Coll. Antropol. **38** (2014) 2: 487–492
Original scientific paper

Efficiency of Alkaline Hydrolysis Method in Environment Protection

Tajana Kričkа[1], Ivan Toth[2], Sanja Kalambura[2] and Nives Jovičić[2]

[1] University of Zagreb, Faculty of Agriculture Zagreb, Zagreb, Croatia
[2] University of Applied Science Velika Gorica, Velika Gorica, Croatia

A B S T R A C T

Development of new technologies for the efficient use of proteins of animal origin, apart from heat treatment in rendering facilities that was used to date, has become the primary goal of the integral waste management system. The emergence of bovine spongiform encephalopathy in Europe and in the World in the 1990s opened up new questions regarding medical safety and use of meat bone meal in the animal feed, which is produced by processing animal waste. Animal waste is divided into three categories, out of which the first category is high-risk waste. Alkaline hydrolysis is alternative method for management of animal by-products not intended for human diet and imposes itself as one of the solutions for disposal of high-risk proteins. The paper will present the analyses of animal by-products not intended for human diet treated in laboratory reactor for alkaline hydrolysis, as one of the two recognized methods in EU for the disposal of this type of material and use in fertilization.

***Key words**: alkaline hydrolysis, animal by-product, bovine brain, high risk material*

Introduction

Animal waste in the overall system of waste management does not represent a special problem. However, pollution of soils, water and air that it can cause if incorrectly disposed in nature classifies it very high on the scale of risky and harmful waste[1]. Through uncontrolled degradation in nature, namely, apart from unpleasant smell, the result is the creation of sulphides, ammoniac, methane, carbon(IV)oxides, mercaptan, etc. Less known but no less important, is that in unsanitary conditions various infectious diseases may arise such as anthrax, Q-fever, brucellosis, rabies, salmonella, trichinosis, etc.[1]. The most affected areas are precisely the areas of intensive livestock and slaughterhouse production. According to the data of the World Organization for Food and Agriculture (FAO) the production of meat has reached in the year 2011 the amount of 11,841,000 tonnes and has been marking steady growth. The number of slaughtered animals in the Republic of Croatia in the year 2011 was 43 million chickens, 262 thousand cattle, 71 thousand goats, 422 thousand sheep and 1.77 million pigs and piglets[2]. Taking into consideration the total mass of the animal, only 50–58% of this mass is intended for human food chain, whereas 25% is non-edible by-product of slaughter[3]. At the same time, the balance of animal waste is increased also by intensive livestock breeding on the farms, where there is daily death toll of animals[1]. The proteins from the components of animal origin until the occurrence of bovine spongiform encephalopathy (BSE) represented an irreplaceable element in animal nutrition[4]. With the discovery of prions in cattle, the spongiform encephalopathies were related to other transmissive encephalopathy's that occur also in people in the form of Creutzfeldt-Jacob disease (CPS). A connection has been set between the prions and the use of animal proteins such as e.g. of meat bone meal (MBM) in animal feeding with the human diet, meat and meat products of infected animals[5]. For the most part this waste is generated by satisfying the human nutritional needs, where the main place belongs to meat and animal products. With the advent of BSE Europe is facing major problems, and this has significantly tightened the criteria for processing of animal waste. The introduction of EC Directive 1774/2002 has ordered a strict implementation of proper and hygienic handling of carcasses and animal waste. A division has been defined into high, medium and low risk category. Although there are no registered cases of BSE,

Received for publication November 29, 2013

in the process of direct accession into the European Union, the methods of disposal in heat treatment facilities – rendering plants, have been defined. This realizes the legal basis for systemic and safe disposal of this type of waste, also giving the possibility to introduce, along with the existing treatment technologies also new disposal methods.

