Agrochemical value of organic matter of fermenter wastes in biogas production

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ABSTRACT

We performed 28-day mesophilic fermentation of a mixture of pig slurry and primary (raw) sludge from the sedimentation stage of a wastewater treatment plant at a 1:1 ratio. The components and the original and fermented mixture of slurry and sludge were subjected to acid hydrolysis. Dry matter of the solid phase of both components and both mixtures was incubated with sandy-loamy Cambisol at a weight ratio 3:1 at 25°C for 20 weeks; in 14-day intervals lipids, crude protein, hemicelluloses, cellulose, lignin, total nitrogen and hot-water-insoluble solids were determined. Changes in ion-exchange and buffering capacity of the test materials were recorded. Labile organic matters were determined after 20 weeks of incubation. Liquid fractions of both components and their mixtures were analysed before and after anaerobic fermentation. It was concluded that beneficial effects of wastes as fertilisers from anaerobic digestion could be attributed to their liquid fraction. After anaerobic digestion the solid fraction of these wastes has relatively increased ion exchange capacity as well as buffering capacity but it is very stable, hardly degradable organic matter, and therefore it cannot play the role of organic matter in soil. This is the reason why it should not be considered as an organic fertiliser.

Keywords: organic fertilisers; wastes from anaerobic digestion; organic matter lability; quality

A prevailing opinion of bio-power engineers as well as in literature is that wastes from fermenters in biogas production are an excellent fertiliser and that anaerobic digestion is to some extent an improvement process in relation to the fertilising value of organic materials used for biogas production. These opinions are apparently based on the fact that in anaerobic stabilisation of sludge the ratio of organic to mineral matters in dry matter is approximately 2:1 and after methanisation it drops to 1:1. Because there is a loss of a part of organic dry matter of sludge in the process of anaerobic digestion, the weight of its original dry matter will decrease by 40%, which will increase the concentration of originally present nutrients. In reality, anaerobic digestion will significantly release only ammonium nitrogen from the original material, which will enrich mainly the liquid phase due to its solubility; the process will not factually influence the content of other nutrients (Institut für Energetik und Umwelt GmbH 2006).

If organic matter is to be designated as organic fertiliser, it has to satisfy the basic condition: it has to be easily degradable microbially so that it will release necessary energy for soil microorganisms. Then a part of this energy from the exothermic process of mineralisation can be transferred to the endothermic process of humification. By their sorption capacity and mainly by their ion-exchange capacity humus substances influence in a decisive way not only the elution of nutrients from soil but also the self-purifying function of soil if it is contaminated by xenobiotic pollutants, formation of organo-mineral complexes of soil aggregates and many other factors that are important for potential soil fertility. The productivity of humification depends on the ratio of free energy originating in aerobic processes of soil organic matter transformation to the production of low-molecular organic compounds that are humus precursors and originate mainly in anaerobic transformation processes (Novák 1966).

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Great attention is currently paid to the lability of soil organic matter characterised by labile fractions of soil carbon while these fractions are considered as a significant indicator of soil quality (Ghani et al. 2003, Haynes 2005, Maia et al. 2007, Majumder et al. 2007, 2008). The labile fraction is characterised in a different way. It may be hot- or cold-water soluble carbon compounds, mineralised organic matters, substances extractable with K_2SO_4 solution, soluble saccharides, proteins and hemicelluloses. The lability of organic matters is derived from basal respiration carbon, content of aminosugar nitrogen, microbial biomass carbon, content of particulate organic matter carbon, from fractions of gradual oxidation with $K_2Cr_2O_7$ in 6M, 9M and 12M of H_2SO_4 or with 6M, 9M and 12M H_2SO_4 and from fractions of labile carbon oxidisable with 15.6, 33 and 333 mmol/l $KMnO_4$, from kinetics of their microbial oxidation as the first-order reaction (Vance et al. 1987, Blair et al. 1995, Chan et al. 2001, Rovira and Vallejo 2002, 2007, Ni et al. 2004, Zhang et al. 2004, 2006, Gelsomino et al. 2006, Jiang and Xu 2006, Kolář et al. 2006, Marriot and Wander 2006, Majumder et al. 2007, 2008, Passos et al. 2007, Soon et al. 2007, Vieira et al. 2007).

