CHEMICAL MARKER AS A SAFE METHOD IN RESEARCH

Sylwester BOROWSKI[1], Sławomir ŻAK[2]

[1] *Faculty of Mechanical Engineering, UTP University of Science and Technology,*
[2]*Faculty of Chemical Technology and Engineering, UTP University of Science and Technology.*

Abstract
In the study there is presented the method of biologically active preparations' marking with n-hexane. Application of the marker allowed efficient conducting of studies concerning irregularity of the preservative's distribution. The material obtained as a result of the experiments' conducting – biomass may be with success applied for normal technological processes in biogas plants and as fodder.

Key words: *biomass, preservatives, marker.*

INTRODUCTION

Studies concerning production of fodders and substrates, should answer the question whether the obtained product is of good quality and what elements of the applied technological process have an impact on that quality.
Many researchers are evaluating the impact of applied technology on the end effects. Zbytek et al. (2016) in his publication analyzes the technologies available on the market, whereas Zastempowski, Borowski & Kaszkowiak (2013) in their publication, they analyzed several selected issues related to the collection of biomass. Zastempowski & Bochat (2014) whether Flizikowski et al. (2015) they focused on the issues involved in constructing machines used during harvesting and processing of biomass. The problem of applied technology is also evident in economic articles (Bojar, 2001).
In the studies performed by the authors concerning the quality of humid hay harvested with the use of a biologically active preservative, the quality of the obtained fodder was determined on the basis of its testing at the assumed time following harvesting. The applied preservative Inoculant 1155, in its composition has lyophilisate of *Bacilus spp* bacteria, which after being added to the harvested hay multiplicated and spread in the harvested mass. For their existential needs they use humidity from hay, and following its utilization (dehumidification of hay) they die. So, it is a natural and fully safe preservation method. However, research of the number of bacteria in individual places of the „silo" several days following harvesting, does not give full information on the preparation's distribution immediately after harvesting, what in case of chemical preparations' use is a very essential information (Bernes et al., 2008, Dulcet et al., 2006).
The condition of correct application of the preservative is supplying of its precisely determined volume to the harvested biomass in such a way as to receive its smooth mixing. As a result of smooth preservative's distribution in the harvested biomass, worsening of its quality may take place. It is particularly evident in case of harvesting biomass of decreased humidity or in places where relocation of silage juices is not possible (Dulcet, Mikołajczak & Olszewski, 2002, Rotz, 2003). There are always losses of it at the time of the preservative's adding at the time of harvesting. Apart from the possible, toxic impact on environment in case of use of chemical preservatives, losses also influence the increased costs of preservation. The use of the chemical preservatives may also adversely influence the plants' growth, what as an effect may result in the yield's decrease (Dulcet et al., 2006, Dulcet, Mikołajczak & Olszewski, 2002).
The literature analysis of the issues concerning harvesting of fodders with the use of additives showed, that in case of some studies there occurred the problem of assessing the evenness of the formulation's mixing with the harvested material. The assessment was conducted on the basis of the analysis of collected samples through:
- pH measurements of quantitative marking of formulation's used in the studies (Dulcet, 2001, Wrzos, 1980),
- analysis of the obtained fodder's quality (Dulcet, 2001, Maškova Holubowa & Luňaček, 1991).
Other methods used for the assessment of evenness of mixing of the formulation with the harvested material are the fluorimetric analysis and irradiation with isotopes. However, these methods, are rela-

tively difficult to be used, and fodders remaining after studies conducted in such a manner may not be fit for feeding (Koch, 1985, Wittenberg 1997).
The purpose of this study is presentation of the method of marking biologically active preparations with the use of n-hexane and the obtained results of the studies.

MATERIALS AND METHODS

In the studies there was used the solid granulated preparation Inoculant 1155 of Pioneer of the following physical properties: bank mass 1040 kg · m^{-3}, relative humidity 2,5 %, average diameter of granules 0,87 mm. The preparation was applied in the volume of 0,1 % (1 kg per ton of humid hay). The preparation comprises lyophilisate of natural bacteria *Bacilus spp.* and calcium carbonate. The guaranteed number of live bacteria is 1 · 108 cfu · g^{-1}.
For the needs of the experiment, the microbiological preparation Inoculant 1155 was marked with a contact method ensuring high efficiency (fig. 1). The marker's odour was passed through the preparation's bed till the moment of the indicator's saturation (Kondo et al., 2001).

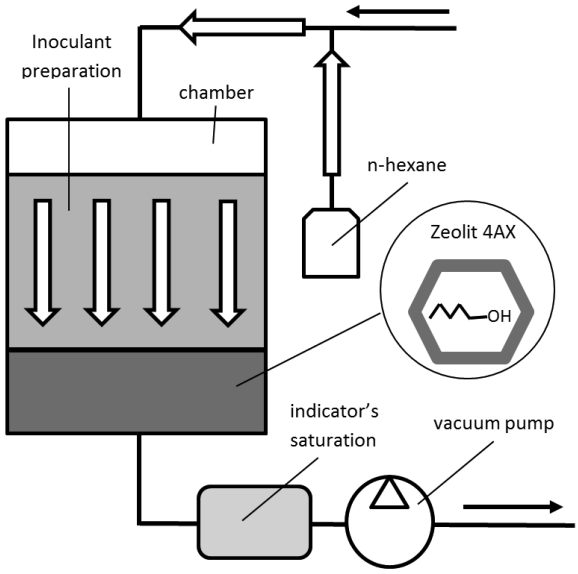

Fig. 1. Marking of Inoculant preparation with the use on n-hexane with the use of the contact method.