With the entry into force of the ban of the use of animal proteins, including the MBM in animal nutrition and at the same time with the significant increase of the consumption of meat and meat products, opens the question of the development of different methods of disposal of this type of waste which might eventually supplement or replace the existing rendering processing. In this regard the previous studies in the area of the development of different technologies such as biogas production[6,14,16] and alkaline hydrolysis, speak in favour of useful exploitation of animal products for energy purposes or about the possibilities of usage in plant fertilization. Regarding the high significance of collection, systemic recycling and recovery of waste in a way that would provide beneficial use of all its components, various technologies for the recovery of animal waste have been developed.

Places of animal waste origin

Animal waste is generated at slaughterhouses, plants for the processing of meat, fish, eggs, milk, intestines, in cold storage facilities, warehouses, one-day chicks hatcheries, markets, meat stores, fish-markets, restaurants and other public food facilities, facilities for breeding and keeping of animals, zoos, hunting industry, port terminals, border crossings, transport of animals and products and other places where animals are kept and bred and where animal food items are produced. Animal waste is produced also in the facilities throughout Croatia in 226 slaughterhouses of hoofed animals, 77 poultry slaughterhouses, 611 meat processing plants, 315 facilities for cooling and freezing of meat and meat products, 38 fresh water fish farming ponds, 33 mariculture facilities for marine fish farming, 75 shellfish farms, 41 fish processing facilities, 67 places to take over the fish from catch, 11 shellfish distribution centres, 3 snail farms, 31 refrigerators for cooling and freezing of fish and fish products, 57 dairies, 44 table egg farms, one facility for egg processing, 26 one-day chick hatcheries and a number of facilities that also produce animal waste[7,15]. There is also a significant number of agricultural households that keep and breed different types of domestic animals, and they are usually not included in the system of organized veterinarian supervision.

Animal waste quantities in the Republic of Croatia

The development of intensive livestock production based on industrial food for animals has created great need for protein animal feed. This has actualized in the history the possibility of processing animal waste in meat and meat bone meal. Based on the annual reports of the Croatian Bureau of Statistics, the number of bred livestock in 2011 marked a slight decline, whereas the number of poultry has been staying already for years at approximately the same values (Table 1).

TABLE 1
NUMBER OF LIVERSTOCK AND POULTRY AS OF 31 DECEMBER 2011 IN THOUSANDS OF HEAD (DZS, 2012)

Number of livestock and poultry expressed in head			
Name	2010	2011	Relation / increase
Cattle	444,000	446,000	2,000
Pigs	1,231,000	1,233,000	2,000
Sheep	630,000	639,000	900
Poultry	9,470,000	9,523,000	53,000

The number of livestock and poultry in 2011 increased in relation to 2010. The growth rate of cattle, sheep and poultry marked an increase and the growth rate of pigs marked a decline. Thus, compared to 2010 the growth rate of cattle in 2011 increased by 19%, the growth rate of pigs decreased by 4%, the growth rate of sheep increased by 18%, whereas the growth rate of poultry increased by 1% (Table 2).

TABLE 2
LIVERSTOCK PRODUCTION IN THE REPUBLIC OF CROATIA FOR 2011 (DZS, 2012)

Livestock production in RH for 2011			
Name	Production in 2010	Production in 2011	Index 2011/2010
Cattle growth rate	82,000	98,000	119
Pigs growth rate	178,000	172,000	96
Sheep growth rate	11,000	13,000	118
Poultry growth rate	122,000	113,000	101

Measuring unit: tonnes

The growth rate is calculated by adding the difference of the volume of import and export to the mass of slaughtered livestock and the difference of the mass of the herd at the end and at the beginning of the observed time period, i.e. in 2011. In 2011 in organized slaughtering of cattle and poultry at the facilities specialized for this purpose 245,944 thousand head of cattle, 48,992 thousand of sheep, 1,253,660 thousand of pigs and 35,348,892 of poultry were slaughtered (Table 3).

