# Digested residue as a fertilizer after the mesophilic process of anaerobic digestion

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**ABSTRACT**

The aim of this paper is to determine the difference in quality of the digested residue after the process of anaerobic digestion by using different input raw materials. The research was conducted in the Republic of Austria on four facilities for biogas production. The raw materials used for biogas production were chicken manure, pig manure, Sudan grass and organic household waste. The research included chemical analysis and bacteriological tests of the samples taken. It was found that the digested residue in all of the samples, all of which are mildly alkaline, contains a low level of dry matter, 70% of which is organic matter. Biogenic elements were present in moderate concentration; the values of heavy metals were within approved limits. This analysis leads to the conclusion that the digested residues of all input materials can be used in agricultural production, especially so in plant production and grassland cultivation. Mesophilic and thermophilic microorganisms were found in the digested residue samples, but there were no cryophilic microorganisms and no pathogenic bacteria.

**Keywords:** anaerobic digestion; input raw material; digested residue; chemical analyses; bacteriological tests

A recent concern over the effects of the gaseous release of hydrocarbons into the atmosphere on the degradation of the ozone layer and consequential health effects and global warming has led to an increased awareness of the release of methane from animal waste production facilities (Hill et al. 2001). Agro industries produce high quantities of organic wastes that are rich in nutrients and that can well be used in agriculture to conserve and recycle nutrients and to reduce waste discharge and use of chemical fertilizers. However, without sufficient treatment these wastes may pose severe health risks, odor, environmental pollution, and visual problems, or their use may be legally banned altogether. Treatments may help to improve the physical and chemical properties of the waste and reduce its phytotoxicity (Marchaim et al. 1991, Vermeulen et al. 1992). As opposed to fertilization by means of non-fermented organic substances in the agriculture, research has proved the fermented, i.e. digested fertilizers to have a more immediate effect, due to the fact that after the process of digestion, the nutrients are already mineralized and thus can be used by plants more effectively. Each plant has a specific need for nutrients with a definite ratio of the amount of nutrients $N:P_2O_5:K_2O$. For instance, the ratio of nutrients necessary in wheat production is 1.2:1:1.5; the ratio of nutrients necessary in potato production is 1:1:1.8; the ratio of nutrients necessary in grass production is 2.4:1:1.4 (Kaltwasser 1980).

Anaerobic digestion converts a major part of organic nitrogen to ammonia, which is then directly available to plants as a nitrogen source, though also potentially phytotoxic (Wang 1991, Tiquia et al. 1996). It was found that, in addition to nitrogen, the digested residue as a fertilizer could satisfy the plants' need for phosphorus, whereas potassium needs to be added through fertilization. However, if different raw materials are used for the production of biogas, i.e. biofertilizer as a byproduct, the question arises whether the above stated is accurate. Consequently, this paper will investigate the quality of the digested residue as a biofertilizer produced from various raw materials in the process of anaerobic digestion. The raw materials most commonly used for biogas production, such as chicken manure, pig manure, Sudan grass and organic domestic waste were used as the input materials.

The potential health risk associated with the digested residue from biogas plants is partly dictated by the substrate that is treated in the plant. It is an established fact that biowaste contains pathogenic bacteria. Consequently, the digested residue must be proven hygienically safe for both people and animals in order to be recycled. If not, a new way of transmission of pathogens between people and animals could be established (Shih 1987, Fukushi et al. 2003, Sahlström 2003). Since the digested residue that can be used as biofertilizer is a byproduct of biogas production, one of the aims of this research is to determine whether it is possible to use this fertilizer safely.

## MATERIAL AND METHODS

The research was conducted on four biogas plants in Austria, two of which are attached to facilities for intensive chicken and pig farming. One of the biogas plants uses Sudan grass stored next to the plant and another plant uses organic household waste stored in plastic containers. All the facilities included in the research used the same technology of anaerobic digestion – the mesophilic process. The samples for analysis were taken immediately after the process of anaerobic digestion.

