

## CHARACTERISTICS, APPLICABILITY AND INFLUENCE ON ENVIRONMENT OF THE PRODUCT "VESTA AGRI"

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### Abstract

In this paper characteristics, applicability and influence on the environment of the product "Vesta Agri" are presented. The product "Vesta Agri" represents mixture of catalytic thermostable enzymes, which are bonded with saprophytic bacteria and relative eutrophic compounds. This combination allows very fast degradation and fermentation of organic matters. Use of this product is for initiating of biological process in the newly constructed lagoons or objects. Too, it is used in the case of big organic load of wastewaters.

The results of physicochemical properties of this product are showing that the product is in the form of fine granules with the smell of yeast, moderate solubility and has pH value 6.20. Microbiological properties are given of the following content: cellulase 2.93 %, protease 1.50 %, amilase 0.43 %, lipase 0.17 % and total bacteria 149.6 million ufc g<sup>-1</sup>. Regarding of radiological properties, the activity concentrations of all investigated radionuclides were less than minimum detectable concentration (MDC).

The obtained results of all analysis showed that the product "Vesta Agri" meets the safety criteria. After all examination of the bacterial enzyme mixture called "Vesta Agri" it is concluded that there is no adverse effects on the environment and it is not harmful to the health of the people who come into the contact with this product.

**Keywords:** *"Vesta Agri"; Enzymes; Radioactivity; Ecological influence*

### Introduction

Available resources of fresh water are exhausting steadily and this presents global catastrophe (Adulkar and Rathod, 2014). Increasing water consumption for anthropogenic activities significantly is affecting the reduction of available water resources. Water pollution due to toxic metals and organic compounds is a serious environmental and public problem (Crini, 2005).

Wastewaters from different processing industries may contain high concentrations of oil and grease, solids and chemical oxygen demand levels, which are difficult to treat through conventional biological treatment system mainly due to slow biodegradability (Jeganathan et al., 2007). If not treated, presence of these substances may cause gross pollution of land and water with their high damage (Cammarota and Freire, 2006). Some conventional methods for wastewater treatment include biological processes. These are mainly aerobic processes, but in the last two decades anaerobic reactors have been increasingly applied (Cammarota and Freire, 2006). Anaerobic treatment processes are considered to be better than aerobic processes because of valuable biogas production, less biomass production, higher organic loading application and less energy consumption (Lettinga, 1996). Conventional biological treatments of wastewater are not given satisfactory results always. Hence, today in focus of research is application of preparation based on enzymes, as enzymes which have been isolated from plants, fungi and bacteria.

Company WEST CHEMIE BGD uses two groups of technology in biological wastewater processing. One of them is technology based on bioaugmentation, while the other is based on selected microorganisms. The main advantage of these methods is better and bigger performance in comparison with biological processes. The aim is simply application on existing systems with infrastructure which is built. These technologies are effectively used in lagoons (both aerobic and anaerobic), as well as in water treatment plants.

The aim of this study is showing of characteristics with special emphasis on the radiological characteristics, applicability and influence on the environment of the product "Vesta Agri", which is used in the above technologies of biological wastewaters processing.

### Materials and methods

The product "Vesta Agri" represents mixture of catalytic thermostable enzymes (cellulase, protease, amylase and lipase), which are bonded with saprophytic bacteria (*Bacillus subtilis*, *Lactobacillus acidophilus* and *Aspergillus oryzae*) and relative eutrophic compounds to provide of organic carbon (dextrin). "Vesta Agri" is used in the next cases: starting of biological processes in new lagoons or objects and big loads in existing lagoons or objects (bigger organic loads of wastewater). This product is not adding only in the case when need to be increased number of microorganisms or degradation of corresponding organic pollution. Addition of this product is performed that to be introduced organisms, which should better adapt to the conditions which are quickly changing (pH, temperature, etc.). The characteristics of the product "Vesta Agri" which is taken on 10 January 2012 are given in Table 1.

**Table 1.** The characteristics of sample of bacterial enzyme mixture named "Vesta Agri".

Characteristic	Value
Form	fine granules with the smell of yeast
Solubility	moderate
pH	6.20
Cellulase	2.93 %
Protease	1.50 %
Amilase	0.43 %
Lipase	0.17 %
Bacteria (total)	149.6 million ufc g <sup>-1</sup>

The preparation of sample for measurement of the gross alpha and beta activity was performed used MARLAP method (MARLAP, 2004). Sample (about 130 mg) was transferred into a stainless-steel planchet and the planchet was placed directly into the detector for measurement of the gross alpha and beta activity. The preparation of sample for gamma spectrometric measurement included weighing and placing into a plastic box of 200 mL in volume (IAEA, 1989). Since the purpose of measurement is screening, the counting of the sample was conducted immediately after preparation.

