THE EFFECTS OF SOME COMPOUNDS OF Mn(II) AND Zn(II) ON THE MULTIPLICATION OF WINE YEAST AND BIOSYNTHESIS OF CARBOHYDRATES IN THE BIOMASS

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Abstract. The aim of investigation was to establish the influence of sulphates, acetates and coordination compounds of Mn(II) and Zn(II) as stimulators of the multiplication and growth of wine yeasts strain *Saccharomyces cerevisiae* CNMN-Y-20 and inducers of carbohydrate biosynthesis in biomass with the following elaboration of new procedures for directed carbohydrate biosynthesis. The study has been revealed that compounds $MnSO_4•4H_2O$, [Mn_2Ac(2PyTCH)-1,5-Bis(piridin)tiocarbohidrazid-dimanganese-acetate] and ZnLP-2 in optimal concentrations can be recommended as effective regulators of the yeast *Saccharomyces cerevisiae* CNMN-Y-20 multiplications, as well as compounds $[Mn(Gly)_2]Cl_2$, $(CH_3COO)_2Zn$ and ZnLP-2 in established concentrations - of the carbohydrates biosynthesis in the biomass of studied wine yeast strain. Stimulatory properties of studied compounds of Mn^{2+} and Zn^{2+} on carbohydrate biosynthesis in biomass of *Saccharomyces cerevisiae* CNMN-Y-20 can be explained both by the action of metal and by the nature of the ligand. The obtained biomass of wine yeast strain with high content of carbohydrates can be utilized for the obtaining of new bioproducts with the following application in the field of medicine and cosmetology.

Keywords: *Saccharomyces cerevisiae,* multiplication, carbohydrates, compounds of Mn (II) and Zn (II)

INTRODUCTION

In recent years, bioactive polysaccharides obtained from natural sources have attracted much attention due to their multiple applications in various industries: food and cosmetic, medical and pharmaceutical, veterinary, ecology and plant protection, extractive industries [16, 19, 25].

Carbohydrates, recognized as biologically active substances have generally beneficial effect on live organisms, due to their antiviral and antibacterial [26], immunomodulatory and immunostimulatory [6, 7, 23, 35, 36, 39, 44], antioxidant [17, 18], antimutagen [20], antitumor effects [24, 37, 38, 40], features that find applications in various fields. In this context great interest presents the obtaining of microbial polysaccharides, which is more advantageous economically to those of plant origin.

Among the microorganisms able to synthesize carbohydrates, the strain *Saccharomyces cerevisiae* is characterized as a superproducer of proteo-mannan and β-glucan, which are the basic substances for complex carbohydrates.

It is possible to use the organic or inorganic molecules that act as inducers, activators or inhibitors in the biosynthetic processes of a variety of bioactive substances such as antibiotics, vitamins, aminoacids. It is known that different taxonomic groups of microorganisms have used different metals in biochemical processes. For example, in cyanobacteria, copper participates in the processes of synthesis of plastocyanin - an important copper-containing protein; zinc - in the processes of synthesis of carboanhydrase; cobalt - in the processes of synthesis of cobalamin; magnesium - in the processes of synthesis of chlorophyll α; iron - in the processes of synthesis of phycobilins; molybdenum in the processes of synthesis of heterocyst and nitrogenase synthesis; the system for the photooxidation of water requires the presence of manganese [1, 5, 27].

Transition metal ions have a great importance for the assimilation of nutrient substrate or for the carbohydrates biosynthesis as cofactors of different enzymes. Some, like those of phosphorus (in excess) act as inhibitors of carbohydrate synthesis; others, like those of iron have a positive effect on carbohydrate biosynthesis at *Pseudomonas aeruginosa* and *Ganoderma oxidans.* Ions Zn^{2+} and Mn^{2+} are required for the biosynthesis of glucan in the biomass of *Rhizobium japonicun* and mannan in the biomass of *Saccharomyces cerevisiae.* Ions of Mg^{2+} and Ca^{2+} have positive impact on dextran synthesis in the biomass of *Leuconostoc mesenteroides* and exopolysaccharides in the biomass of *Azotobacter vinelandii* [43].

