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EFFECT OF BIOACTIVE ADDITIVES ON BIOMASS FERMENTATION FROM AGRO-INDUSTRIAL SECTOR

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Abstract. Wastes from the agro-industrial sector, due to their toxic effects with regard to plants and living organisms, cannot be dumped in the soil. However, they can serve as renewable source of value-added products, following the specific digestive treatment. This research was focused on studies of vinasse fermentation processes in the presence of bioactive substances introduced directly into the digested biomass. The results obtained testify that the substances of natural origin used as additives, demonstrate the pronounced effects on alcoholic fermentation of vinasse under the mesophilic conditions. The comparative assessment of different additives action in the studied processes have demonstrated that the dihydroxyfumaric acid caused the emission of 266 cm^3 CO_2 in 76 hours, aescinum – 251 cm^3 in 55 hours, tomatin – 233 cm^3 during 78 hours, sclareol – 232 cm^3 during 55 hours, vanillin – 229 cm^3 during 69 hours, whereas catechin – 180 cm^3 during 61 hours of fermentation, until the fermentation process was completed. Thus, the study of the effects of bioactive substances with possible antioxidant properties on biomass from winemaking sector with identification of these processes mechanisms can be a perspective direction, suggesting the new ways of wastes valorisation.

Keywords: *Agro-industrial wastes, vinasse, fermentation, bioactive additives.*

Rezumat. Deșeurile din sectorul agroindustrial, datorită efectelor toxice asupra plantelor și organismelor vii, nu pot fi aruncate în sol, dar pot servi drept sursă regenerabilă de produse cu valoare adăugată. Prezenta cercetare s-a axat pe studiul procesului de fermentare a vinasei în prezența substanțelor bioactive, introduse în biomasa digerată. Rezultatele obţinute mărturisesc că substanţele de origine naturală utilizate ca aditivi demonstrează efectele pronunţate asupra fermentaţiei alcoolice a vinasei în condiţii mezofile. Evaluarea comparativă a acțiunii aditivilor a demonstrat că acidul dihidroxifumaric a determinat emisia de 266 cm^3 CO_2 în 76 ore, aescinum – 251 cm^3 în 55 ore, tomatin – 233 cm^3 în 78 ore, sclareol – 232 cm^3 în 55 ore, vanilină – 229 cm^3 timp de 69 de ore, în timp ce catechina – 180 cm^3

pe parcursul a 61 de ore de fermentație, până la finalizarea procesului de fermentație. Astfel, studiul efectelor substanțelor bioactive cu posibile proprietăți antioxidante asupra biomasei din sectorul vitivinicol cu identificarea mecanismelor acestor procese poate fi o direcție de perspectivă, sugerând noi modalități de valorificare a deșeurilor.

Cuvinte cheie: *Deșeuri agroindustriale, vinasă, fermentație, aditivi bioactivi.*

1. Introduction

The problem of wastes management from agro-industrial sector, to prevent the environmental pollution with toxic components and at the same time, to obtain the series of value-added products, is an important issue requiring a smart and complex approach. The wastes treatment technology is should be selected on the base of the initial wastes nature, composition and amounts, which, on their turn, are functions on the main production cycle, raw materials, process technology and conditions applied.

Specifically, in the agricultural regions having the wine, spirit, beer and juice-producing industries, the specific types of liquid wastes are generated in the state of continued digestion, which implies the strict prevention of their discharges/dumping directly into the landfills, natural water bodies or other environmental compartments as they can destroy the chemical composition of soil, violate the natural balance of microorganisms, plants, and other living organisms. Existing methods of the solid organic wastes management involve their application in agriculture, burning (although the rather costly process associated with air emissions), anaerobic digestion, composting, etc. The liquid wastes from agro-industrial sector can be treated by sedimentation, decanting in stabilization ponds, anaerobic fermentation, etc.

To produce ethanol from carbohydrate-containing raw materials, the fermentation process is applied commercially [1]. Alcohol is obtained by fermentation of the sugars present in the different raw materials, which are metabolized by microorganisms belonging to the genera *Saccharomyces, Zymomonas, Kluyveromyces,* and *Zygosaccharomyces* [2]. The structural carbohydrates in biomass componence are broken down into the sugars using enzymes. Subsequently, the sugars released are transformed by microorganisms into the alcohols, hydrocarbons or some organic acids during the fermentation process under the atmospheric pressure and temperature 25-70^{o}C. Intermediate sugars can be also utilized to obtain certain useful chemicals [3].

