Nauka Przyroda Technologie

2008
Tom 2
Zeszyt 3

Dział: Melioracje i Inżynieria Środowiska
ISSN 1897-7820 http://www.npt.up-poznan.net/tom2/zeszyt3/art_13.pdf

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ESTIMATION OF CORRELATION BETWEEN TOTAL MICROORGANISM COUNTS AND DEHYDROGENASE ACTIVITY MEASUREMENT IN COMPOST FROM ANAEROBIC SEWAGE SLUDGE

Summary. The study presents a measurement method of dehydrogenase activity in composts from anaerobic sewage sludge. The results of measurements were later compared with the data obtained from assays of the total numbers of microorganisms (mesophilic and thermophilic bacteria, moulds and yeasts) using the method of flood culture. The authors examined the correlation between the two methods employed to assess the activity and the physiological status of microorganisms settling compost. The performed statistical analysis failed to show any correlations between the total number of microorganisms determined by the traditional and plate methods and the measurement of dehydrogenase activity. The determination of the total number of microorganisms does not provide a full answer to the question of the physiological status of microorganisms carrying out the composting process. Neither of the methods of dehydrogenase activity in composts can be used separately and optionally to complete activity and the physiological status of microorganisms settling compost estimate.

Key words: dehydrogenases, compost, anaerobic sewage sludge, microorganisms

Introduction

Classical measurement methods of the microbiological activity in composts involve the determination of the numbers of mesophilic and thermophilic bacteria as well as the determination of the numbers of moulds and yeasts. However, the above methods say nothing about the physiological activity of microorganisms in composts but only about their numbers. In addition, in some cases microorganisms determined as mesophilic exhibit thermo-tolerant properties and inflate the results of thermophilic bacteria. It is

not very uncommon that the sum of meso- and thermophilic bacteria exceeds significantly the maximum theoretical number of bacteria in a given unit of volume. Another problem concerns the determination of moulds which grow in the form of mycelium; therefore it is difficult to talk about numbers. One more issue worth mentioning is the production of spore forms by microorganisms settling composts (JERIS and REGAN 1973) because majority of bacteria living in composts are rod-like bacteria capable of producing endospores in unfavourable conditions. On the other hand, fungi produce huge quantities of spores. Therefore, all dormant forms occurring in composts may develop into vegetative forms on the culturing media and inflate the results of determinations considerably (KUNICKI-GOLDFINGER 2000).

The most important changes that take place in composts comprise changes of carbon and nitrogen compounds. These are complex enzymatic processes. Following the decomposition of organic nitrogen (ammonification), ammonia nitrogen is formed which is either used by microorganisms to synthesise their biomass or can be oxidised in the nitrification process.

Ammonia formed during this process is the main cause of nitrogen losses during composting. In addition, nitrogen losses can also be caused by the presence of anaerobic centres in the compost heap in which microbiological nitrate reduction (denitrification) takes place (JETER and INGRAHAM 1981, KRZYWY et AL. 2000).

From the point of view of the final product, the humification process is the second (after mineralisation) most important process occurring during composting. It is a biochemical process during which the organic components of the biomass undergo synthesis into humus compounds. These compounds, due to their ion-exchange sorption properties, contribute to the improvement of soil properties (DROZD et AL. 1996). Therefore, in order to evaluate the physiological condition of microorganisms, it is essential to determine the activity of oxidation-reduction enzymes.

The purpose of the research was to examine the correlation between the two methods employed, to assess the activity and the physiological status of microorganisms settling compost: the method of dehydrogenase activity in composts and the method of flood culture to total microorganism counts (mesophyllic and thermophilic bacteria, moulds and yeasts) estimation.

Materials and methods

The principle of the method of determination of dehydrogenase activity (TTC test)

Triphenyltetrazole chloride (TTC) is a colourless compound. As a hydrogen acceptor, it is reduced – under the influence of hydrogen – into red-coloured triphenylformazan (TF). The intensity of colour is directly proportional to the amount of dehydrogenases in the compost or, to be more precise, in the internal cellular mass of the microorganisms making up the compost (Figs. 1, 2). In other words, the results of this determination should be strongly correlated with the numbers and activity of bacteria contained in the compost (HERMANOWICZ 1999).