The yeast *Saccharomyces cerevisiae* response to availability of Zn^{2+} ions has been investigated in chemostats culture with the addition of various sources of carbon, nitrogen and aeration. The results have demonstrated a substantial reduction in levels of glycogen and trehalose in cultures limited by Zn^{2+}. An interesting fact was the regulation of Zn^{2+}-dependent genes involved in storage and metabolism of carbohydrates. These data suggest an prominent involvement of ions Zn^{2+} in the biogenesis and functions of yeast cell, than was revealed so far [10].

The extensive researches conducted on the cultivation of some strains of microorganisms from different taxonomic groups (bacteria, yeast, fungi, algae) confirms the efficiency of utilization of some coordinative compounds of transition metals to regulate the productivity and biosynthesis of bioactive principles by microorganisms. Recent researches of moldavian scientists have demonstrated the stimulatory effect of coordinative compounds [28], particularly of manganese and zinc on the synthesis of carbohydrates by cyanobacteria [9], the superoxide dismutase by cyanobacteria and microalgae [15], lipid components in yeast [21, 32], hydrolytic enzymes in fungi [46].

In this context the aim of investigation was to establish the influence of sulphates, acetates and coordination compounds of Mn(II) and Zn(II) as stimulators of the multiplication and growth of wine yeasts strain *Saccharomyces cerevisiae* CNMN-Y-20 and inducers of carbohydrate biosynthesis in biomass with the following elaboration of new procedures for directed carbohydrate biosynthesis.

MATERIALS AND METHODS

The strain of yeast *Saccharomyces cerevisiae* CNMN-Y-20, active producer of carbohydrates, was isolated from pure culture of yeasts sediment microflora of red (Cabernet) wines producing [8].

Cultivation of yeast was effectuated on the nutritive medium with the following composition, g/l: glucose – 10.0; peptone – 5.0; yeast extract – 3.0; malt wort – 3.0; distilled water - 1 liter [11] and medium Rieder with the composition, g/l: glucose – 30.0; $(NH_4)_2SO_4$ – 0.7; $MgSO_4*7H_2O$- 3.0; NaCl – 0.5; $Ca(NO_3)_2$ – 0.4; KH_2PO_4 – 1.0; autolisate of yeast – 10.0 ml; drinkable water - 1 l, pH- 5.0-6.0 [2].

The selected strains of yeast were cultivated on rotary shaker (200 rpm.), at temperature of 23-25°C, time of 120 h. The experimental samples were taken at 24, 48, 72, 96 and 120 hours of cultivation of yeast .

The following chemical compounds were selected as regulators of biosynthetic processes at yeast: mineral salts - manganese sulphate ($MnSO_4·4H_2O$) and zinc sulphate ($ZnSO_4·7H_2O$); acetates – manganese acetate $(CH_3COO)_2Mn$ and zinc acetate $(CH_3COO)_2Zn$; coordinative compounds of Mn(II): [Mn_2Ac(2PyFX)–1,7-Bis(piridin)malonodihidrazid-dimanganese-acetate], [Mn_2Ac(2PyTCH)-1,5-Bis(piridin)tiocarbohidrazid-dimanganese-acetate], [Mn_2Cl_2(2PyFX)–1,7-Bis(piridin)malonodihidrazid-dimanganese-dichloride)], [$Mn(Gly)_2]Cl_2$; coordinative compounds of Zn(II): zinc chloride monocloroacetate (ZnLP-1), zinc chloride tricloroacetate (ZnLP-2), zinc chloride tricloroacetate γ, γ′ dipiridil (ZnLP-3), zinc tartrate imidazol (ZnLP-4).

To select compounds perspective, the level of multiplication and productivity of *Saccharomyces cerevisiae* CNMN-Y-20 strain was compared to the amount of carbohydrates in the obtained biomass.