The lability of plant and other organic material as a potential degradable substrate for organic manuring is evaluated in a similar way. Separation into 3 fractions according to lability in acid hydrolysis with 1M and 2.5M H_2SO_4 at 105°C and with 0.5–12 h reaction time is recommended (Rovira and Vallejo 2000, 2002, Shirato and Yokozawa 2006). To estimate lability other authors use oxidisable carbon of the material in neutral 33 mmol/l $KMnO_4$ (Tirol-Padre and Ladha 2004) or separation into 4 fractions according to the oxidisability of C-compounds with $K_2Cr_2O_7$ in 6M, 9M and 12M H_2SO_4 (Chan et al. 2001).

MATERIAL AND METHODS

A mixture of pig slurry and primary (raw) sludge from the sedimentation stage of a municipal waste water treatment plant at a 1:1 volume ratio was treated in an experimental unit of anaerobic digestion operating as a simple periodically filled Batch-system with mechanical agitation, heating tubes with circulating heated medium at a mesophilic temperature (40°C) and low organic load of the fermenter (2.2 kg org. dry matter/m^3) and 28-day fermentation. Table 1 shows the analysis of raw materials (sludge and pig slurry) and their

Table 1. The content of selected organic matters (%) and ion exchange and buffering capacity of the solid phase of primary sludge (A), pig slurry (B), sludge and pig slurry mixture at a ratio 1:1 before fermentation (C) and after fermentation (D) before and after 20 weeks of incubation with sandy-loamy Cambisol topsoil at a ratio 3:1 at 25°C in dry matter

	Before incubation (25°C)				After incubation (25°C, 20 weeks)			
	A	B	C	D	A	B	C	D
Lipids (petroleum ether extractable compounds) (%)	8.60 ± 0.69	14.27 ± 1.14	10.82 ± 0.86	2.01 ± 0.15	7.97 ± 0.65	13.50 ± 1.09	10.39 ± 0.85	2.08 ± 0.17
Proteins (Berstein) (%)	13.43 ± 1.30	17.95 ± 1.62	15.31 ± 1.60	8.50 ± 0.93	11.81 ± 1.20	16.10 ± 1.53	13.89 ± 1.42	8.50 ± 0.98
Hemicelluloses (%)	1.82 ± 0.19	5.03 ± 0.73	3.32 ± 0.61	0.70 ± 0.60	1.43 ± 0.11	4.23 ± 0.51	2.89 ± 0.30	0.69 ± 0.10
Cellulose (%)	7.45 ± 0.92	11.18 ± 1.33	9.61 ± 1.05	6.03 ± 0.95	5.42 ± 0.82	9.27 ± 0.98	7.96 ± 0.94	6.05 ± 0.83
Lignins (%)	4.84 ± 0.62	5.16 ± 0.84	4.99 ± 0.75	5.18 ± 0.92	4.83 ± 0.91	5.18 ± 1.07	4.98 ± 0.84	5.20 ± 0.91
Total N (%)	1.59 ± 0.06	2.70 ± 0.11	2.29 ± 0.10	1.07 ± 0.04	1.51 ± 0.06	2.50 ± 0.11	2.14 ± 0.09	1.08 ± 0.05
Hot-water insoluble dry matter (%)	98.25 ± 2.94	98.26 ± 2.95	98.25 ± 2.95	98.23 ± 2.92	89.05 ± 2.67	85.17 ± 2.60	87.26 ± 2.58	98.20 ± 2.93
Ion exchange capacity (mmol chem. eq./kg)	48 ± 3	55 ± 3	53 ± 3	145 ± 9	50 ± 3	61 ± 4	55 ± 4	168 ± 10
Buffering capacity (mmol chem. eq./kg)	62 ± 4	69 ± 4	65 ± 4	157 ± 9	65 ± 4	72 ± 4	70 ± 4	179 ± 11

Sample size $n = 4$ (hot-water-soluble dry matter $n = 7$); interval of reliability of the mean for a significance level $\alpha = 0.05$

mixture before and after anaerobic fermentation while Table 2 shows the analysis of their liquid fraction.