Currently, the operation of the most of processing industries in agriculture, including those dealing with the grain processing into the alcohol, remain unsafe for the environment [4, 5]. Meanwhile, closed ecologically safe production cycles will make it possible to resolve fundamental issues such as rational use of natural raw resources, environmental protection and improving the quality of final product [6]. The alcohol industry affects first of all the water resources, then air and soil.

Specific consumption of fresh water makes 195-325 m^3/t alcohol (10-17 L water/ 1 L alcohol produced), depending on the raw material type and processing technology, namely, recycled water supply [7, 8]. Such high amounts of water used trigger the high volumes of liquid wastes thus formed [9, 10]. The admixtures contained in the waste waters contain mineral and organic substances of vegetable origin. Such wastes are poorly filtrated, quickly rotting, releasing unpleasant odors.

Therefore, the indicator of technology efficiency is important, as it determines the degree of transformation of the initial raw material. Amount of substrate that can be transformed in ethanol by microbiological synthesis, depends first of all on the type of raw material and selected production technology [11]. Basically, from 5 to 60% of substrate, in average 32-36%, depending on its type and ethanol production method, are transformed into the production wastes [12]. Thus, conversion degree of glucose in ethanol makes 95-100%, fructose – 95-100%, xylose – 60-90%, sucrose – 94-100%, hemicellulose – 40-81%, cellulose – 90-100%. The wastes generated from the alcohol production include yeasts, vinasse, carbon dioxide, ester-formaldehyde fraction, fuel oils (0.94 kg/t grain). Distilled grain residue is the main solid waste which contains carbohydrates, proteins, lipids and some microbial metabolites, which is used as feed, fertilizer or culture substrate for edible mushrooms [13].

Among these residues, the main type is alcohol vinasse (or post-distillery vinasse), which is the biomass separated in the distillation process and represents a liquid with suspended particles, acid pH and high contents in organic matter.

On average, the vinasse production makes about 10-15 L vinasse/L ethanol [14]. Vinasse represents a liquid solution containing the suspended particles, with acid pH The liquid wastes from food industries have high amounts of organic matter, and, as a consequence, rather high contents of chemical oxygen demand (COD), biochemical oxygen demand (BOD) and suspended solids (SS) values: wastes from alcohol production from grain have on average 130-160 g O_2/L BOD, 200-220 g O_2/L COD, 340-360 g/L SS and pH= 6.5-8.8, whereas the liquid wastes from sugar industry (sugar beet) have around 2370 g O_2/L BOD, 7540 g O_2/L COD, 21320 g/L SS and pH=8.0 [15].

In some cases, vinasse can be discharged to sewerage system, reaching the wastewater treatment plant, thus significantly impairing its operation. Vinasse discharges into the environment as a rule, case serious environmental problems. As an alternative, it could be used as a liquid food additive for cattle or poultry [16-18], biogas production [19-21] or for fertilization of agricultural fields [22-24]. But the direct suing of vinasse for animals is not always safe or rational, and cannot resolve the issue of its complete utilization. Some standardized methods have been developed to evaluate generation of byproducts and residues in alcohol production cycle, including the entire supply chain, and minimize negative impacts from crop establishment to consumption of fuel ethanol [25-27].

High water contents in the organic wastes with high fermentable sugar contents and wet biomass implies their obligatory treatment or recovery which is however rather costly [28]. Therefore, the development of useful valorization of this waste to obtain the other value-added products is a perspective approach.

To break down the waste biomass into sugars that can be further converted into the gas or liquid biofuels, or other useful products, the biochemical conversion by microorganisms and enzymes is often used [29-31], although the other approaches such as coagulation and oxidation have been also proposed [32]. Generally, biochemical digestion is rather slow process that requires some time for biomass transformation into the products. Among these technologies, anaerobic digestion and fermentation are most popular processes, resulting in producing the biogas which mainly contains biomethane, carbon dioxide, and in a smaller amounts biohydrogen, hydrogen sulfide [33-35]. During the anaerobic digestion, bacteria are applied to hydrolyze carbohydrates into the sugars that are digestible by other, methanogenic bacteria, which in their turn, produce biogas from the digestible components of biomass. In dependence on the biochemical digestion conditions, biogas with high contents in methane [36-38] or hydrogen [39, 40] may be produced under the oxygen-free conditions, or under the elevated pressure [41, 42], or using the other approaches such as adding of bioactive compounds accelerating the biochemical digestion and making it possible to obtain much higher amounts of biomethane of biohydrogen per shorter time [43-45]. Biogas can be burnt to produce the heat, or can be converted to energy, or both energy and heat, using the cogeneration devices [46, 47].