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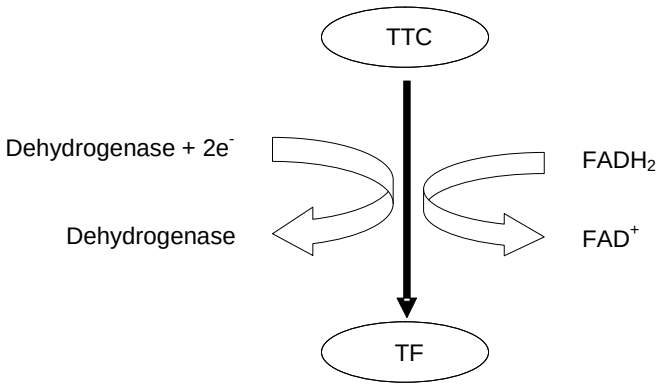

Fig. 1. Scheme of dehydrogenase enzyme and triphenyltetrazolium chloride reaction
Rys. 1. Schemat przebiegu reakcji enzymu dehydrogenazy z chlorkiem trifenylotetrazoliowym

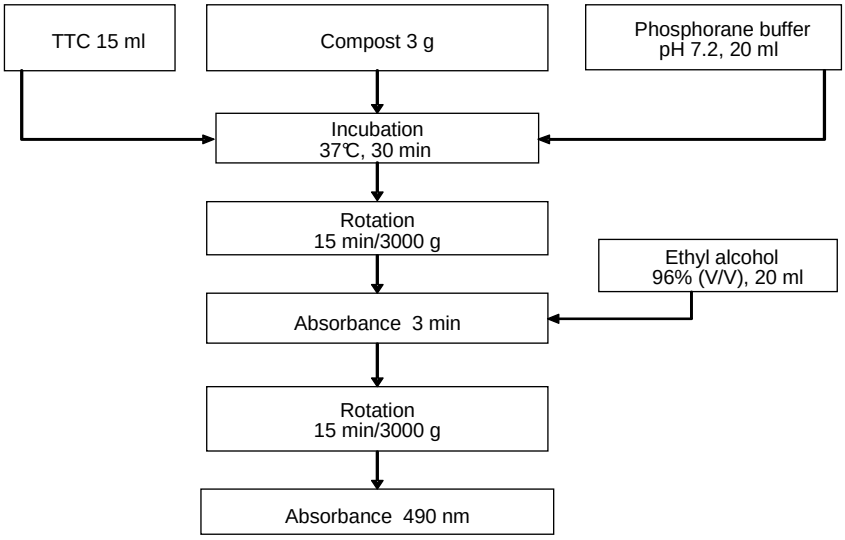

Fig. 2. Method of dehydrogenases activity in compost estimation
Rys. 2. Metoda oznaczania aktywności dehydrogenaz w kompoście

The experimental material comprised three variants of composts made of anaerobic sewage sludge, obtained from the sugar industry manufacture in Kościan: **I –** with addition of wheat straw, **II** – with addition of farmyard manure and **III –** anaerobic sewage sludge.

Methodological assumption of the composting experiment

The oxygen method was adopted to perform the experiment supplemented with the addition of structure-forming organic materials (wheat straw and farmyard manure). Experiments were carried out for the period of 180 days.

The piles were covered by a special hydrophobic, porous material, which was air permeable, in this way the humidity of compost was kept on the same level. The initial moisture content in the individual container ranged from 57 to 65%. The water content in individual compost was regulated in the case of water losses caused by evaporation (albeit limited, due to the covering of compost with hydrophobic material) every 10 days on the basis of dry matter determination and the observation of the compost internal structure. The compost material was mixed during the process of compost formation and it was additionally aerated twice on day 10 and 20 of the composting process.

The experimental composts were composed on the basis of weight ratios calculated per the amount of carbon and nitrogen brought into the compost volume in each of their components. In the course of the performed experiment, samples were collected nine times from each compost pile according to the following schedule: on the day of pile formation and on days 10, 20, 30, 45, 60, 90, 120 and 180.

The prepared piles had the following weight composition (kg DM):

– pile I – anaerobic sewage sludge (209 kg) + farmyard manure (143.4 kg),
– pile II – anaerobic sewage sludge (209 kg) + straw (6.9 kg),
– pile III – anaerobic sewage sludge (209 kg).

The total content of microorganisms was determined by the Koch plate method (TROJANOWSKA et AL. 1996).