The applied marker creates a physical bond with the preparation (fig. 2) which disintegrates under the influence of an extraction solvent.

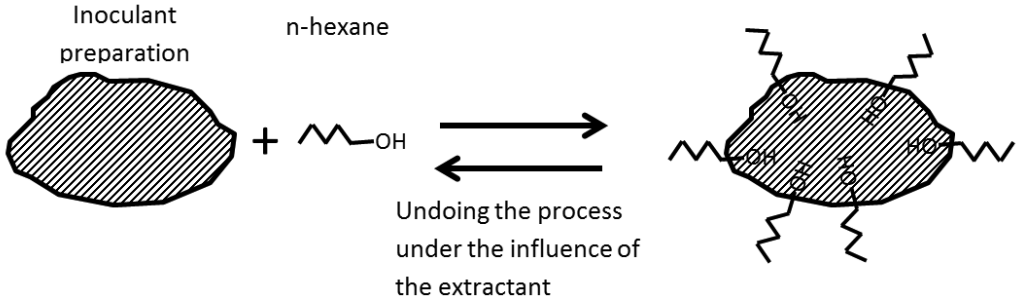

Fig. 2. Scheme of the marker molecules' connections with a microbiological preparation.

In order to obtain repeatable results, the algorithm of procedure was developed. The taken sample, after separation of coarse impurities is subject to be dried to solid mass in order to mark the content of water. Then, the dried mass is homogenised just to be fragmented to the level below 150,0 μm. A

fragmented sample is subject to extraction (extraction solvent – 150,0 ml) in the arrangement liquid (acetone) – solid body in the temperature of 20±1,0OC with the use of centrifuge for 24 hours in a tightly closed conical flasks of capacity of 250,0 ml. Having spinned the solid bodies on the centrifuge, take with a microsyringe from the decantate 2,0-3,0 μl of acetone sample for the purposes of analyte's marking with the use of the gas chromatograph and a mass spectometer.
For the tests there was used the gas chromatograph HP 5890 series II Hewlett Packard (column HP-1, of the length – 30,0 m, diameter φ – 0,53 mm, phase Hypersil ODS Shandon) with detector AED and ECD and a mass spectometer (MS 5972 series Mass Selective Detector – column: Pona of the length 25,0 m, diameter φ – 0,33 mm). The analysis was conducted in the mode of temperature programming: 20-120OC/10 min., 120-180OC/20 min. and 180-260OC/20 min.

RESULTS AND DISCUSSION

For the purposes of the developed method's verification, preliminary studies have been conducted. Pursuant to the assumed algorithm, the quantitative marking of the external standard was conducted. The level of n-hexane's recovery obtained in the studies amounted to 89,1 - 94,6% at the time of that alcohol's retention TR – 11,2 - 11,4 min. The obtained results are presented in figure 3.

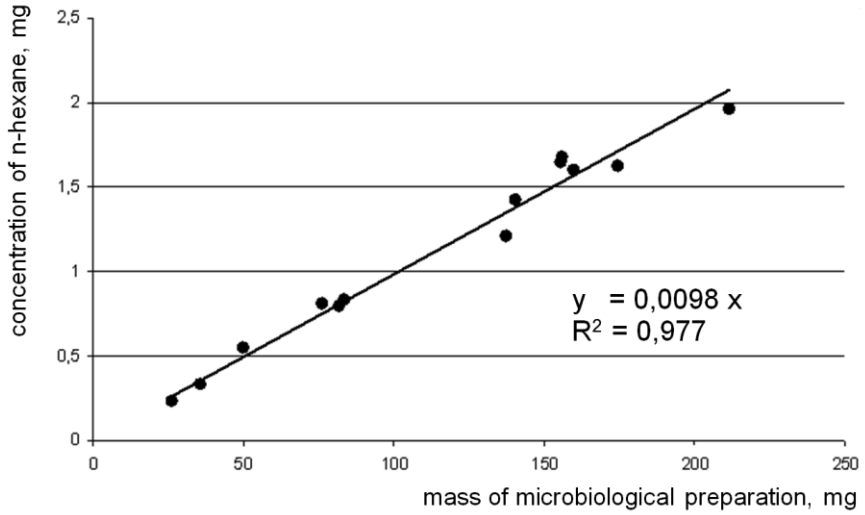

Fig. 3. Dependency of the concentration of n-hexane marked in the preliminary studies.

As a result of the conducted analysis, the recourse equation (1) at the significance level p = 0,05 has been determined. It is a linear function and is characterized by high determination coefficient $R^2 = 0,977$, what proves good determination of the functional dependencies.

$$y = 0,0098 \cdot x \qquad (2)$$

CONCLUSIONS

Application of the new method of microbiological preparation's marking with the use of h-hexane makes it possible to obtain information of the microbiological preparation's distribution directly following harvesting. This method allows conducting of the experiment in the conditions identical to those occurring in agrarian practice.
Application of the marker allows application of quick methods of analysis with the use of gas chromatograph and mass spectometer. It is an environment-friendly method, and fodder that remained after taking of samples is safe for microorganisms occurring in fermentative bed and for animals.

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Corresponding author:
Sylwester Borowski, Ph.D., Faculty of Mechanical Engineering, UTP University of Science and Technology, Al. prof. S. Kaliskiego 7, 85-796 Bydgoszcz, Poland, phone: +48 52 3408132, e-mail: sylwester.borowski@utp.edu.pl