The determination of multiplication was effectuated photocolorimetrically [45].

The determination of productivity was effectuated gravimetrically [48].

The determination of total content of carbohydrates was effectuated according to spectrophotometrical method using antron reagent, standard-D-glucose [14].

Regression and statistical analysis of data obtained in three series was carried out by the methods proposed by Maximov [49] and Dospehov [47].

RESULTS

The study of scientific data enabled us to establish the importance of trace elements in the processess of growth of microorganisms. Most of the key enzymes in metabolic chains contain metal atoms as active center, especially of those transition. So, metals and their complexes significantly regulate the metabolic processes in the cell. Conform to the study of the effect of transition metals, such as manganese and zinc on the productivity of wine yeasts and biosynthesis of carbohydrates in the biomass, three groups of compounds: Mn(II) and Zn(II) sulphates, Mn(II) and Zn(II) acetates and some coordination compounds of Mn(II) and Zn(II) were selected.

The effects of Mn(II) and Zn(II) sulphates on multiplication, the productivity of wine yeasts and biosynthesis of carbohydrates in the biomass.

Usually, the composition of the medium of cultivation includes microelements in the form of inorganic salts, especially in the form of sulphates or acetates, which present sources of cofactors of the enzyme systems. Some authors indicate that mineral and organic components of groundwater can be used as a new nutrient source for yeast cultivation. The use of groundwaters to cultivate yeast leads to the increasing of productivity, permeability of cytoplasm membrane, to the stabilization of cellular structures and activation of enzymes of maltase and zymase complexes, of amino acids, proteins, carbohydrates biosynthesis [41, 42].

Due to of the importance of trace elements in the process of microorganisms cultivation, described in scientific sources, the influence of manganese and zinc sulphates on the biosynthetic activity of *Saccharomyces cerevisiae* strain CNMN-Y-20 have been studied in this investigation. The salts were included in the nutrient medium MGYP in the optimal concentration of 5.0 mg/l, equivalent to that of the corresponding metal of compound.

Initially, it was investigated the effect of manganese and zinc sulphates on the dynamics of yeast multiplication for 120 hours. The results are demonstrated in Fig. 1. It was established that the maximum of multiplication of yeast is recorded after 72 hours of cultivation in the presence of $MnSO_4•4H_2O$ and after 96 hours of cultivation in the presence of $ZnSO_4•7H_2O$. Subsequent phase of the study was to establish the effect of salts on the carbohydrate content of yeast biomass (Fig. 2). It was found that carbohydrate synthesis virtually is not influenced by investigated compounds. Moderate effect both of $MnSO_4•4H_2O$ and $ZnSO_4•7H_2O$ in the studied concentration on the productivity of yeast *Saccharomyces cerevisiae* strain CNMN-Y-20 and accumulation of carbohydrates in the biomass has demonstrated that these compounds can not be included in the list of factors limiting the cultivation of wine yeast.

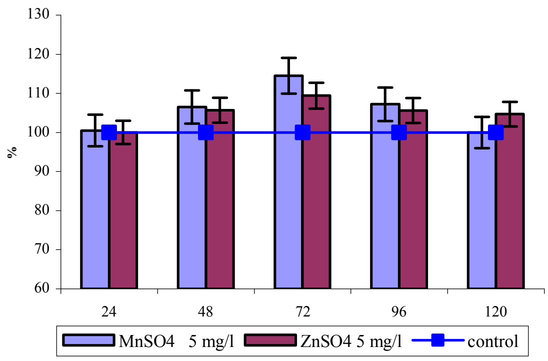


Figure 1. The influence of sulphates of Mn^{2+} and Zn^{2+} on the dynamics of yeast multiplication.