In order to determine organic matter, after the determination of dry matter, the sample was incinerated at the temperature of 550°C in a muffle furnace and cooled down in a desiccator and weighed (HERMANOWICZ 1999).

The total nitrogen was estimated by the Kjeldahl method Kjeltec System 1026 Distilling Unit Tecator (KOROL and KOROL 1992).

Total carbon was assessed by the Tiurin method (POŁEĆ 1999).

Results and discussion

The value of the C:N ratio of approximately 15 confirms the stability of the compost mass, while the value below 12 indicates a high level of maturity of the compost (DROZD and LINCZAR 1996, EILAND et AL. 2001).

The result of transformations of carbon and nitrogen compounds was a steady narrowing of C:N ratios. Particularly significant changes were observed ($p < 0.034$) in the compost made up of anaerobic sludge + farmyard manure, where the C:N ratio narrowed from 27:1 to 10.5:1. On the other hand, in the case of the compost supplemented with straw, the C:N ratio narrowed from 27:1 to 12:1 (Fig. 3).

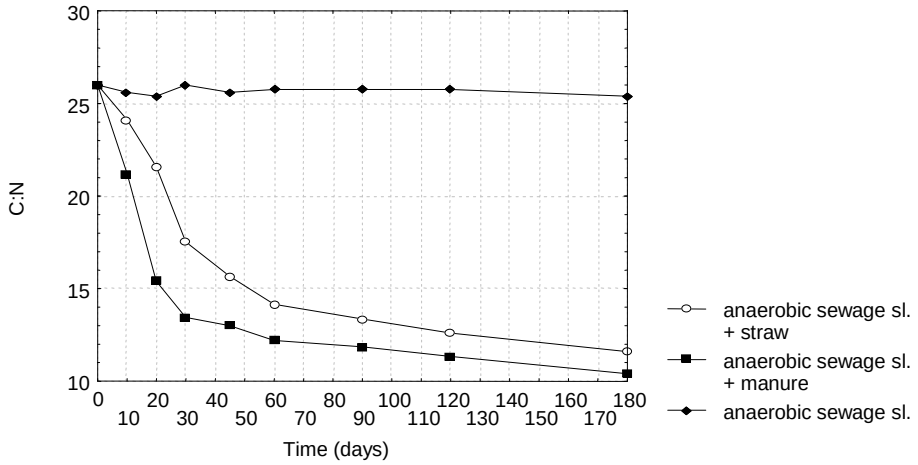

Fig. 3. Changes of C:N ratio in composts during biodegradation process
Rys. 3. Zmiany stosunku C:N w kompostach podczas procesu biodegradacji

The analysis of the content of organic matter revealed that ($p < 0.018$) in the case of the compost made up of anaerobic sludge + farmyard manure, this process was more intensive. The greatest difference was recorded from day 30 of composting onwards and this condition remained until the end of composting. The content of organic matter in this compost decreased by 25%, while in the compost supplemented with straw – by 19% (Fig. 4).

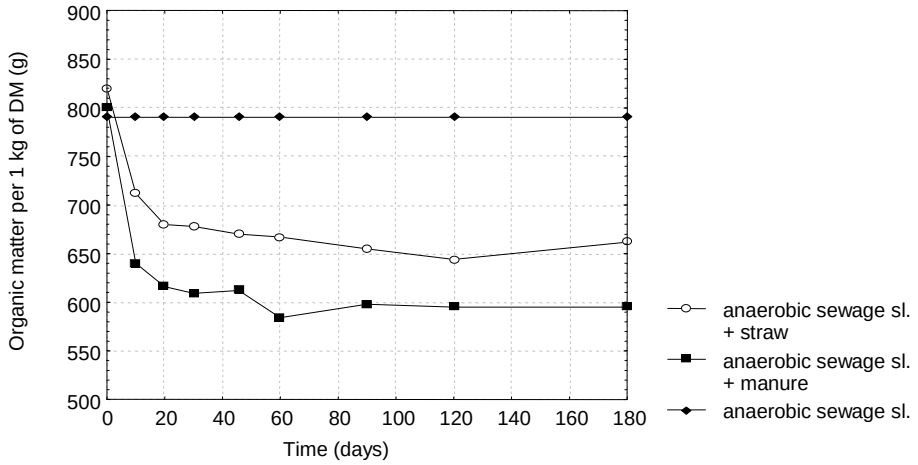

Fig. 4. Changes of organic matter content in composts during biodegradation process
Rys. 4. Zmiany zawartości materii organicznej w kompostach podczas procesu biodegradacji