The gross alpha and beta activity were determined by  $\alpha/\beta$  low-level proportional counter Thermo Eberline FHT 770 T (ESM Eberline Instruments GmbH, Erlangen, Germany). The counting time was 3600 s. Calibration was performed by using a standard source of <sup>90</sup>Sr (EM 145, Prague, Czech Republic) with an activity of 189.4 Bq on the day 1 August 2011 for beta activity and a standard source of <sup>241</sup>Am (EM 445, Prague, Czech Republic) with an activity of 224 Bq on the day 1 August 2011 for alpha activity. The counting gas was a mixture of 90 % argon and 10 % methane. The counting efficiencies for

the system were 26 % for alpha and 35 % for beta. The background of each detector was determined by counting an empty planchet for 3600 s.

Minimum detectable the gross alpha and beta activity concentration was calculated by the *Equation (1)* (Sarap et al., 2014):

$$MDC = \frac{LLD}{m} \quad (1)$$

where *LLD* is the detection limit (1/s) and *m* is the mass of the sample (kg).

Gamma spectrometric measurement was performed using a HPGe Canberra detector (Canberra Industries, Meriden, Connecticut, USA) with counting efficiency of 20 %. Counting time interval was 9000 s. The spectar was analyzed using the program GENIE 2000 (Canberra Industries, Meriden, Connecticut, USA). The activity of <sup>226</sup>Ra and <sup>232</sup>Th was determined by their decay products: <sup>214</sup>Bi (609 keV, 1120 keV and 1764 keV), <sup>214</sup>Pb (295 keV and 352 keV) and <sup>228</sup>Ac (338 keV and 911 keV), respectively. <sup>235</sup>U was determined via 186 keV corrected for <sup>226</sup>Ra. <sup>238</sup>U was determined via <sup>234</sup>Th (63 keV) or by <sup>234</sup>Pa (*t*<sub>1/2</sub>=1.17 min, 1000 keV). The activities of <sup>40</sup>K and <sup>137</sup>Cs were determined from its 1460 keV and 661.6 keV, respectively.

The calibration of detector for measurement of items of general use in powder form was performed using a plastic box of 200 mL in volume. Secondary reference material was obtained from the primary reference liquid radioactive material (9031-OL-427/12, type ERX, Czech Metrological Institute, Prague, Czech Republic) spiked with a series of radionuclides (<sup>241</sup>Am, <sup>109</sup>Cd, <sup>139</sup>Ce, <sup>57</sup>Co, <sup>60</sup>Co, <sup>203</sup>Hg, <sup>88</sup>Y, <sup>113</sup>Sn, <sup>85</sup>Sr, <sup>137</sup>Cs and <sup>210</sup>Pb) with total activity of 72.4 kBq on the day 31 August 2012. Energy calibration is checked in whole region before applying usual quality control (QC) procedure for gamma spectrometry measurement. The total activity of calibration source is used to check the efficiency calibration and the general operating parameters of the gamma spectrometry system (source positioning, contamination, library values, and energy calibration). The detector – shield background, detector efficiency, peak shape, and peak drift are measured and verified if they are within the warning and acceptance limits. For that purpose <sup>60</sup>Co and <sup>133</sup>Ba sources were used.

Minimum detectable concentration (MDC) of gamma emitters was calculated by the *Equation (2)* (Sarap et al., 2014):

$$MDC = \frac{LLD}{t \times P_{\gamma} \times E_f \times m} \quad (2)$$

where *LLD* is the detection limit,  $LLD = 2.71 + 4.65\sqrt{B}$ , *B* is count of the background, *t* – counting time (s), *P<sub>γ</sub>* – probability of gamma decay (%), *E<sub>f</sub>* – detector efficiency (%) and *m* – mass of the sample (kg).

### Results and discussion

*Table 2* shows the result for gross alpha and beta activity concentration for analyzed sample "Vesta Agri". The gross alpha activity was <120 Bq kg<sup>-1</sup>, while the gross beta activity was <171 Bq kg<sup>-1</sup>. The Republic of Serbia has no regulations about recommended levels of the gross alpha and beta activity for items of general use in powder form.

**Table 2.** The gross alpha and beta activity of the product "Vesta Agri".

Gross alpha activity (Bq kg <sup>-1</sup> )	Gross beta activity (Bq kg <sup>-1</sup> )
< 120	< 171

The results of gamma spectrometric measurement in the same sample are presented in *Table 3*. The activity concentrations of all investigated radionuclides were below the MDC. The limits of the radionuclides content, whose half-life is longer than 60 days, in powder substances intended for general use and for which limit values are not set by the Rulebook on registration and notification of sources of ionizing radiation, are equal to the values that are ten times greater than the limits of radionuclide content in drinking water (Official Gazette of the Republic of Serbia, 2011). Volume of 1 L of drinking water is replaced by a mass of 1 kg. Derived concentrations of individual radionuclides in drinking water expressed in Bq L<sup>-1</sup> are 2.9, 3.0, 0.49, 0.59 and 10 for <sup>235</sup>U, <sup>238</sup>U, <sup>226</sup>Ra, <sup>232</sup>Th and <sup>137</sup>Cs, respectively (Official Gazette of the Republic of Serbia, 2011). Based on the results presented in *Table 3*, it can be seen that the criteria of recommended levels of each radionuclides in investigated product are met.