The chemical analysis of the digested residue was conducted by means of several different chemical procedures. pH was measured directly in the sample by means of pH-meter with the combined electrode. Electrical conductivity (EC) was determined by the conductometer MA5964 with a combined electrode. Total nitrogen was measured according to Kjeldahl (Kjeltec system 1026 Distilling Unit); ammonia nitrogen ($NH_3$-N) was measured by means of Nesler reagent method according to Jackson (1958) spectrophotometrically at the wavelength of 436 mm. Phosphorus was measured by means of the molybdate-blue method on a UV/VIS spectrophotometer PU 8600, potassium and sodium were measured flame-photometrically, and all the other elements (Ca, Mg, Mn, Zn, Cu, Fe, Pb, Cd) were measured by means of atomic absorber spectrometrically (Jackson 1958).

At the same time, the following bacteriological tests were performed: general test, pathogen bacteria test for *Salmonella* spp. and *Listeria* spp.; the number of bacteria in the samples was recorded as well – Colony Forming Units (CFU).

The incubation of the digested material was done at the temperatures of 4°C, 35°C and 55°C on culture media (XLD, neutral blood agar). At the temperatures of 4°C and 55°C, the incubation lasted for 72 hours, and at the temperature of 35°C, the CFU was made (the number of increased colonies in a 1 ml sample) due to the increase of various bacterial colonies. Isolating the pathogenic bacteria was accomplished by the selective broth culture method. Because the digested residue was intented to be applied in the agriculture, it was sterilized in the autoclaves for a period of 15 min at 121°C; after that, the samples were subjected to a repeated series of bacteriological tests.

Analysis of variance (ANOVA) and correlation analysis was performed by using the SAS software package (SAS Institute 1989). The least significant differences (*LSD*) among mean values were calculated at $\alpha < 0.05$ confidence level. A value of $P$ less than or equal to 0.05 was considered to indicate statistically significant differences.

## RESULTS AND DISCUSSION

After the preparation of samples from the four different biogas production facilities using chicken manure, pig manure, grasses and organic household waste as the input raw materials, they were subjected to the series of suggested chemical analyses. Table 1 displays the mean results of the chemical analyses performed on the digested residues.

If our purpose is to effect an optimal production of the methane bacteria as well as the quick and proper degradation of the digested material into methane and carbon dioxide, we need to make sure that the digested material is pH neutral (7–7.4) (Barlaz et al. 1989). Since the four of our investigated samples showed a mildly alkaline reaction of the digested residue (7.92–8.45) ($P > 0.05$), which was probably caused by an increased calcium (Ca) quantity, it can be concluded that the pH value is within boundaries.

The measuring of the electro conductivity was aimed at determining the total salt content in the solution. The values of the electro conductivity ($P < 0.01$) ranged from 16.96 mS/cm for the samples produced out of grasses to 46.04 mS/cm for the samples produced from the household waste. In the rest of the samples, the electro conductivity ranged from 45.61 mS/cm (chicken manure) and 26.00 mS/cm (pig manure). The cause of the increased value of the electro conductivity in the digested residue produced from the household waste can be traced back to the assumption that the input substrate in the biogas production consisted of food scraps rich with minerals added to the food. This is the reason why the quantity of the most important biogenic elements was measured in the investigated substrates: the quantity of calcium, magnesium and sodium. The quantity of calcium in the digested residue samples ($P < 0.01$) ranges from 2.72% for the grass to 3.2% for the household waste. The quantity of magnesium ($P < 0.01$) in the digested residue was also measured. The highest quantity was found in the sample produced by household waste digestion – 1.15%, and the lowest was found in the sample produced from chicken manure (1.01%). Sodium content ($P < 0.01$) was, as expected, highest in the sample produced out of household waste (0.77%), and the lowest in the sample from chicken manure (0.51%). The found values for biogenic elements in the investigated digested residue were moderate, so they can be used as fertilizer in the agricultural production. After these elements were mutually correlated

Table 1. The means of the chemical analyses results of the digested residue samples with chicken manure, pig manure, grass and organic household waste as the raw material