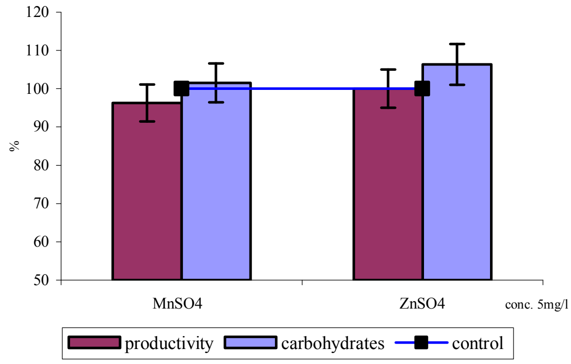


Figure 2. The influence of sulphates of Mn^{2+} and Zn^{2+} on the productivity of *Saccharomyces cerevisiae* CNMN-Y-20, cultivated at medium MGYP, and content of carbohydrates in the biomass.

The effects of Mn(II) and Zn(II) acetates on the multiplication, productivity of wine yeasts and biosynthesis of carbohydrates in the biomass.

It is known that acetates usually act as inducers of some processes of the biosynthesis of bioactive substances. The influence of manganese and zinc acetate, added to the nutrient medium Rieder in concentration of 10.0; 20.0; 30.0 mg/l on the multiplication, productivity of yeast strain and synthesis of carbohydrates in the biomass has been established.

The analysis of obtained results has demonstrated that the effect of acetates is variable and depends on the metal and concentration. Zinc acetate in concentration of 10.0 and 20.0 mg/l contribute to the increasing of carbohydrate content in the biomass of *Saccharomyces cerevisiae* strain CNMN-Y-20 with 26 and 14% respectively compared with blank. The increasing of the carbohydrate content is held on the background of decreasing of yeast multiplication and productivity (Fig. 3).

The positive effect of chemical compounds in the form of acetates of Zn(II) on the process of carbohydrates synthesis can be explained both by including of the ions Zn^{2+} as cofactors in structure of key enzymes of glucans and mannans biosynthesis in microorganisms and, in particular, in yeasts and by involving of Zn^{2+}- dependent genes in carbohydrates storage and metabolism [10], and by the including of acetates in the biosynthesis of acetyl coenzyme A, which is an important component of various metabolic processes [43].

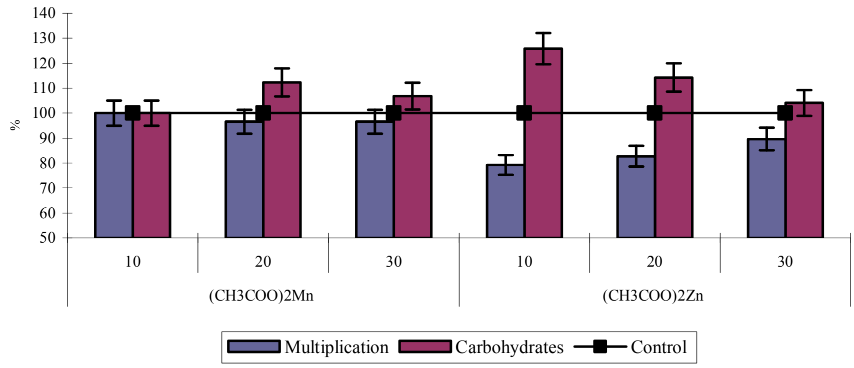


Figure 3. The influence of acetates of $Mn^{2+}$and Zn^{2+} on the multiplication of *Saccharomyces cerevisiae* CNMN-Y-20 and content of carbohydrates in the biomass at the cultivation on medium Rieder.

The effects of coordination compounds of Mn(II) and Zn(II) on the multiplication, productivity of wine yeasts and biosynthesis of carbohydrates in the biomass.