Acid hydrolysis of sludge, slurry and their mixture was done before and after anaerobic fermentation. The hydrolysis of samples was performed with the dry matter of examined sludge and its mixture with pig slurry including the liquid fraction after screening the material through a 250-μm mesh sieve. The method of hydrolysis according to Rovira and Vallejo (2000, 2002) as modified by Shirato and Yokozawa (2006): 300 mg of homogenised sample is hydrolysed with 20 ml of 2.5M H_2SO_4 for 30 min at 105°C in a pyrex tube. The hydrolysate is centrifuged and decanted, the residues are washed with 25 ml water and the wash water is added to the hydrolysate. This hydrolysate is used to determine Labile Pool I (LP I).

The washed residue is dried at 60°C and hydrolysed with 2 ml of 13M H_2SO_4 overnight at a laboratory temperature and continuous shaking. Such an amount of water is added that the concentration of the acid will be 1M, and the sample is hydrolysed for 3 h at 105°C at intermittent shaking. The hydrolysate is isolated by centrifugation and decantation, the residue is washed again with 25 ml of water and the wash water is added to the hydrolysate. This hydrolysate is used for the determination of Labile Pool II (LP II). The residue from this hydrolysis is dried at 60°C and Recalcitrant Pool (RP) is determined from this fraction. C_{tot} is determined in all three fractions.

Degradability of organic matter of the test materials was studied by modified methods of Leblanc et al. (2006) used to examine the decomposition of green mulch from *Inga samanensis* and *Inga edulis* leaves. These authors conducted their study in outdoor conditions (average annual temperature 25.1°C) and we had to modify their method in the cold climate of this country. At first, the liquid phase of sludge, slurry and mixture was separated by centrifugation; the solid phase was washed with hot water several times and separated from the solid phase again. By this procedure we tried to separate the solid phase from the liquid one, which contains water-soluble organic matters and mineral nutrients. Solid phases of tested organic materials were mixed with sandy-loamy Cambisol at a weight ratio 3:1 to provide for inoculation with soil microorganisms and volume ventilation of samples with air. After wetting to 50% of water retention capacity the mixtures at an amount of 50 g were put onto flat PE dishes 25 × 25 cm in size. The material was spread across the surface of the dish. Cultivation was run in a wet thermostat at 25°C, and in the period of 2–20 weeks dishes were sampled in 14-day intervals as subsamples from each of the four experimental treatments. The agrochemical analysis of the used topsoil proved that the content of available nutrients P, K, Ca and Mg according to Mehlich III is in the category "high" and pK_{KCl} = 6.3. After drying at 60°C for 72 h the content of lipids, crude protein, hemicelluloses, cellulose, lignin, total nitrogen and hot-water-insoluble dry matter was determined in the dish contents.

After twenty weeks of incubation organic matters were determined in the dish contents by fractionation into 4 degrees of lability according to Chan et al. (2001).

The content of hemicelluloses was calculated from a difference between the values of neutral detergent fibre (NDF) and acid detergent fibre (ADF), lignin was calculated from ADF by subtracting the

Table 2. The fractionation of organic carbon (g/kg) of primary sludge, pig slurry, and sludge and slurry mixture at a ratio 1:1 before fermentation (A) and after fermentation (B) in a mixture with sandy-loamy Cambisol (3:1) in dry matter after 20 weeks of incubation at 25°C by the modified Walkley-Black method according to Chan et al. (2001) with a change in H_2SO_4 concentration. The values given in brackets are % of the C fraction of total dry matter carbon

Fraction	Unfermented primary sludge	Unfermented pig slurry	Mixture A	Mixture B	Soil only
1 12N H_2SO_4	59.84 ± 7.18 (32.00)	55.38 ± 6.52 (28.40)	54.09 ± 6.50 (30.05)	2.65 ± 0.30 (2.60)	1.30 ± 0.17 (7.22)
2 12N–18N H_2SO_4	42.45 ± 5.13 (22.70)	35.76 ± 4.26 (18.34)	34.22 ± 4.10 (19.01)	9.28 ± 1.10 (9.07)	0.80 ± 0.09 (4.44)
3 18N–24N H_2SO_4	27.34 ± 3.28 (14.62)	20.18 ± 2.53 (10.35)	20.30 ± 2.42 (11.28)	11.13 ± 1.33 (10.91)	3.70 ± 0.44 (20.56)
4 TOC = 24N H_2SO_4	57.37 ± 6.85 (30.68)	83.67 ± 10.01 (42.91)	71.39 ± 8.55 (39.66)	78.97 ± 9.40 (77.42)	1.22 ± 1.42 (67.78)

