g ww	pfu/g ww
4	0	4 t0 A	09/10/2012	<1	.1	1,00E+02	1 165,00	<1	1
4	U	4 t0 B	09/10/2012	<1	N 1	1,31E+02	1,101+02	<1	N 1
4	E	4 t5 A	15/10/2012	<1	-1	2,00E+02	1 705 02	<1	-1
4	S	4 t5 B	15/10/2012	<1	<1	1,40E+02	1,70E+02	<1	<1
4	20	4 t20 A	30/10/2012	<1	-1	1,00E+02	1 225,02	<1	-1
4	20	4 t20 B	30/10/2012	<1	< <u>1</u>	1,43E+02	1,220+02	<1	51
	40	4 t40 A	19/11/2012	<1	87 3 4	1,00E+02	1 205 . 02	<1	- 1
4	40	4 t40 B	19/11/2012	<1	<1	1,40E+02	1,20E+02	<1	<1
4	60	4 t60 A	11/12/2012	<1	- 21	1,28E+02	1 425:02	<1	-21
4	00	4 t60 B	11/12/2012	<1	<1	1,57E+02	1,43E+02	<1	<1

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Enumeration of Salmonella

	Sample 1	Sample 2	Sample 3	Sample 4	Sample 5
Time (d)	(thermophilic)	(mesophilic)	(thermophilic)	(compost)	(mesophilic)
0	>0,48	>0,48	0,48	<0,018	>0,48
5	>0,48	>0,48	0,046	<0,018	>0,48
20	0,046	0,48	<0,018	<0,018	>0,48
40	0,046	0,046	<0,018	<0,018	0,48
30	<0,018	0,046	<0,018	<0,018	0,019

Comments

Data show that only compost (sample #4) is always complying with the hygienic requirements set up by the 3rd draft of April 2000, i.e. *E. coli* lower than 500 CFU/g dm and *Salmonella* absent in 50 g of final product (wet weight). Thermophilic digested sludge (samples #1 and #3) sometimes is complying, while mesophilic digested sludge is always not complying.

Kinetics during storage at 22±2°C



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Kinetics during storage at 37±1°C





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Eco-toxicity assessment

Selected terrestrial biotests were:

a) The test for inhibition of enzyme activity in the soil bacterium *Arthrobacter globiformis*

The endpoint of the *A. globiformis* test is the inhibition of dehydrogenase, a key enzyme of many organisms. A dilution series with five dilutions (between 0.1 % and up to 50% sludge added to the substrate quarz sand) was tested to estimate the median effect concentration (EC_{50} in g sludge dry weight/kg quartz sand dry weight). The maximum tested sludge concentration was 250 g sludge kg⁻¹ substrate (in two cases 500 g sludge kg-1 substrate).

b) The test for avoidance behaviour of the earthworm *Eisenia fetida*. Due to the limited amount of available sludge it was not possible to test a full range of dosages suitable to derive an EC_{50} estimate for avoidance, but only to conduct tests at very few different dosages. Sludge samples were applied at maximum with 25 g dry sludge kg⁻¹ soil dm in the test.

Eco-toxicity assessment

- ⇒ The ecotoxicological results were compared to the application rates of sludge to agricultural land in order to determine the resulting safety margin.
- ⇒ Both the usual application rate in Europe, i.e. 2 t ha⁻¹ (EC 2010), and the maximum allowed application rate in Ontario, Canada, i.e. 22 t ha⁻¹ (OR 2009) are here considered.
- ⇒ Assuming a ploughing depth of 20 cm and a soil bulk density of 1.3 g cm⁻³, these application rates result in 0.8 g sludge kg⁻¹ soil (Europe) and 8.5 g sludge kg⁻¹ soil (Ontario).

Results by the Arthrobacter globiformis



Comments on sludge samples from TT tests

- In both the two tests, the toxicity toward *A. globiformis* was hardly reduced by the pre-treatment (TT-FU versus TT-FP).
- The thermophilic digestion step reduced toxicity only when carried out with pre-treated sludge where the toxicity was reduced by a factor of about 7 (only for the test #1 at high loading rate).
- No reduction of toxicity was observed with untreated sludge.
- For the samples of test 2, the avoidance test evidenced that, only the feed (TT-FU) but none of the final digested sludge samples caused a limited habitat function (85% avoidance) when amended to soil, confirming the findings obtained by the *A. globiformis* test.

Comments on sludge samples from UMT tests

- The sonication pre-treatment apparently did not reduce toxicity as there was little difference between the FU and FP sample.
- The mesophilic digestion (MDU versus FU and MDP versus FP) decreased A. globiformis toxicity by factor 4 to 5, while the subsequent thermophilic digestion increased toxicity again.
- Overall, the complete treatment process achieved little reduction of toxicity, independently of the pre-treatment of the secondary sludge.
- The EC₅₀ values of the final digested sludge samples (MTDU and MTDP) provided a safety margin of about factor 100 to the usual application rate in Europe and of about factor 10 to the one in Ontario.
- None of the sludge samples indicated a limited habitat function (i.e., avoidance of 80% or more) in the earthworm avoidance tests.

Comments on sludge samples from AA tests

- A significant reduction of toxicity toward *A. globiformis* from the feed (AA-FU) to the treated sludge (AA-DAA) was observed in both tests.
- The mixed sludge was more toxic than the secondary feed sludge and any other sludge tested in this study.
- The degree of toxicity reduction by AA was greater in the test with mixed sludge rather than in the one with secondary sludge by factor 19 compared to factor 7, respectively.
- The remaining toxicity of the final sludge reached a safety margin of more than factor 100 only for secondary but not for mixed sludge as feed in relation to usual European application rates.
- No earthworm avoidance behaviour of sludge-amended soil was observed for the samples of the sludge #1 (not enough sludge sample was available for testing sludge #2 for earthworm avoidance).