Coordination compounds [Mn_2Ac(2PyFX)–1,7-Bis (piridin) malonodihidrazid – dimanganese - acetate], [Mn_2Ac(2PyTCH) - 1,5 – Bis (piridin) tiocarbohidrazid – dimanganese - acetate] and [Mn_2Cl_2(2PyFX)–1,7-Bis (piridin) malono dihidrazid – dimanganese-dichloride)] were added to the medium of yeast cultivation MGYP in four concentrations to reveal their effect of the dynamics of the process of multiplication of yeast strains *Saccharomyces cerevisiae* CNMN-Y-20 (Fig. 4). It was established that the maximum multiplication was obtained after 48-72 hours of yeast cultivation in the presence of compound [Mn_2Ac(2PyTCH)-1,5-Bis(piridin)tiocarbohidrazid-dimanganese-acetate]. It was demonstrated that the phase of accelerated growth occurs earlier than the phase established in the case of use of sulphates of manganese or zinc.

It was demonstrated that coordination compound [Mn_2Ac(2PyTCH)-1,5-Bis(piridin)tiocarbohidrazid-dimanganese-acetate] in concentrations of 10.0 -15.0 mg/l had significant effect on the content of carbohydrates in the biomass of yeast strain *Saccharomyces cerevisiae* CNMN-Y-20, being more with 7-11% than the blank (Fig. 5), other compounds had not any stimulatory effect on the yeast productivity and carbohydrate content in the biomass.

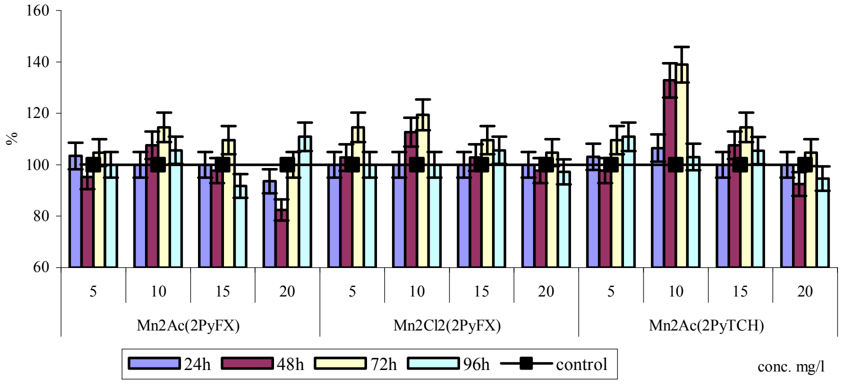


Figure 4. The influence of coordination compounds of Mn^{2+}on the dynamics of multiplication of *Saccharomyces cerevisiae* CNMN-Y-20 at the cultivation on medium MGYP.

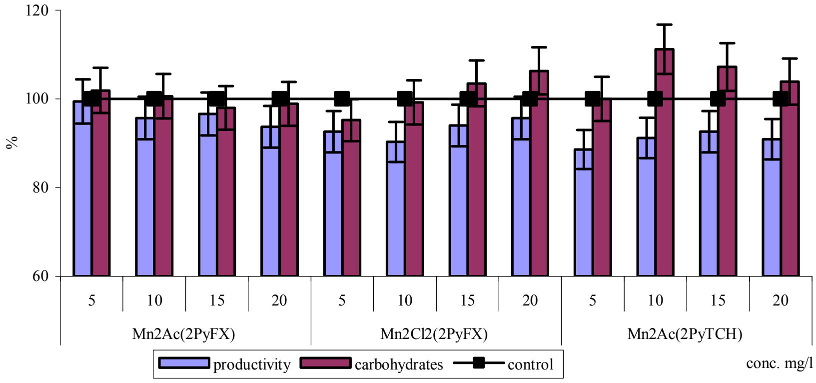


Figure 5. The influence of coordinative compounds of Mn^{2+}on the productivity of *Saccharomyces cerevisiae* CNMN-Y-20 and content of carbohydrates in the biomass at the cultivation on medium MGYP.

Another coordination compound of manganese with amino acid glycine $[Mn(Gly)_2]Cl_2$, added to the medium Rieder in concentration of 5.0 – 20.0 mg/l, had been characterized by the specific effect depending on the species of yeast (Fig. 6). It was established the stimulatory effect (by 18% compared to the blank) for carbohydrate biosynthesis in the biomass of *Saccharomyces cerevisiae* CNMN-Y-20. The positive effect of coordination compound of manganese on the process of carbohydrate biosynthesis can be explained by the synergistic action of the metal and ligand (glycine), which is involved in the synthesis of mannan-protein complexes, a key component of carbohydrates of wine yeasts [31].