However, not only the biofuels but also a series of value-added products such as sugar alcohols (xylitol) can be obtained by using the combined technologies such as chemical or biochemical process combined with the thermochemical method [48, 49].

Production of alcoholic and non-alcoholic beverages generates the wastes and by products that may be recovered, thus allowing not only to reduce their disposal costs and minimize the environmental pollution, but also to develop the new useful products, in addition to the traditional uses of such wastes as animal feed or soil fertilizers. Dumping in the environment of such residues can provoke the problems such as rotting, higher soil acidity, phytotoxicity, methane gas production, etc. [50].

Wine-and juice production is a large source of wastes in agriculture [51]. Grape marc is generated in the grapes processing in rather large amounts in Moldova [52]. Bioactive compounds are among the potential value-added products than can be extracted from the organic wastes. They could be isolated and structurally investigated, and subsequently used for the development of innovative products [53, 54]. Thus, the grape marc along with the other solid and liquid wine-production residues is used for feeding the cattle and poultry in agricultural sector, although having a low nutrition value [55, 56], and is also applied as fertilizer in agricultural fields [57]. But, according to the previous studies, the grape marc is a prospective raw material for obtaining of series of bioactive substances, specifically, fatty acids, pectins, phenols, etc. to be further used in the food production, supplements, medical products, cosmetics, colorants [58-61].

According to numerous works, polyphenols and other components contained in the grapes, wine or juice have beneficial effects on human health, such as inhibiting the development of certain cancer cells acting as free radical scavengers [62], antihyperglycemic, cardioprotective, anti-hyperlipidemic, etc. effects [63, 64]. Apart from the phenolic compounds, grape marc also contains significant amount of such potentially useful substances, some of them having the pronounced antioxidant activity as catechin, epicatechin, hydroxytyrosol, tyrosol, cyaniding glycosides, a series of acids like gallic, caffeic, procatechinic, syrignic, vanillic, *o*-coumaric, *p*-coumaric [65]. A solid-liquid extraction method [66, 67], hydrothermal treatment [68] and other approaches have been applied to extract the bioactive components from grape pomace, considering the fluctuating composition of its solid fraction for further using in food production. As waste biomass from agro-industrial sector can serve as a raw material for not only the energy production, but also for food and animal feed production, as a source of bioactive and other substances with useful properties for industry, its conversion efficiency needs to be improved. For this reason, current research is focused on increasing the product yields, improving the biochemical conversion technologies, increasing the conversion degree of biomass, improving the entire biochemical conversion system. Biochemical digestion of waste biomass using the microorganisms has an important advantage that no high temperatures are needed. In addition, the wet biomass of organic wastes has high water contents, making it necessary to apply the effluent treatment.

Our studies are focused on investigation of an original approach in enhancing the conversion rate and conversion degree of organic wastes biomass, using the small amounts of biologically active substances introduced into the fermented mixture, to accelerate the process rate and obtain the valuable products and, at the same time, to prevent the discharges of harmful mixtures into the environment. It was of special interest to study the effect of various additives of natural vegetal origin, introduced into the digested mixture and un type of biomass used.

2. Materials and Methods

The overall scope of the work was to elaborate processes of liquid wastes treatment from the agri-food sector, with obtaining the value-added products and avoid discharges into environment of toxic substances which may provoke unfavorable effects on soil, water, air and living organisms.

The following instruments and equipment were used for the research: thermostat PURA, digital pH-meter PH-3CU, refractometer Brix, titration equipment, bench-scale biochemical reactor. The studies of the effect of biologically active substances (BAS) on the digestion process have been performed using the vinasse resulted from the grains distillation at the" Garma Grup" company (Hâncești, Republic of Moldova). Also, the other types of wastes biomass have been taken for the research, such as wastes biomass from corn distillation.
Bench-scale technological experiments have been performed using the laboratory set-up in which the nutrition mixture and various types of bioactive additives have been introduced. The nutrition mixture has been prepared using 30 mL fresh grape juice with 10 % fermentation yeast 10^{10} CFU/g), 20 mL inversed sugar (42 %), 150 mL vinasse and 2 mL of BAS (3 g/L).