| No. | Designation of chemical analysis | | Chicken manure | Pig manure | Household waste | Sudan grass | *P*-value |
|---|---|---|---|---|---|---|---|
| 1. | pH directly | | $8.1^{ab} \pm 0.16$ | $7.9^{b} \pm 0.18$ | $8.5^{a} \pm 0.34$ | $8.1^{ab} \pm 0.12$ | > 0.05 |
| 2. | E.C. mS/cm | | $45.6^{a} \pm 2.56$ | $26.0^{b} \pm 2.60$ | $46.0^{a} \pm 1.01$ | $17.0^{c} \pm 1.10$ | < 0.01 |
| 3. | % (dry matter105°C) | | $7.6^{b} \pm 0.35$ | $3.8^{c} \pm 0.24$ | $29.3^{a} \pm 3.21$ | $4.3^{c} \pm 0.31$ | < 0.01 |
| 4. | % $H_2O$ | | $92.4^{b} \pm 0.35$ | $96.2^{a} \pm 0.24$ | $70.7^{c} \pm 3.21$ | $95.8^{a} \pm 0.31$ | < 0.01 |
| 5. | % burning residue (550°C) | | $22.7^{c} \pm 0.27$ | $32.1^{ab} \pm 2.35$ | $33.3^{a} \pm 2.02$ | $29.2^{b} \pm 1.81$ | < 0.01 |
| 6. | %. burning loss | | $77.4^{a} \pm 0.27$ | $67.9^{bc} \pm 2.35$ | $66.7^{c} \pm 2.02$ | $70.8^{b} \pm 1.81$ | < 0.01 |
| 7. | % organic matter | | $75.0^{a} \pm 0.07$ | $65.9^{c} \pm 0.78$ | $64.6^{d} \pm 0.27$ | $68.8^{b} \pm 0.64$ | < 0.01 |
| 8. | % C organic | | $43.3^{a} \pm 0.11$ | $38.0^{c} \pm 0.22$ | $37.4^{d} \pm 0.43$ | $39.8^{b} \pm 0.33$ | < 0.01 |
| 9. | | in the natural sample | $0.4^{b} \pm 0.03$ | $0.3^{c} \pm 0.02$ | $0.9^{a} \pm 0.02$ | $0.3^{c} \pm 0.03$ | < 0.01 |
| 10. | % N | total in dry matter | $5.4^{b} \pm 0.25$ | $6.5^{a} \pm 0.06$ | $3.1^{c} \pm 0.46$ | $6.6^{a} \pm 0.11$ | < 0.01 |
| 11. | | $NH_3$-N | $1.6^{a} \pm 0.21$ | $0.1^{b} \pm 0.02$ | $0.2^{b} \pm 0.05$ | $1.5^{a} \pm 0.18$ | < 0.01 |
| 12. | % $P_2O_5$ | | $1.7^{a} \pm 0.02$ | $1.7^{a} \pm 0.02$ | $1.7^{a} \pm 0.06$ | $1.8^{a} \pm 0.15$ | > 0.05 |
| 13. | % $K_2O$ | | $3.6^{a} \pm 0.03$ | $3.6^{b} \pm 0.03$ | $3.2^{c} \pm 0.06$ | $2.6^{d} \pm 0.01$ | < 0.01 |
| 14. | % Ca | | $3.1^{a} \pm 0.09$ | $2.8^{b} \pm 0.13$ | $3.2^{a} \pm 0.09$ | $2.7^{b} \pm 0.03$ | < 0.01 |
| 15. | % Mg | | $1.0^{b} \pm 0.03$ | $1.0^{b} \pm 0.04$ | $1.2^{a} \pm 0.05$ | $0.9^{c} \pm 0.03$ | < 0.01 |
| 16. | % Na | | $0.5^{c} \pm 0.01$ | $0.6^{b} \pm 0.02$ | $0.8^{a} \pm 0.03$ | $0.5^{c} \pm 0.05$ | < 0.01 |
| 17. | mg/kg Mn | | $181.7^{bc} \pm 27.54$ | $158.3^{c} \pm 17.56$ | $281.7^{a} \pm 10.41$ | $213.3^{b} \pm 10.41$ | < 0.01 |
| 18. | mg/kg Zn | | $72.3^{a} \pm 2.52$ | $65.3^{a} \pm 3.22$ | $55.7^{b} \pm 4.16$ | $68.3^{a} \pm 5.69$ | < 0.01 |
| 19. | mg/kg Cu | | $38.3^{a} \pm 2.08$ | $38.3^{a} \pm 2.08$ | $35.3^{a} \pm 2.52$ | $28.7^{b} \pm 2.52$ | < 0.01 |
| 20. | mg/kg Fe | | $647.7^{a} \pm 11.68$ | $599.7^{ab} \pm 38.28$ | $592.7^{b} \pm 33.56$ | $425.0^{c} \pm 21.79$ | < 0.01 |
| 21. | mg/kg Pb | | $2.5^{a} \pm 0.19$ | $2.0^{b} \pm 0.14$ | $2.2^{b} \pm 0.11$ | $1.1^{c} \pm 0.05$ | < 0.01 |
| 22. | mg/kg Cd | | $0.3^{b} \pm 0.06$ | $0.3^{b} \pm 0.01$ | $0.4^{a} \pm 0.03$ | $0.1^{c} \pm 0.03$ | < 0.01 |