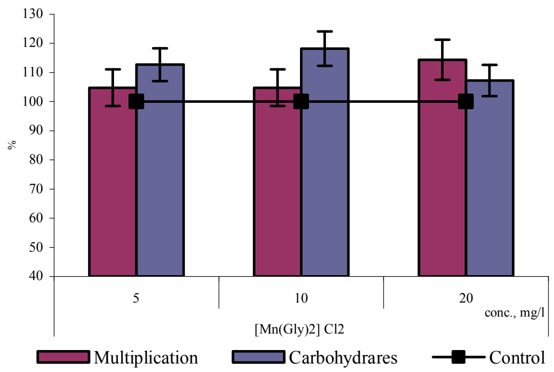
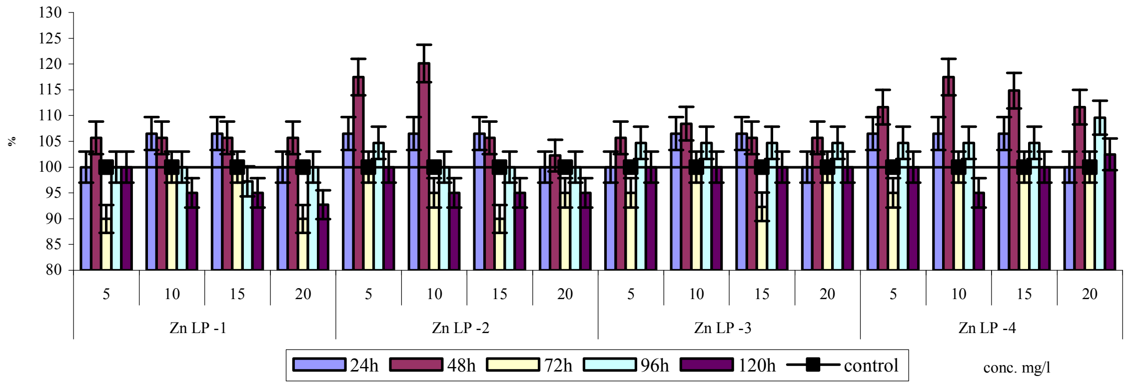


Figure 6. The influence of coordinative compound $[Mn(Gly)_2]Cl_2$ on the multiplication of *Saccharomyces cerevisiae* CNMN-Y-20 and content of carbohydrates in the biomass at the cultivation on medium Rieder.

The four of coordination compounds of zinc had the benefic effect on the multiplication of studied yeast strains. The compound ZnLP-2 - chloride of the trichloroacetate of zinc in concentrations of 5.0 and 10.0 mg/l, after 48 hours of *Saccharomyces cerevisiae* CNMN-Y-20 cultivation had the positive influence on cell multiplication (increasing by 17-20%, respectively, compared with blank) (Fig. 7).