The biomass mixed with the nutrition mixture was left for alcohol fermentation under the mesophilic conditions (20-32^{o}C). The digestion process was followed by the volume of emitted gas (CO_2) which replaced NaOH, determined by standard method (titration with phenolphthalein). Composition and characteristics of initial vinasse used to study the fermentation process in the presence of bioactive additives, is shown on Table 1 and 2.

Table 1

Composition of initial vinasse* [69, 70]

	Component	*Corn vinasse*	*Grain vinasse*	*Barley vinasse*	*Rye vinasse*
1.	Dry matter, %	8.5	4.2	26.0	8.0
2.	Proteins (% of dry matter)	25.5	34.8	31.3	42.4
3.	Lipids (% of dry matter)	11.7	2.2	10.2	3.5
4.	Fibers (% of dry matter)	10.6	3.4	13.7	5.9
5.	Ash (% of dry matter)	4.7	8.6	2.1	3.5

* P⩾0,95

Table 2

Physico-chemical indices of media study mediums

	Indicator	*Vinasse*	*Grape juice*
1.	Sugar content, g/L	26.27 ± 0.91	124. 4 ± 1.6
2.	pH	3.91 ± 0.07	3.77± 0.12
3.	Titratable acidity, g/L sulfuric acid	3.417± 0.241	2.74± 0.322
4.	Contents in amine nitrogen, mg/L	492.2± 0.63	140.8± 1.2

3. Results

Digestion process was studied with introduction of biologically active substances of natural origin into the fermented biomass, which included the waste biomass and nutrition mixture. As can be seen from Figure 1, additives of aecium and betuline suppress the CO_2 emission, whereas using of tomatine ensures higher amount of gas evolved, or more intensive fermentation process, as compared to the witness test.

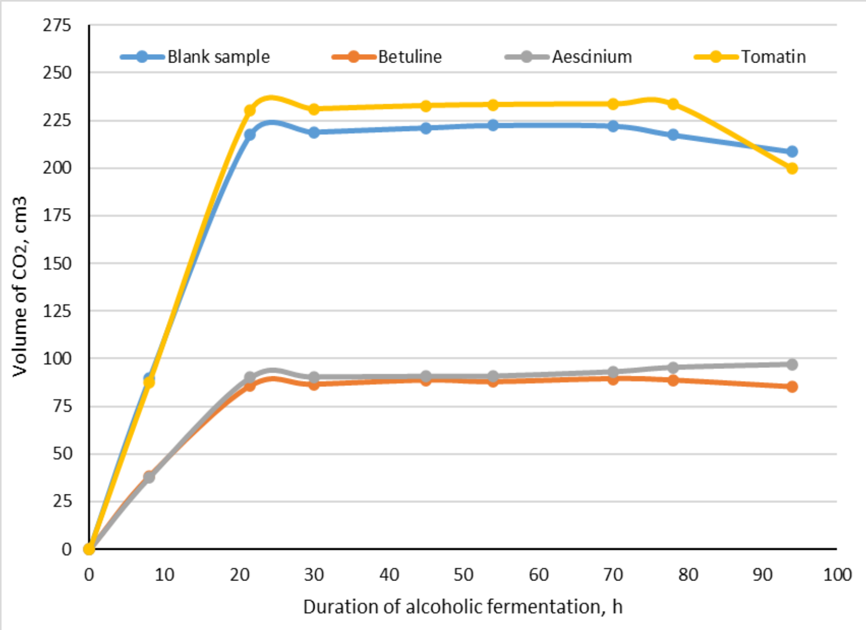

Figure. 1. Kinetics of carbon dioxide (CO_2) emission during the vinasse alcoholic fermentation in the presence of 0,006 g additives /L.

Practical experiences have shown that introduction of sclareol accelerates the biomass digestion, whereas addition of aescinum suppresses this process (Figure 2). Unlike these two bioactive substances, sclareol at the first stages of digestion significantly suppresses the CO_2 emission, and after 30 hours of fermentation, it makes this process somewhat more intensive as compared to the witness test.