Sample size n = 5; interval of reliability of the mean for a significance level α = 0.05

result after lignin oxidation with $KMnO_4$. Because ADF contains lignin, cellulose and mineral fraction, it was possible to determine the cellulose content by ashing the residue in a muffle furnace and by determination of mineral fraction. These methods were described by Van Soest (1963), modifications used by Columbian authors (Leblanc et al. 2006) were reported by López et al. (1992).

Ion exchange capacity (mmol chem. eq./kg) was determined in the dry matter of examined materials according to Gillman (1979), buffering capacity was determined in samples induced into the H^+-cycle with HCl diluted with water at 1:1 and washed with water until the reaction to Cl disappears. In the medium of 0.2M KCl the samples were titrated to pH = 7 with 0.1M NaOH and buffering capacity was calculated from its consumption.

Lord's test and other methods suitable for few-element sets and based on the range R of parallel determinations (Eckschlager et al. 1980) were used for the mathematico-statistical evaluation of analytical results including the computation of reliability interval of the mean.

RESULTS AND DISCUSSION

The average content of nutrients in pig slurry and in primary (raw) sludge basically corresponds with data reported by Pitter (1981) and Vaněk et al. (2007). Obviously, pig slurry usually differs from sewage sludge by more than twofold content of nitrogen and phosphorus and more than tenfold content of potassium if related to fresh matter; in dry matter these ratios will change only slightly. The content of organic matters in fresh and dry matter of pig slurry is slightly higher than is the upper limit of their content in sewage sludge. After anaerobic digestion sludge (digested sludge or anaerobically stabilised sludge) loses about 23% of nitrogen on average but its content of all other nutrients is higher than in primary sludge. During methanisation the organic matter of sludge is reduced by 45–65%, there is a decrease mainly in the content of lipids and a smaller reduction in proteins and polysaccharides including cellulose. This is the reason why the content of nutrients in the dry matter of sludge expressed in % apparently increases. It would be more suitable to express concentrations in values of g/l or mg/l.

In the sludge liquor of primary sludge % contents of N and P are practically the same but anaerobic digestion will increase them more than 5 times in nitrogen while the increase in phosphorus is negligible. If the content of N and P in the sludge liquor of primary and anaerobically stabilised sludge is recalculated in relation to the content of these nutrients in original, primary sludge, we will get that anaerobic digestion increases nitrogen content in sludge liquor 6.4 times while it is only 1.3 times in phosphorus. Particularly, a considerable amount of nitrogen passes from sludge to sludge liquor – up to 55% of nitrogen from the original nitrogen content in primary sludge.

In the course of anaerobic digestion degradation of lipids, which are mostly the main component of primary sludge, is the highest, while after methanisation proteins are the main component of anaerobically stabilised sludge; naturally lignin undergoes the lowest degradation.

Table 3 shows the analyses of a mixture of pig slurry and primary sludge used in the experiment. Obviously, compared to the values reported in literature (Pitter 1981, Vaněk et al. 2007) our ex-

Table 3. The analysis of experimental pig slurry and primary sludge, mixture of pig slurry and primary sludge before methanisation in a fermenter and after methanisation in % of dry matter (pig slurry and primary sludge were mixed for anaerobic digestion at a volume ratio 1:1)

		Pig slurry	Primary sludge	Mixture of slurry and sludge before methanisation	Mixture of slurry and sludge after methanisation
Organic matters		65.1 ± 2.6	62.7 ± 2.4	64.1 ± 2.4	36.9 ± 1.5
Total nutrients	N	6.2 ± 0.2	2.6 ± 0.1	3.9 ± 0.2	3.1 ± 0.1
	P	1.6 ± 0.1	0.7 ± 0.0	1.1 ± 0.0	1.3 ± 0.1
	K	2.3 ± 0.1	0.2 ± 0.0	1.2 ± 0.0	1.2 ± 0.0
	Ca	2.8 ± 0.1	2.6 ± 0.1	2.5 ± 0.1	2.8 ± 0.1