Comments on sludge samples from Canada

- The biosolids samples from Canada showed very low and quite similar toxicity with safety margins to assumed application rates in Europe and Ontario of a factor of about 1000 and 100, respectively.
- In accordance with the finding of little toxicity toward *A. globiformis*, biosolids induced no avoidance by earthworms when added to soil.

General comments

- The *A. globiformis* toxicity of sludge samples is quantifiable and can be used for comparing the efficiency of various sludge treatment processes.
- The earthworm avoidance test requires a rather large volume of sludge sample and could therefore only be performed at a single dosage, which did not allow quantifying the toxicity toward earthworms.
- The earthworm avoidance test measures the response of a key soil organism at an integrative organismal level, which allows a more straightforward extrapolation to the field.
- The final digested sludge samples exhibited toxicity to the soil bacterium *A. globiformis* at concentrations that were always higher than the usual application rate of sludge to soil in Europe and the maximum allowed application rate in Ontario. In the avoidance tests, a safety margin of factor 30 was generally achieved for the final digested samples.
- The thermophilic digestion process achieved among the three processes the least toxicity reduction (at least when operated at low organic load), while the double stage AA process appeared as the most effective process as it could greatly reduce the considerable toxicity of the mixed sludge.
- The toxicity exerted by the Canadian biosolids was very low in both terrestrial tests. Interestingly, a similar safety margin (about factor 100) was obtained for the biosolids with regard to the maximum allowed application rate of Ontario as for the European sludge with regard to the European application rate.

Correlation coefficients between toxicity to A. *globiformis* (EC₅₀) and characteristics of sludge samples

	R
Stability index VS/TS	-0.61
Carbamazepine	-0.56
Triclocarban	-0.57
Naphthalene	-0.54
Soluble COD	-0.16
Soluble N-NH ₄	0.32
Sum of PAHs	0.13
Sum of PCBs	-0.13
Sum of phthalates	-0.38
Sum of QACs	-0.02
Sum of pharmaceuticals	-0.34
Sum of biocides and fungicides	-0.31
EOX	-0.20

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Conclusions on technical performance of the stabilization processes

Volatile solid removal:

- ☆ UMT process 45-55% (low and high loading rate);
- ☆ TT process 38-45% (low and high loading rate);
- ☆ AA process 50 + 45% with mixed sludge;
- AA process 40 + 33% with secondary sludge (performance in the aerobic reactor depends on temperature, 20-25% at room temperature, 33% at 37°C)
- ☆ Conventional MAD 30-32% (low and high loading rate);

Conclusions Technical performance of the stabilization processes

Biogas production:

- ☆ UMT process 0.35-0.33 Nm³/kg VS fed (low and high organic rate);
- ☆ TT process 0.32-0.30 Nm³/kg VS fed (low and high organic rate);
- AA process 0.41 (mixed sludge) 0.31-0.35 (secondary sludge) Nm³/kg VS fed
- ☆ Conventional MAD 0.26-0.25 Nm³/kg VS fed (low and high organic rate)

Conclusions Effects of the enhanced stabilization processes on digested sludge quality

- ⇒ Enhanced processes caused an increase in soluble COD and ammonia in anaerobic supernatants, with respect to conventional MAD.
- ⇒ Enhanced processes caused also worse dewaterability of digested sludge.

Conclusions Effects on chemical contaminants

- Positive effects of AA process on PAH, PCB, phthalates, non ionic and anionic surfactants (only for secondary sludge);
- Positive effects of TT process on PAH;
- Positive effects of UMT process on phthalates.

Conclusions Effects on pathogens and their indicators

- ⇒ The assumed standards were: 2log removal of *E. Coli, E. Coli* < 500 CFU/100 g dm, absence of *Salmonella* in 50 g wet weight, *Somatic Coliphages* (SOMCPH)< 10⁴ PFU/g dm.
- ⇒ MAD was compliant only for *Salmonella*;
- ⇒ AA was compliant on 2log removal of *E. Coli* and on *Salmonella*;
- ⇒ TAD and UMT were compliant on both the two criteria for *E. Coli* and on *Salmonella*;
- ⇒ Thermal pretreatment at 135°C was compliant on 2log removal of *E. Coli*, on *Salmonella* and on SOMCPH;
- ⇒ Sonication was never compliant;
- ➡ Compost with sludge displayed much better quality than all the other sludge samples;
- ⇒ No regrowth occurred in all the sludge samples after at least 20 d of storage at 22 and 37°C.

Conclusion Effects on ecotoxicity

- Only AA showed a clear reduction of ecotoxicity;
- UMT process displayed a reduction of ecotoxicity only after the 1st mesophilic step of digestion. Ecotoxicity increased after the 2nd thermophilic step.
- The toxicity exerted by the Canadian biosolids was considerably lower than that of the European samples even after enhanced stabilization processes. The toxicity of sludge seems to be more related to the source than to the treatment, with "source" meaning the origin (and thereby contamination) of the wastewater from which the sludge was produced. This was confirmed by some tests on mixed sludge (AA process) which was much more ecotoxic than secondary sludge.
- The stability of the sludge, as measured by the VS/TS ratio, significantly correlated with the toxicity to *A. globiformis* in 18 samples: the more stable the sludge the lower the toxicity was. Ammonium released from the less stabilized sludge may cause the toxicity in *A. globiformis*.
- Concentrations of only three of the measured individual pollutants (carbamazepine, triclocarban and napthalene) exhibited significant correlations with toxicity to *A. globiformis*.

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