The total net weight of the slaughtered livestock and poultry amounted in the year 2011 to 203,438 tonnes whereas the meat yield of the slaughtered livestock and poultry, expressed as a percentage ratio of the mass of slaughtered livestock and poultry (net weight) and mass of live cattle and poultry (gross mass), has been specified separately for each species. Based on the obtained data the total quantity of animal waste, generated in the

TABLE 3
SLAUGHTERING OF CATTLE AND POULTRY IN SLAUGHTERHOUSES IN 2011 (DZS, 2012)

Slaughtering of livestock and poultry in slaughterhouses in 2011			
Name	Number of head	Net weight/ tonnes	Meat yield
Cattle	245,944	53,800	55
Sheep	48,992	578	47
Pigs	1,253,660	88,182	78
Poultry	35,348,892	60,878	71

slaughtering facilities, is estimated at more than 125,000 tonnes which includes all three categories.

Animal waste and mad cow disease

It is well-known today that the infection of cattle by BSE occurs in the very early stage of their life, but after birth[8]. It seems that BSE was present to a large extent in the animal feed after 1980, and that the livestock got relatively easily infected. The incubation phase of this disease takes about five years, but in case of some animals it may take even up to ten years. Since Great Britain was the first that had this big problem with BSE the ban of using meat bone meal in Great Britain came into force in July 1988, but large quantities of MBM remained on the agricultural farms also after this date. However, in 1996 strict measures came into force and they included the ban on using meat bone meal in the livestock diet, and ordered that all of the material that could contain MBM be removed from the households and be destroyed. Furthermore, since that year, MBM has been allowed exclusively as fertilizer on soils that are not intended for animal breeding. The export of MBM from Great Britain has never been prevented. Regarding bans that occurred in Great Britain in 1988, large quantities of MBM were exported into the EU countries, which eventually resulted also in the ban on export of MBM into EU countries. However, except for the USA and Canada (that signed an agreement on non-export of this material), the meat bone meal continued to be exported into the countries not members of the EU. As response to this, in mid-1990s FAO suggested the ban on using MBM in all the countries in the world. After banning the usage of meat bone meal, even for the low-risk raw materials, in the EU occurred the need for adequate form of recovering of large quantities of MBM.

In the period from 1988 to 1996, Great Britain brought several regulations:

- 1988: The Bovine Spongiform Encephalopathy Order 1988 (SI 1988/1039), which bans the sales of certain raw materials for feeding the ruminants,
- 1990: The EC Decision to ban exports of SBO and other tissues from the UK (90/200/EEC), which bans the export of animal tissue from Great Britain,
- 1996: The Bovine Spongiform Encephalopathy (Amendment) Order 1996 (SI 1996/962), which bans sales or purchase of meat bone meal or any other raw material for feeding of animals that include feeding of domestic animals, horses and fish,
- 1996: The Fertiliser (Mammalian Meat and Bone Meal) Regulations 1996 (SI 1996/1125), which bans the usage of MBM as fertilizer or as component of fertilizer on farmlands,
- 1996: The Feed Recall Scheme, which has the aim of collecting and disposing of meat bone meal or food for animals that contains MBM, and which are still found on the farms,
- 1996: The Bovine Spongiform Encephalopathy Order 1996 (SI 1996/2007), supplement to regulations from 1991, which stipulates new measures regarding animals exposed to BSE, then ban on keeping MBM in the places where animals are kept, as well as the ban on disposal of MBM and cleaning and disinfection of places, vehicles and equipment where MBM is produces, stored or used.

Numerous measures regarding the usage of meat bone meal have been introduced in the meantime in EU. First, these came into force in certain member countries, but over time the European Commission modified them and implemented them itself. The biggest problem was that many EU countries have not implemented adequate measures regarding the usage of MBM. For instance, in 1996 French Government brought strict measures and implemented them, whereas German Government brought only negligible precautionary measures in certain parts of the country that came into force as late as in 2000.