According to TIQUIA et AL. (2002), the activity of dehydrogenases allows monitoring of the physiological condition of microorganisms in the compost. Already at the beginning of the experiment, the activity in the compost made up of anaerobic sludge + farmyard manure and anaerobic sludge + straw was recorded at the level appropriately of 1.4 and 1.3 mM TF per 1 kg DM. As the biodegradation of the composted material advanced, the activity of dehydrogenases clearly increased.

In 30 days in compost with addition of manure to 3.5 mM TF per 1 kg of DM of compost, and in variant with straw to 2.8 mM TF per 1 kg of DM of compost increased. Then gradually decrease in dehydrogenase activity in composts was indicated (Fig. 5). The activity of dehydrogenase in compost with addition of manure was stronger than in compost with straw ($p < 0.05$).

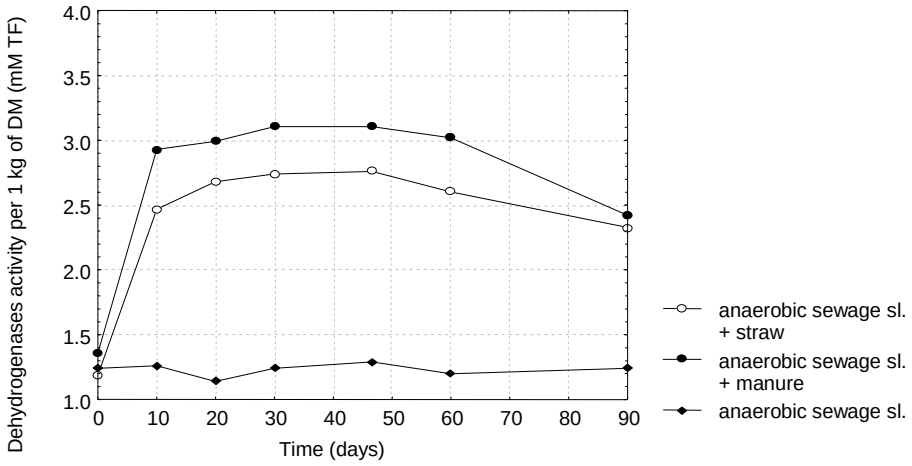

Fig. 5. Influence of composting duration over the changes of dehydrogenases activity from anaerobic sewage sludge composts
Rys. 5. Zmiany aktywności dehydrogenaz w kompostach podczas procesu biodegradacji

Graphic conclusion – correlation of total amount of microorganisms and dehydrogenase activity

Recapitulating, it should be stated, that no statistically significant correlation was found between the total number of cells of meso- and thermophilic bacteria and the activity of dehydrogenases in any of the examined variant of compost ($p > 0.23$) (Figs. 6, 7, 8).

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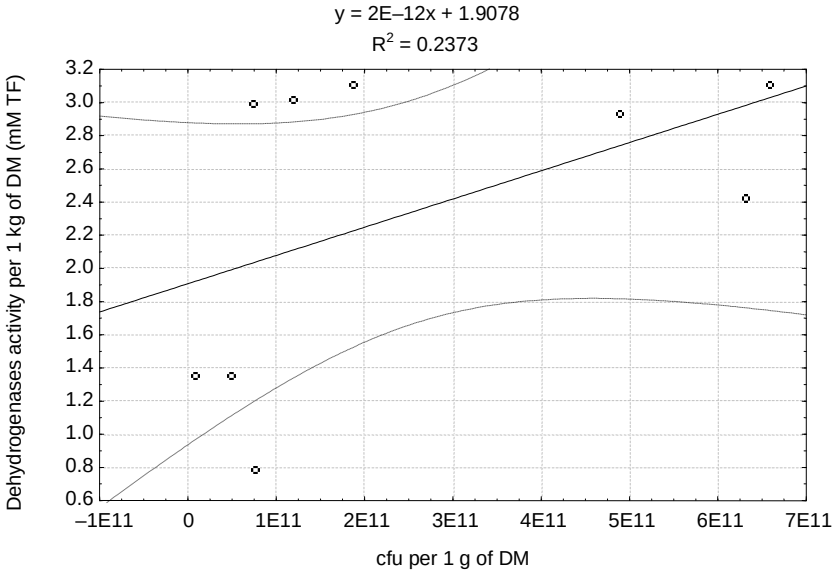