**Table 3.** The activity concentration of individual radionuclides in the product "Vesta Agri".

Radionuclid	<sup>235</sup> U	<sup>238</sup> U	<sup>226</sup> Ra	<sup>232</sup> Th	<sup>40</sup> K	<sup>137</sup> Cs
Bq kg <sup>-1</sup>	< 0.8	< 8	< 3	< 0.5	< 12	< 0.5

In addition to determination of the radionuclides content in the product "Vesta Agri", physico-chemical characteristics of wastewater that is treated with this product were determined, too. The results of physico-chemical laboratory examinations of wastewater that was treated with enzymatic product "Vesta Agri" are presented in *Table 4*. Sample of wastewater was taken in December 2013.

**Table 4.** A physico-chemical characteristics of wastewater that was treated with the product "Vesta Agri".

Parameter	Value	Maximum allowed value	Standard/Method
pH	7.6	6.0–9.0	ISO/10523:1994
Consumption of KMnO <sub>4</sub> (mg L <sup>-1</sup> )	189.1	/	PRI <sup>a</sup> /P-IV-9a
Chemical consumption of O <sub>2</sub> (from K <sub>2</sub> Cr <sub>2</sub> O <sub>7</sub> ) (mg L <sup>-1</sup> )	183	500	VDM 0181 <sup>b</sup>
Biochemical consumption of oxygen (BPK <sub>5</sub> diluted) (mg L <sup>-1</sup> )	76	400	SRPS EN/1899-1:09
NH <sub>4</sub> <sup>+</sup> (mg L <sup>-1</sup> )	131.8	15	ISO/14911:1998
NO <sub>2</sub> <sup>-</sup> (mg L <sup>-1</sup> )	0.065	35	EPA/300.1
NO <sub>3</sub> <sup>-</sup> (mg L <sup>-1</sup> )	< 0.1	50	EPA/300.1
Cl <sup>-</sup> (mg L <sup>-1</sup> )	122.3	500	EPA/300.1
SO <sub>4</sub> <sup>2-</sup> (mg L <sup>-1</sup> )	18.5	300	EPA/300.1
P (mg L <sup>-1</sup> )	6.31	/	EPA/200.7Rev 5
Total nitrogen (mg L <sup>-1</sup> )	136.4	/	SRPS EN/12260:2008
Total inorganic nitrogen (mg L <sup>-1</sup> )	131.87	/	/
Conductivity at 20 °C (µS cm <sup>-1</sup> )	1880	/	SRPS ISO/27888:2009
Dry residue at 105 °C (mg L <sup>-1</sup> )	776	/	SMEWW <sup>c</sup> 19 <sup>th</sup> /m 2540 B
Suspended solids at 105 °C (mg L <sup>-1</sup> )	84	500	SMEWW <sup>c</sup> 19 <sup>th</sup> /m 2540 D
Sedimentary materials by Inhoff after 2 hours	< 0.1	2	SMEWW <sup>c</sup> 19 <sup>th</sup> /m 2540 F

(mL L <sup>-1</sup> )			
Fats and oils by Soxhlet (mg L <sup>-1</sup> )	9.2	40	SMEWW <sup>c</sup> 19 <sup>th</sup> /m 5520 D

<sup>a</sup> Drinking water, Standard methods for hygienic safety, SZZZ, Belgrade, 1990.

<sup>b</sup> Manufacturer instructions for equipment Lovibond.

<sup>c</sup> Standard methods for Examination of Water and Wastewater, 19<sup>th</sup> Edition, 1995.

As can be seen from *Table 4*, the obtained values of investigation of physico–chemical characteristics of bacterial enzymatic product are in the range of allowed values, except for content of amonium ion.

### Conclusion

Catalytic thermostable enzymes in the composition of the product "Vesta Agri" perform degradation of proteins, carbohydrates, fats and derivates of cellulose which are found in wastewater. Enzymes are used in wastewater treatment to develop a remediation processes that are environmentally less aggressive in compared to conventional techniques. The obtained results of analysis of physico–chemical characteristics are showed that the product "Vesta Agri" met the safety criteria. In Serbia, based on the regulation (Official Gazette of the Republic of Serbia, 2011) limit values of activity concentrations of certain radionuclides are defined for different materials, inter alia for items for general use in powder form. On the obtained results of determination of radionuclides content, it can be concluded that enzymatic preparat "Vesta Agri" is met the criteria for radiological safety.

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