Alkaline hydrolysis method

Alkaline hydrolysis as a method of animal waste disposal, of animals infected by transmissive encephalopathies, has been implemented in the USA since 1993 at the Faculty of Veterinary Medicine Alabany[9], and the majority of the set units was used in the beginning for disposal of CPS infected deer[9]. Alkaline hydrolysis gives positive results in harmless destruction of prions and as such it proved very efficient in the disposal of high-risk animal waste. This is a process in which, in alkaline medium, bigger molecules are degraded into smaller ones, and it represents an important step in splitting of protein chains like prions, causes of BSE. The alkaline hydrolysis process can be catalyzed by enzymes, metal salts, acids and alkalis – potassium and sodium hydroxide water solutions. The hydrolysis process is accelerated when the system is heated, and the optimal results are achieved at a temperature of 150 °C. Amino acids, as the basic building units of all proteins are interconnected by peptide links creating the polypeptide chains. During the alkaline hydrolysis process, the peptide links are broken down leaving small peptide chains and amino acids in the form of potassium and sodium salts[3].

Depending on the final purpose, hydroxide is added in the proportional ratio with the quantity of waste and the entire mixture is continuously heated and mixed from three to six hours at a pressure of four bars in adequate stainless steel reactors[10].

Infectious tissues and alkaline hydrolysis

Alkaline hydrolysis is a method which is used in the USA for destruction of infected, infectious tissues[3]. The method efficiency has been studied at Alabany Medical College. The tested samples of tissue from pigs, sheep, rabbits, dogs, rats, and mice included the analyses to the following causes: *Staphalacocus aureus, Mycobacterium fortuitum, Candida albicans, Bacillus subtilis, Pseudomonas aeruginosa, Asperigillus fumigatus, Mycobacterium bovis BCG, MS-2 bacetriofage* and *Giardia muris*. The obtained results have shown that after alkaline hydrolysis the mass is sterile to bacteria and fungi, including *Giardia*. This remained so even after the growth in a culture medium. The sample was completely destroyed and solved except for the inorganic parts from the teeth and bones. The alkaline hydrolysis destroys completely the causative agents as peptide links of virus protein coating, the prions[9].

The aim of research is to perform the whole process of alkaline hydrolysis of animal waste, from the treatment in the autoclave to final disposal and harmless usage of hydrolysed material in soil fertilization.

Material and Methods

The samples analysed were of the first category of animal waste – brain, from the slaughterhouse Kudelić, Bedenica, cattle 22 months old. The brain samples were not tested for BSE because the age of the cattle for the mentioned sample was less than 30 months which was also the precondition for testing.

For the preparation of samples the alkaline hydrolysis reactor was used, 175 mm in height and 165 mm in diameter. Ten mm wide space between inner and exterior wall is used for cooling the reactor by circulating plain water of ϑ=15 °C temperature at device's entry point. The needed reaction mixture temperature was maintained through indirect cooling of reactor wall, without measuring water exit temperature and quantity of heat driven away. Built onto reactor's lid is a manometer with accurate measuring division for continued monitoring of the pressure of reaction mixture, as well as a safety valve for stabilising possibly excessive working pressure during the course of experiment. The reactor is calibrated for 7 bars. At the bottom of the reactor, there is an opening with duct for driving hydrolysed substances away, which significantly facilitates the discharging of the experimental device and reaction mixture. The reactor is mounted on a specifically shaped electric heater with magnetic stirrer, which is equipped with thermometer for permanent measuring and monitoring of the reaction mixture temperature. There is a magnet in the reaction mixture, which stirs the reaction mixture with the use of magnetic stirrer (Figure 1).