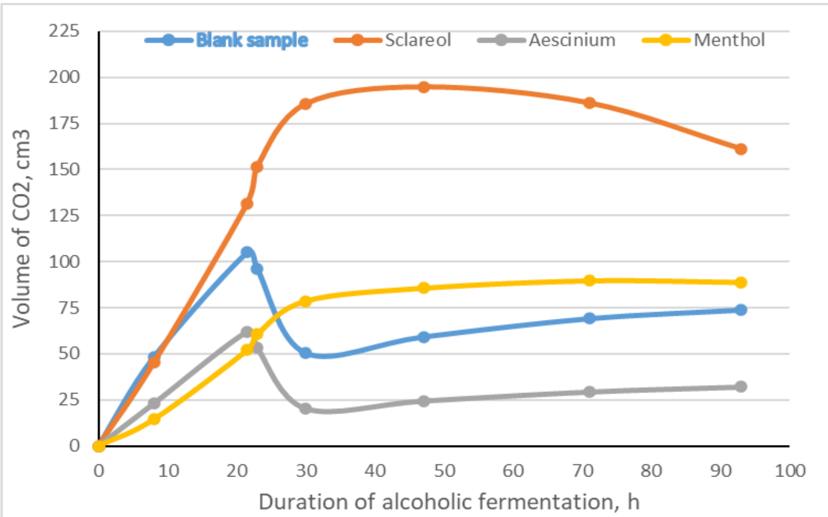

Figure. 2. Kinetics of the vinasse fermentation in the presence of additives of aescinum, menthol and sclareol of 0.006 g /L biomass.

At the same time, introduction into the alcohol fermentation of sclareol and menthol in different amounts (0.003-0.012 g/L biomass) have shown that the amount of additive introduced has no significant effect on the fermentation rate, although the higher amounts (0.012 g/L) of both additives did not demonstrate higher efficiency; on the opposite, the fermentation rate was lower as compared to first 3 addition (Figure 3). As compared to menthol, sclareol provokes more intensive fermentation at a much lower concentration.

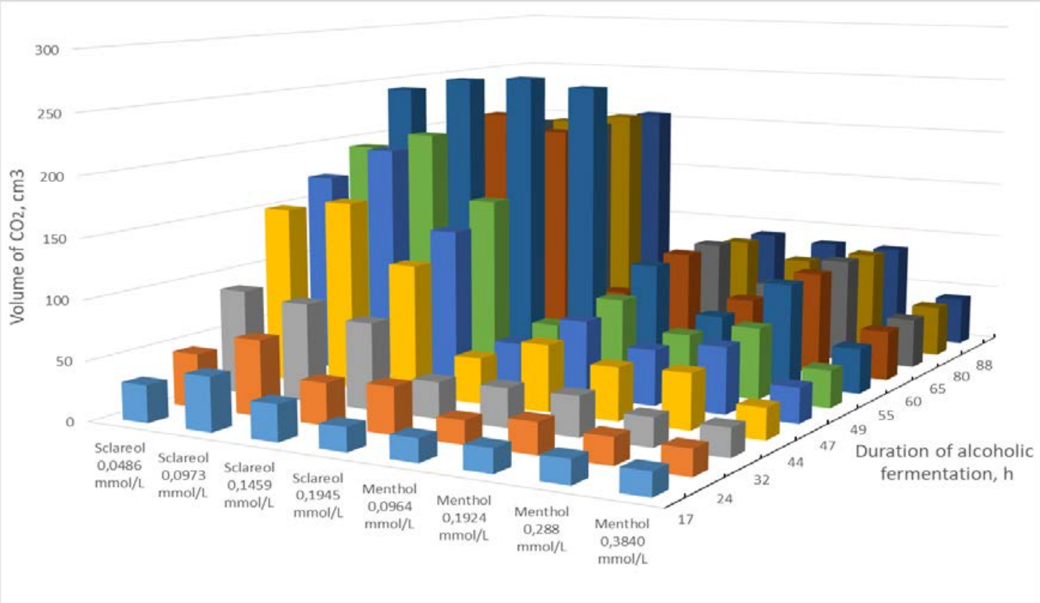

Figure. 3. Kinetics of the vinasse fermentation in the presence of sclareol and menthol additions.

In the presence of dihydroxyfumaric acid the vinasse fermentation proceeds somewhat more easily as compared to witness test, being approximately similar in case of introduction of additive in various concentrations ($0.1013 \div 0.4052 \cdot 10^{-6}$ mol/L), although with the lesser amount of addition $0.1013 \cdot 10^{-6}$ mol/L the fermentation was accelerated significantly (Figure 4).