Sample size n = 6; interval of reliability of the mean for a significance level α = 0.05

Table 4. The analysis of the liquid fraction (sludge liquor) of a mixture of pig slurry and primary sludge from a wastewater treatment plant (1:1) before fermentation and after fermentation in mg/l. The values A and B express % in the liquid phase of the total amount of sludge before and after fermentation

	A (%)	B (%)	Before fermentation (mg/l)	After fermentation (mg/l)
Total N	8.40	55.20	246.2 ± 14.7	994.7 ± 59.6
Ammonia N	52.60	90.80	153.7 ± 8.4	907.2 ± 48.2
Total P	12.20	25.30	134.5 ± 8.7	176.3 ± 11.6
Total K	19.90	28.10	172.9 ± 10.4	184.1 ± 11.0

Sample size n = 5; interval of reliability of the mean for a significance level α = 0.05

perimental materials had a somewhat lower content of organic matters in dry matter, and perhaps this is the reason why anaerobic fermentation reduced the content of organic matters by 39% only although the usual reduction by 45–65% for primary sludge was expected as reported in literature (Pitter 1981) and by 40–50% for pig slurry (Institut für Energetik und Umwelt GmbH 2006). As a result of the organic dry matter reduction the content of nutrients in sludge after anaerobic fermentation is higher, nitrogen content is by about 20% lower. In this process organic nitrogen is converted to $(NH_4)_2CO_3$, which partly decomposes into NH_3 + H_2O + CO_2 and partly passes into sludge liquor. Roschke (2003) reported that up to 70% of total nitrogen might pass to the ammonium form at 54% degradation of organic matters of dry matter. Even though concentrations of the other nutrients in the dry matter of aerobically stabilised sludge increased as a result of the organic dry matter reduction, their content in sludge liquor also increased (Table 4).

Taking into account that the amount of water-soluble nutrients in sludge liquor and organic forms of N and P dispersed in sludge liquor in the form of colloid sol (but it is a very low amount) is related not only to the composition of the substrate but also to technological conditions of anaerobic digestion, fermenter load and operating temperature, it is evident that the liquid fraction of anaerobically stabilised sludge contains a certain amount of mineral nutrients, approximately 1 kg N/m^3, besides the others; differences in the concentration of P and K in the liquid fraction before and after fermentation are however generally negligible. It is a very low amount, and there arises a question whether the influence of the liquid fraction on vegetation is given by the effect of nutrients or water itself, particularly in drier conditions.

After anaerobic digestion the solid phase of sludge still contains a high amount of proteins and other sources of organic nitrogen that could be a potential pool of mineral nitrogen if the degradation of sludge after fermentation in soil is satisfactory.

The results of hydrolysis in Table 5 prove that pig slurry has 59% of its total carbon in LP I, which indicates great lability, corresponding to the hy-

Table 5. Proportions of the three pools of carbon in experimental materials, as determined by the acid hydrolysis approach of Rovira and Vallejo (2002)

Material	Proportion		
	LP I	LP II	RP
Primary sewage sludge	68 ± 5	23 ± 2	9 ± 1
Pig slurry	59 ± 5	15 ± 2	26 ± 2
Mixture of primary sludge and pig slurry at a volume ratio 1:1	63 ± 5	20 ± 2	17 ± 1
Mixture of primary sludge and pig slurry at a volume ratio 1:1 after methanisation	18 ± 2	16 ± 1	66 ± 5

LP – labile pool; RP – recalcitrant pool; materials including the liquid fraction were used; sample size n = 4; interval of reliability of the mean for a significance level α = 0.05

drolysability of cereals and grasses according to Shirato and Yokozawa (2006). Primary sewage sludge is still better from this aspect, having almost 70% C in LP I. The degree of lability of the sludge and slurry mixture is relatively high and corresponds to the component ratio. After methanisation carbon content in LP I of the sludge and slurry mixture decreases to less than a third of the original amount and carbon of non-hydrolysable matters increases even almost four times in the RP fraction. The sum of LP I and LP II, i.e. the labile, degradable fraction of carbon compounds of the sludge and pig slurry mixture, was reduced by anaerobic digestion from 83 to 34%, that means approximately by 50%. These are enormous differences and they prove that mainly very labile organic matters are heavily destroyed by the anaerobic process, even though a reduction in the content of organic matters during anaerobic fermentation is lower (by 39% in our experiment).