Values are mean ± *SE*; means with the same letter are not significantly different

(Table 2), it was obvious that all of the comparisons of elements are in a positive correlation.

Furthermore, the residue was also tested for the amount of manganese ($P < 0.01$). The highest level of manganese was discovered in the digested residue of household waste as well, amounting to 281.67 mg/kg, and the lowest level in the sample obtained by digesting pig manure, where it amounted to 158.22 mg/kg. We can conclude that, regarding all elements included in the research, the household waste proved to be the raw material with the best mineral value for producing digested residue for fertilization.

Chemical analysis of all digested residue samples showed a low content of dry matter, except in the sample produced from the household waste. The total mass of samples contains 90–95% of water, except for the sample produced from the household waste, which contains 70% ($P < 0.01$). 65–75% of the total dry matter is organic matter, the highest content in the samples where chicken manure was used as the raw material. The high content of organic matter results in the high content of organic carbon – as much as 43% in the sample produced from chicken manure, whereas it is somewhat lower in other samples, ranging between 37% and 39%.

The research of burned residue was conducted in order to determine the content of organic and mineral matter in the samples and it showed that the percentage of burned loss ranged between 22.65% in the digested residue produced from chicken manure, to 33.33% in the digested residue produced from household waste ($P < 0.01$).

The literature offers information that the ratio of N:P:K in the digested residue originating from various raw materials is approximately 3:1:0.3. This ratio indicates that the digested residue can satisfy

Table 2. Correlation values for the samples of the digested residue

| The researched elements in the digested residue | | Correlation coefficient *r* | *P*-value |
|---|---|---|---|
| Ca | Mg | 0.84 | 0.0006 |
| Ca | Na | 0.58 | 0.048 |
| Mg | Na | 0.77 | 0.0036 |

the plants' needs for nitrogen and phosphorus, whereas potassium must be additionally supplied. In order to determine the feasibility of utilization of the researched digested residues in agriculture as fertilizers, the relative ratios of basic biogenic elements, nitrogen ($P < 0.01$), phosphorus ($P < 0.01$) and potassium ($P < 0.05$) were calculated for chicken manure N:P:K = 3.09:1:2.17; for pig manure N:P:K = 3.83:1:2.09; for grasses N:P:K = 3.65:1:1.42 and for household waste N:P:K = 1.88:1:1.94.

Any substance found in the arable land in the concentration that temporarily or permanently endangers its primary role of a good habitat for crops or natural herbal life is considered harmful. The use of digested residues is allowed on crop land, meadows and lowland pastures whose soils contain one of the heavy metals and persistent organic harmful matter under 50% of the limit values set by the Regulations on ecological production of plants and plant products in the Republic of Croatia (The Public Gazette 91/2001). Table 1 shows that the quantities of the heavy metals determined by analysis in all samples of digested residues are in concentrations lower than those prescribed, and thus satisfy the requirements of the Republic of Croatia (as well as the European Union) and can be used in the agricultural production. The comparison of correlations of heavy metals in the researched samples is also interesting. If the values of heavy metals in the researched samples are correlated, the resulting values show that they are positively correlated with a high level of significance (Table 3).

The analysis of the digested residue with chicken manure as the raw input material showed that after the period of 72 hours and at the temperatures 4°C and 55°C there was no bacterial increase, whereas the same sample showed a large increase of bacterial colonies at the temperature of 35°C. Consequently, the procedure for determining CFU was performed. The tested samples' CFU value was approximately 120 grown mesophilic colonies at the rarefaction of $10^{-8}$. The isolated bacteria belonged to the genera *Escherichia*, *Bacillus* and *Enterococcus*. No bacteria from the genera *Salmonella* and *Listeria* have been isolated by means of the selective broth cultures method.