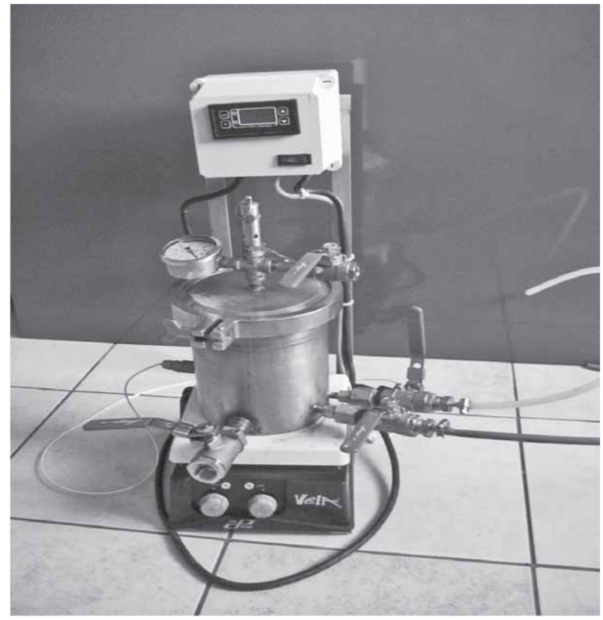

Fig. 1. Schematic display of alkaline hydrolysis reactor.

High-risk animal waste treatment experiments were conducted using the reactor and through hydrolysis process, at standardised temperature ϑ=150 °C, pre-pressure p=4 bar and duration t=2 hours, as well as at temperatures higher or lower than the standardised (given) ones.

The samples of high-risk first category of animal waste were used, i.e. bovine brains for the purposes of the experiment and research into efficiency of alkaline hydrolysis method at different temperatures and hydrolysation duration. Hand blender was used for mixing and chopping up the needed quantity of waste until the mixture became homogenous. Homogenous mixture of waste was placed in the reactor, and 45% NaOH solution and water were added. The obtained reaction mixture contained 400 grams of experimental brains sample, 30 mL of 45% NaOH and 600 mL of water. Homogeneity of reaction mixture during the running of each experiment was obtained by using a magnetic stirrer and through continuous stirring. Prior to starting to heat up the reaction mixture, the reactor is hermetically closed and there is no contact with the environment. Maintaining of experimental temperature of the reaction mixture was obtained through continuous supply of cold water between double walls and by driving away the heat with the same quantity of water from the cooled surface area of the reactor.

Hydrolysis was conducted under the following testing conditions: hydrolysation duration t_1=2 hours, temperature ϑ_1=150 °C and pre-pressure p_2=4.78 bars.

Statistical analysis took place in accordance with randomly selected experimental design, i.e., three replications and values that represent $\overline{X}\pm SD$ of three replications were calculated.

Results

Chemical analyses serve to determine the chemical properties and the presence of heavy metals of hydrolysed animal waste. The chemical analyses included determination of:

- pH, directly from samples on pH-meter with combined electrode[11];
- electro-conductivity (E.C.), by means of conduct meter MA5964 with combined electrode[12];
- total nitrogen, determined by the method according to Kjeldahl[13];
- phosphorus, determined spectrophotometrically at 420 nm;
- potassium and sodium, determined by flame emission photo metrically, whereas all the other microelements (Ca, Mg, Mn, Zn, Cu, Fe, Pb, Cd) were determined by means of atomic absorption spectrometry (AAS).

Table 4 shows the results of chemical analyses of hydrolysed animal waste. The data from the table show that for pH of fertilizers in the alkaline area, an increased content of salts and sodium has been determined, whereas the values of other macro- and microelements are within limits that allow their implementation in the fertilization of certain cultures. In relation to manure, this fertilizer has more nitrogen, phosphorus, potassium, calcium and magnesium and a higher content of microelements. Thus, the fertilizer can be used for fertilization, but this should be preceded by the analysis of soil and determining of the requirements of certain cultures for macro- and microelements.