Fig. 6. Correlation of total amount of microorganisms and dehydrogenases activity in compost of anaerobic sewage sludge + manure
Rys. 6. Zależność pomiędzy ogólną liczbą komórek bakterii a aktywnością dehydrogenaz w kompoście z beztlenowego osadu ściekowego + obornik

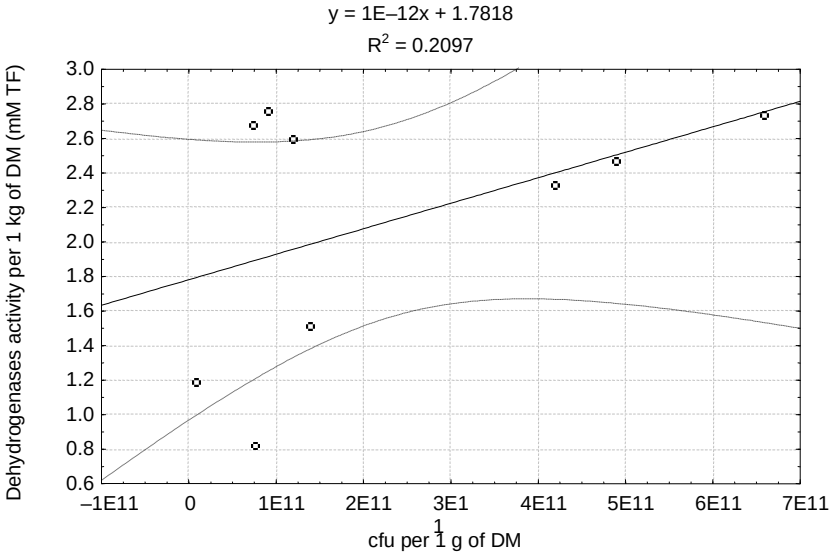

Fig. 7. Correlation of total amount of microorganisms and dehydrogenases activity in compost of anaerobic sewage sludge + straw
Rys. 7. Zależność pomiędzy ogólną liczbą komórek bakterii a aktywnością dehydrogenaz w kompoście z beztlenowego osadu ściekowego + słoma

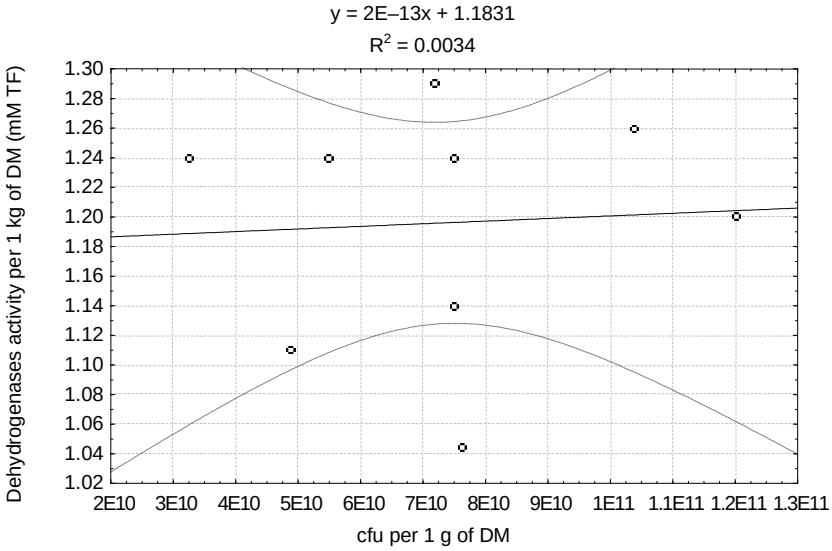

Fig. 8. Correlation of total amount of microorganisms and dehydrogenases activity in compost of anaerobic sewage sludge
Rys. 8. Zależność pomiędzy ogólną liczbą komórek bakterii a aktywnością dehydrogenaz w kompoście z beztlenowego osadu ściekowego

Conclusion

The results of the assessed research concerning the activity and the physiological status of microorganisms settling compost, presented that, neither the method of dehydrogenase activity in composts nor the method of flood culture to total microorganism counts (mesophilic and thermophilic bacteria, moulds and yeasts) estimation, can be used separately and optionally to complete activity and the physiological status of microorganisms settling compost estimate.