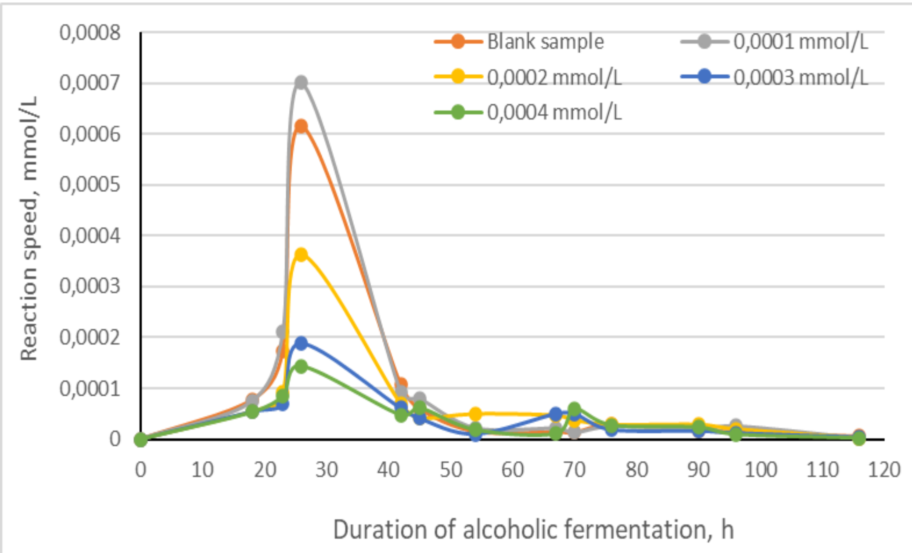

Figure. 4. Reaction rate dynamics of the vinasse alcoholic fermentation in the presence of dihydroxy fumaric acid addiction.

In case of vanillin introduction into the fermented mixture, likewise, the fermentation process was suppressed in case of introduction of higher amount of additive (0.19 ÷ 0.39 $\cdot 10^{-3}$ mol/L), whereas the insignificant acceleration of carbon dioxide emission, as compared to the witness test, was observed in case of the using of 0.098 mmol/L amount of vanillin addition (Figure 5).

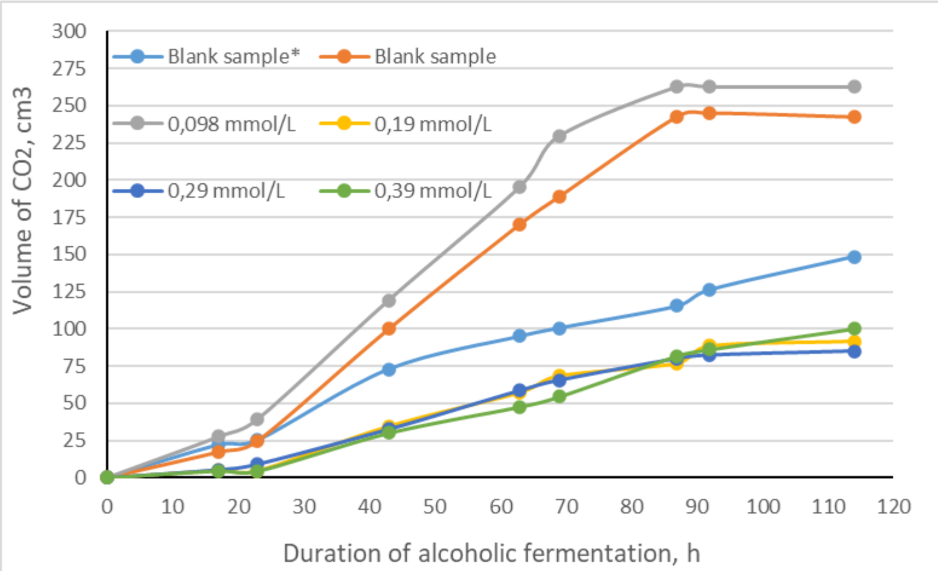

Figure. 5. Kinetics of the vinasse fermentation in the presence of vanillin additive.

Different amounts of catechin introduced in biomass have shown a difference in carbon dioxide gas emission: smaller amounts of additive provoked the lower efficiency of fermentation, and the higher concentration made the process more intensive as compared to witness test (Figure 6).