TABLE 4
THE RESULTS OF MEAN VALUES OF CHEMICAL ANALYSIS OF ANIMAL WASTE SUBJECTED TO ALKALINE HYDROLYSIS TREATMENT

Chemical Analysis	Animal waste
pH directly	7.98
E.C. mS/cm	37.10
% s.tv. (dry matter 105 °C)	17.31
% H_2O	82.70
% @.O. (550 °C)	21.43
% G.@. (burning loss)	78.60
% C organic	40.02
% N – in natural sample	1.62
– total on s.tv.	9.42
– other forms (105 °C)	9.28
– NH_3-N	0.12
% P_2O_5	2.81
% K_2O	1.96
% Ca	6.24
% Mg	1.53
% Na	1.90
mg/kg Mn	94.52
mg/kg Zn	68.13
mg/kg Cu	12.22
mg/kg Fe	441.30
mg/kg Pb	0.72
mg/kg Cd	0.11

Based on the results of the chemical analysis it can be seen that the hydrolysed slaughterhouse waste contains a large quantity of water (82.7%), which means that the share of dry matter is very low (17.3%). The content of total nitrogen ranges in the concentration of 9.4%. Furthermore, the N:P:K ratio in the sample has been calculated and it is 3.35:1:0.70 and C/N ratio which is 4.25:1 and is thus below the usual values for bio-fertilizers. The content of phosphorus, calcium and magnesium determined by analysis ranges within the normal concentrations for this type of matter. The total content of microelements and heavy metals ranges in concentrations which do not exceed the maximally allowed concentrations (MDK) stipulated by the Regulations on the Protection of Agricultural Land against Pollution by Harmful Matter (NN 15/1992). Based on this, one may conclude that the hydrolysed animal waste is not burdened by harmful matter that could pollute the environment.

Conclusion

Hydrolysed animal waste indicates lower concentration of feedstuff, but still possible implementation of hydrolysed material as fertilizer for agricultural areas. The results have been compared and interpreted according to the Regulations on the Protection of Agricultural Land against Pollution by Harmful Matter (NN 15/92) and the Regulations on Ecological Production (NN 91/01). It may be determined that the hydrolysed material contained a larger quantity of water (82.7%), which means that the share of dry matter was very low 17.3%. Alkaline reaction has been determined at pH 7.98 which has to be taken into consideration in case of possible implementation in other branches of agriculture. The electro-conductivity is higher than in common organic fertilizers, which indicates a higher content of salts in the sample. Very high values of ash indicate medium high to low share of organic matter, whereas the content of total nitrogen ranged in the concentration of 9.4. The analysed samples were rich with organic carbon, whereas the ratio of carbon and nitrogen was 4.25 which is within the limits expected for this type of material. The content of phosphorus, calcium and magnesium as macro-feedstuffs determined by analysis are lower than usual. The content of potassium does not increase with the application of KOH alkali in reaction compared to NaOH alkali, i.e. there is no enriching of the material by potassium. The total content of microelements and heavy metals ranged in concentrations that do not exceed the maximally allowed concentrations stipulated by the Regulations on the Protection against Pollution by Harmful Matter. Based on the visual inspection, the mentioned material was determined to belong to light-brown liquid products, of odour similar to soap. By mixing with water there is no development of temperature nor are gases released and no subsequent chemical reactions have been noticed.

Regarding the problems of using the processed animal waste by the method of alkaline hydrolysis as fertilizer and the possibility of its application for agricultural areas, these have been compared and interpreted according to the Regulations on the Protection against Pollution by Harmful Matter, which indicates harmful matter for the soil, as well as permitted quantities of harmful matter in the soil. According to these Regulations, the by-materials of production, as in this case the hydrolysed material can be used on the agricultural land only provided that this has been analysed and that it is under the supervision of experts. The maximal quantities of applied organic fertilizer amount to up to 60 m^3/ha annually in the vegetation period and 30 m^3/ha in off-vegetation period (October – April). The hydrolyzed material is not suitable for areas exposed to high risk of pollution, on soil saturated with water, covered with snow, nor for frozen agricultural land. It is also forbidden to use it in coastal areas, along water flows at distances less than 10 m, standing waters up to 70 m, nor in the production of vegetables, strawberries and herbal plants within 30 days from maturity and harvest. Apart from the fertilizer analysis it is necessary to perform also the analysis of soil in order to determine the quantity of the applied liquid fertilizer.