References

DROZD J., LINCZAR M., PATORCZYK-PYTLIK B., RABIKOWSKA B., 1996. Zmiany w składzie chemicznym kompostów z odpadków miejskich w czasie ich kompostowania. Zesz. Probl. Post. Nauk Roln. 437: 131-137.

EILAND F., KLAME M., LIND A.M., BAATH E., 2001. Influence of initial C/N ratio and microbial composition during long term composting of straw. Microb. Ecol. 41: 272-280.

HERMANOWICZ W., 1999. Fizyczno-chemiczne badanie wody i ścieków. Arkady, Warszawa.

JERIS L., REGAN E., 1973. Controlling environmental parameters for optimum composting. J. Compost Sci. 14: 10-15.

JETER R.M., INGRAHAM J.L., 1981. The denitrifying prokaryotes. In: The prokaryotes. Vol. 1. Ed. M.P. Starr. Springer, Berlin: 913-925.

KOROL J., KOROL R., 1992. Badanie składu i właściwości osadów ściekowych. Wyd. PIOŚ, Dębe.

KRZYWY E., WOŁOSZCZYK C., IŻEWSKA A., 2000. Przemiany zawartości azotu amonowego i azotanowego zachodzące w kompostach z komunalnych osadów ściekowych podczas procesu ich rozkładu. Folia Univ. Agric. Stetin. 84: 211-216.

KUNICKI-GOLDFINGER W., 2000. Życie bakterii. Wyd. Nauk. PWN, Warszawa.

POŁEĆ B., 1999. Proekologiczna gospodarka wodno-ściekowa cukrowni. Część III. Metody badań wód, ścieków i procesów unieszkodliwiania zanieczyszczeń. Fundacja „Rozwój SGGW", Warszawa.

TIQUIA S., JUDY WAN H.C., NORA TAM F.Y., 2002. Dynamics of yard trimmings composting as determined by dehydrogenase activity, ATP content, arginine ammonification, and nitrification potential. Process Biochem. 37: 1057-1065.

TROJANOWSKA K., GIEBEL H., GOŁĘBIEWSKA M., 1996. Mikrobiologia żywności. Wyd. AR, Poznań.

OKREŚLENIE ZALEŻNOŚCI POMIĘDZY LICZEBNOŚCIĄ MIKROORGANIZMÓW A POMIAREM AKTYWNOŚCI DEHYDROGENAZ W KOMPOŚCIE Z BEZTLENOWEGO OSADU ŚCIEKOWEGO

Streszczenie. W pracy przedstawiono metodę pomiaru aktywności dehydrogenaz w kompostach z beztlenowego osadu ściekowego i porównano otrzymane wartości z danymi uzyskanymi z oznaczenia ogólnej liczby mikroorganizmów (bakterii mezofilnych i termofilnych, pleśni i drożdży) metodą posiewu zalewowego. Zbadano korelację pomiędzy dwiema zastosowanymi metodami w celu sprawdzenia aktywności i stanu fizjologicznego mikroorganizmów zasiedlających kompost. Na podstawie przeprowadzonej analizy statystycznej wykazano brak jakiejkolwiek zależności pomiędzy oznaczeniem ogólnej liczby mikroorganizmów metodą tradycyjną – płytkową – a pomiarem aktywności dehydrogenaz. Oznaczenie ogólnej liczby mikroorganizmów nie daje pełnej informacji na temat stanu fizjologicznego mikroorganizmów prowadzących proces kompostowania, podobnie jak samo oznaczenie aktywności dehydrogenaz w kompoście nie prezentuje pełnego obrazu aktywności mikrobiologicznej kompostu.

Słowa kluczowe: dehydrogenazy, kompost, beztlenowy osad ściekowy, mikroorganizmy

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Accepted for print – Zaakceptowano do druku:
27.06.2008

For citation – Do cytowania:
Piotrowska-Cyplik A., Cyplik P., 2008. Estimation of correlation between total microorganism counts and dehydrogenase activity measurement in compost from anaerobic sewage sludge. Nauka Przyr. Technol. 2, 3, #13.