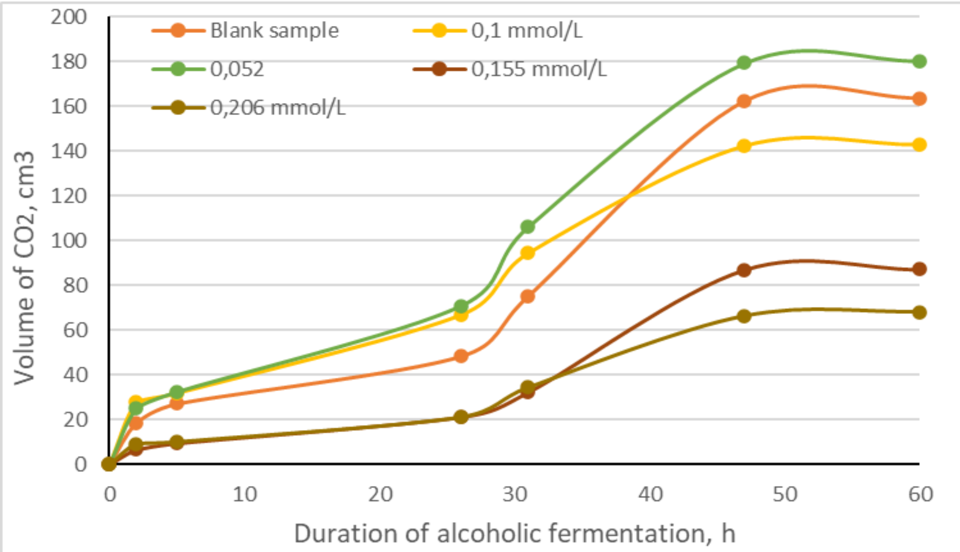

Figure. 6. Kinetics of the vinasse fermentation in the presence of catechin additive.

The results obtained demonstrate that the substances of natural origin with bioactive / antioxidant properties show the pronounced effects on the alcohol fermentation of vinasse under the mesophilic conditions. The most efficient concentrations of studied additives are summarized on Table 3.

Table 3

Comparative efficiency of different types of additives of bioactive substances in the vinasse fermentation process at concentration of 0,003 g/L biomass.

Nr. crt.	Bioactive substance used as an additive to the fermented biomass	Total volume of CO_2 emitted gas, cm^3	Fermentation time, h
1.	Dihydroxy fumaric acid	266.00	76
2.	Aescinum	251.01	55
3.	Tomatin	233.46	78
4.	Sclareol	232.50	55
5.	Vanillin	229.00	69
6.	Catechin	180.00	61
7.	Betuline	250.00	80
8.	Menthol	200.00	70

The results demonstrate that introduction of higher compounds of additives into the fermented biomass is not rational, as generally does not accelerate the fermentation process. It can be suggested that the ability of studied additives to accelerate the fermentation of liquid wastes from agro-industrial sector are connected with their molecular structure and antioxidant, antihypoxant, antimutagen, etc. properties.

4. Conclusions

The study of the influence of additives of biologically active substances on waste biomass fermentation from agro-industrial sector, along with the revealing of these processes' mechanisms can open a new perspective research direction in this area. This would allow not only to manage the fermentation processes, accelerating or suppressing them for the specific scopes, but also to obtain a series of value-added products under the more favorable conditions, as well as to prevent the discharges of harmful wastes into the environment. It was found that in case of tomatin application, higher amounts of emitted gas were observed, whereas in case of menthol, dihydroxyfumaric acid, sclareol, vanillin the rate of the gas emission was lower. At the same time, it became obvious that introduction of 2-4 times higher amounts of additives in many cases makes no sense as it does not provoke further acceleration of alcoholic fermentation process.

Products of alcohol fermentation can have the added value in agriculture and adjacent fields. Thus, carbon dioxide emitted during the biomass digestion can be used, for instance, in the microalgae cultivation basins, which in their turn, could serve as a valuable feed for poultry/cattle in zootechnical sector, while the water separated from solid fraction can be used for technical scopes at the industries (cleaning machines and apparatuses, cleaning and washing up the production halls and territory, etc.), or watering the plants in the city parks, flower beds, etc. The solid fraction after the fermentation, with the condition of its controlled composition, can be used as fertilizer for technical crops.

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