REFERENCES

1. VUČEMILO M, VINKOVIĆ B, GRLIĆ D, GRLIĆ I, VRABAC N, TOFANT A, MATKOVIĆ K, Zbornik radova Veterinarski dani, (Veterinarski dani, Šibenik, 2003). — 2. CROATIAN BUREAU OF STATISTICS (2012) — 3. KALAMBURA S, KRIČKA T, JURIŠIĆ V, JANJEČIĆ Z, Cereal Res Comm, 36 (2008) 179. — 4. FRANCO DA, SWANSON W, Orig Recycl, (1996) 150. — 5. COLLINGE J, Hum Mol Genet, 6 (1997) 1699. — 6. KRIČKA T, VOĆA N, JUKIĆ Ž, KALAMBURA S, KALAMBURA D, In: Proceedings (International Congress Flour-Bread ž03, Osijek, 2003). — 7. WASTE MANAGEMENT STRATEGY REPUBLIC OF CROATIA, (Official Gazette 130, Zagreb, 2005). — 8. GLIBOTIĆ A, Goveđa spongiformna encefalopatija; Bolest lude krave, upravni i inspekcijski postupci u Bavarskoj, usmeno predavanje (Veterinarski fakultet, Zagreb, 2000) — 9. KAYE G, Personal communication to H. L. Thacker regarding alkaline hydrolisis (WR2, Indianapolis 2003). — 10. COMMISSION REGULATION (EC) No 92/2005 15/92. — 11. DIN EN 12506:2003. — 12. ISO 7888: 1985. — 13. ISO 1871: I 975. — 14. VOĆA N, KRIČKA T, JANUŠIĆ V, JUKIĆ Ž, MATIN A, KIŠ D, Stroj Vestn-J Mech E, 54 (2008) 232. — 15. KALAMBURA S, Air Hig Rada Toksikol 57 (2006) 267. — 16. AMON T, AMON B, KRYVORUCHKO V, MACHMUELLER A, HOPFNER-SIXT K, BODRIOZA V, HRBEK R, FRIEDEL J, POERSCH E, WAGENTRISTL H, SCHREINER M, ZOLLITSCH W, Bioresour Technol, 98 (2006) 1. — 17. Regulations on the Protection of Agricultural Land against Pollution (OG 032/2010.)

S. Kalambura

University of Applied Sciences Velika Gorica, Zagrebačka Cesta 5, 10410 Velika Gorica, Croatia
e-mail: sanja.kalambura@vvg.hr

EFIKASNOST METODE ALKALNE HIDROLIZE U ZAŠTITI OKOLIŠA

SAŽETAK

Razvoj novih tehnologija za efikasno zbrinjavanje nus proizvoda životinjskog podrijetla koji nisu za prehranu ljudi postao je jedno od vrlo važnih ciljeva integriranog sustava gospodarenja otpadom. Pojava goveđe spongiformne encefalopatije u Europi i Svijetu 90-tih godina, otvorila je nova pitanja oko zdravstvene ispravnosti i upotrebe mesnog koštanog brašna u ishrani domaćih životinja, koji nastaje preradom nus proizvoda životinjskog podrijetla. Nusproizvodi se dijele na tri kategorije, od čega prva kategorija čini visoko rizičan otpad. Alkalnom hidrolizom učinkovito se zbrinjavaju nus proizvodi visokog rizika. Rad predstavlja prikaz metode alkalne hidrolize, jedne od dvije priznate metode u EU za obradu nusproizvoda te upotrebu hidroliziranog ostatka u poljoprivredi.