The same results (Table 1) are provided by the incubation of the solid phase of sludge, pig slurry and their mixture before and after anaerobic fermentation when incubated with soil at 25°C and by the contents of lipids, crude protein, hemicelluloses, cellulose, lignin, total nitrogen and hot-water-insoluble dry matter; the same explicit conclusion can be drawn from the results of the fractionation of organic matter lability of the experimental treatments after 20-week incubation with soil according to Chan et al. (2001) shown in Table 2. A comparison of the results in Tables 2 and 5 indicates that as a result of the activity of microorganisms of the added soil in incubation hardly hydrolysable organic matter was also degraded – differences between the most stable fraction 3 and fraction 4 in Table 2 are larger by about 73% after anaerobic fermentation while in the course of acid chemical hydrolysis the content of non-hydrolysable fraction was worsened by anaerobic fermentation because it increased by about 290%. But it is a matter of fact that the soil microorganisms are not able to stimulate anaerobically fermented sludge to degradation as proved by more than ¾ of total carbon in fraction 4.

The table results document that 20-week incubation decreased more or less the percent content of examined organic matters except lignin (total N 5–8%, cellulose 17–25%, hemicellulose 13–22%, proteins 9–12%, lipids 4–7%, and the content of hot-water-insoluble dry matter by 10–15%); the decrease was observed factually in all experimental treatments except the treatment of anaerobically fermented mixture of primary sludge and pig slurry, where a reduction in these matters is low or nil. Hence, primary sludge, pig slurry and their mixture can be considered as organic fertilisers but only before anaerobic fermentation. We recorded a substantially lower degree of degradation of selected organic matters in sludge, pig slurry and their mixture during incubation with 25% of sandy-loamy soil (5–25%) than did Leblanc et al. (2006) with phytomass of *Inga samanensis* and *Inga edulis* leaves, who reported about 50% degradation of total mass, hemicelluloses and nitrogen in mass. We are convinced that it is caused by a very different content of hemicelluloses in our materials compared to the materials used by the above-mentioned authors. No easily degradable hemicelluloses are present in sewage sludge or in pig slurry any longer; obviously, only more stable forms pass through the digestive tracts of animals and humans. It is also interesting that after anaerobic fermentation and after 20-week aerobic cultivation at 25°C only the compounds (lipids + proteins + hemicelluloses in mixture II D account roughly for 11%) that could be considered as labile remained in the mixture of slurry and sludge. These are apparently their more stable forms as confirmed by the results in Table 2, which illustrate that to approximately 11% of organic carbon compounds it is necessary to add the % proportions of the first and second fraction on the basis of oxidisability according to Chan et al. (2001). Literary sources report that in anaerobically stabilised sludge from municipal waste water treatment plants the sum of lipids, proteins and hemicelluloses amounts to 13–39.6% of dry matter, so it is quite a general phenomenon.

The ion exchange capacity of sludge, pig slurry and their mixture before fermentation, before incubation and after incubation is very low and does not reach the values that are typical for sandy soil. It is increased by anaerobic fermentation along with incubation markedly; still, the level of significance is practically little compared to the level typical for medium-textured soils. The same relations were observed for buffering capacity, which is not surprising. The results document that degradability of the organic part of anaerobically stabilised sludge worsened substantially and that it cannot be improved markedly by the use of soil microorganisms and soil.

We have to draw a surprising conclusion that sludge as a waste from the processes of anaerobic digestion is mineral rather than organic fertiliser; considering its use as organic fertiliser, it is a material of much lower quality than the

original materials. We cannot speak about any improvement of the organic material by anaerobic digestion at all. Their liquid phase, rather than the solid one, can be considered as a fertiliser. If it is taken as a fertiliser in general terms, we do not protest because besides the slightly higher content of mineral nutrients available to plants (mostly nitrogen) it has the higher ion exchange capacity and higher buffering capacity than the material before anaerobic fermentation, but this increase is generally little significant. As the content of available nutrients is so low especially in wet processes of anaerobic digestion with a large amount of liquid fraction and with respect to the present cost of fuels and transportation costs, the use of this waste will be complicated.

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