The tests performed on the digested residue where the input raw material was pig manure indicated that in the period of 72 hours there was no increase in the bacterial colonies in the culture media at 4°C. A small number of bacteria from genera *Bacillus* and *Micrococcus* grew in the culture media in the period of incubation at 35°C. The number of bacteria approximated $22 \times 10^4$ (CFU). During the incubation period, some thirty colonies of *Bacillus* genus members grew at the temperature of 55°C. The methods of enriching and oversaturation in the selective broth culture media did not prove the presence of the bacteria from the genera *Salmonella* and *Listeria*.

The tests performed on the digested residue where the input raw material was grass indicated that in the 72-hour incubation period there was no increase in the bacterial colonies in the culture media at 4°C. A large number of bacterial colonies from genera *Bacillus*, *Micrococcus* and *Nocardia* grew at 35°C. The number of bacteria (CFU) ranged from $15 \times 10^6$ to $20 \times 10^7$. A large number of colonies of *Bacillus* genus members grew during the incubation period at the temperature of 55°C. The methods of enriching and oversaturation in the selective broth culture media did not prove the presence of the bacteria from the genera *Salmonella* and *Listeria*.

The tests performed on the digested residue with domestic waste as the input raw material indicated that in the period of 72 hours there was no increase in the bacterial colonies in the culture media at 4°C. A large number of bacteria from genera *Bacillus*, *Nocardia*, *Escherichia*, and *Micrococcus* grew in the culture media in the period of incubation at 35°C. The number of bacteria approximated $30 \times 10^6$ (CFU). A large number of colonies of *Bacillus* genus members grew during the incubation period at the temperature of 55°C. The methods of enriching and oversaturation in the selective broth culture media did not prove the presence of the bacteria from the genera *Salmonella* and *Listeria*.

Table 3. Correlation values for the analyzed samples regarding the heavy metals

| Heavy metals in the digested residue | | Correlation coefficient *r* | *P*-value |
|---|---|---|---|
| Fe | Pb | 0.93 | < 0.0001 |
| Fe | Cu | 0.92 | < 0.0001 |
| Fe | Cd | 0.79 | < 0.0001 |
| Pb | Cu | 0.81 | 0.0012 |
| Pb | Cd | 0.82 | 0.0011 |
| Cu | Cd | 0.70 | 0.011 |

However, the optimal pH for the development of pathogenic bacteria *Salmonella* spp. is 6.2–7.2 (Fields 1979). Therefore, in order to prevent the development of pathogenic bacteria and the recontamination, the digested material should be sterilized (Sahlström 2003). The digested residue samples of the effluent were sterilized in the autoclave after the tests were conducted. A repeated bacteriological test of all samples was performed afterwards. The culture media did not show any bacterial growth; i.e. the samples of the effluent manure were sterile. A procedure of this kind (sterilization) should be obligatory when utilizing the effluent manure in order to prevent possible infections of people and animals.

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**ABSTRAKT**

**Využití fermentovaných odpadů po mezofilním procesu anaerobní digesce**

Cílem publikace bylo stanovit rozdíl v kvalitě fermentovaných odpadů v procesu anaerobní digesce za využití různých vstupních surovin. Výzkum proběhl v Rakousku na čtyřech zařízeních pro výrobu bioplynu. Jako surovina pro výrobu bioplynu byla použita kejda drůbeže, kejda prasat, sudanská tráva a organické domovní odpady. Výzkum zahrnuje chemické analýzy a bakteriologické testy odebraných vzorků. Bylo zjištěno, že vyhnilé odpady všech vzorků jsou mírně alkalické, mají nízký obsah sušiny, přičemž 70 % tvoří organická hmota. Biogenní prvky byly přítomny v poměrně nízkých koncentracích. Hodnoty obsahů těžkých kovů se pohybovaly v rámci povolených limitů. Analýzy vedou k závěru, že fermentované odpady všech sledovaných vstupních surovin mohou být použity v zemědělství, zejména v rostlinné výrobě, na lukách a pastvinách. Ve vzorcích vyhnilých odpadů byly nalezeny mezofilní a termofilní mikroorganismy. Nebyly však zjištěny žádné kryofilní mikroorganismy ani patogenní bakterie.



**Klíčová slova:** anaerobní digesce; vstupní surovina; fermentovaný odpad; chemická analýza; bakteriologické testy



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