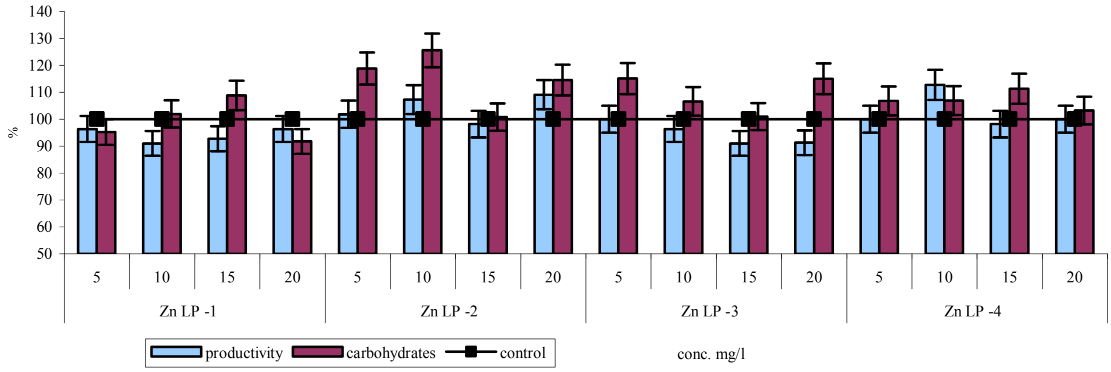
The ability of yeast strains to synthesize carbohydrates was subsequently studied. As it is evident from the results demonstrated in Fig. 8, the compound ZnLP-2 is characterized by properties of stimulating of the carbohydrates synthesis. The compound ZnLP-2 in concentrations of 5.0 and 10.0 mg/l have a beneficial effect on intracellular synthesis of carbohydrates, which values have increased by 18 and 25% respectively compared to the blank.

The obtained results demonstrate the need of the utilization of some quantities of coordination compounds for the stimulating of carbohydrates synthesis. Can be assumed, also, that the accumulation of studied coordination compounds in the biomass of wine yeast occurs as a result of metabolism transformation of carbohydrates, interspersed with other important metabolic pathways in the cell.



ZnLP-1 - zinc chloride monochloroacetate; ZnLP-2 - zinc chloride trichloroacetate;
ZnLP-3 - zinc chloride trichloroacetate γ, γ´ dipyridyl; ZnLP-4 - imidazole zinc tartrate

Figure 7. The influence of coordination compounds of Zn(II) on the dynamics of multiplication of the yeast *Saccharomyces cerevisiae* CNMN-Y-20 at cultivation on the medium MGYP.



ZnLP-1 - zinc chloride monochloroacetate; ZnLP-2 - zinc chloride trichloroacetate;
ZnLP-3 - zinc chloride trichloroacetate γ, γ´ dipyridyl; ZnLP-4 - imidazole zinc tartrate

Figure 8. The influence of coordination compounds of Zn(II) on the productivity of the yeast *Saccharomyces cerevisiae* CNMN-Y-20 and content of carbohydrates at cultivation on the medium MGYP.

DISCUSSION

In recent years the numerous biological and chemical investigations are focused on the studying of capacity of different compounds of transition metals (zinc, manganese, copper) to stimulate the growth and biosynthetic activity of microorganisms, including yeasts [3, 13, 30]. At the same time, inorganic forms of these metals often possess toxicity to living organisms [22]. In this context an important role have coordination compounds of transition metals due to the low toxicity and high degree of their assimilation by microorganisms. The possibility of using of coordination compounds of transition metals as regulators of biosynthetic activity of microorganisms was confirmed by different researches [12, 29, 33, 34]. However, there are a limited number of studies of the effects of coordination compounds on the synthesis of carbohydrates in the biomass of yeast.

In present investigation the influence of sulphates, acetates and coordination compounds of Mn(II) and Zn(II) on multiplication and growth of wine yeasts strain *Saccharomyces cerevisiae* CNMN-Y-20 and on the carbohydrate biosynthesis in biomass was established.

The beneficial effect of $MnSO_4 \bullet 4H_2O$ in optimal concentration of 5.0 mg/l and of coordination compound [Mn_2Ac(2PyTCH) - 1,5 - Bis (piridin) tio-carbohidrazid – dimanganese - acetate] in concentration of 10.0 mg/l (15 and 39% respectively, compared to the reference sample) on the multiplication of studied yeast strain has been recorded after 72 hours of cultivation. The inclusion of the coordination compound ZnLP-2 (chloride of the trichloroacetate of zinc) in medium of yeast cultivation in concentrations of 5.0 and 10.0 mg/l has demonstrated a maximum increase of multiplication (17 and 20%, respectively, compared to the reference sample) of *Saccharomyces cerevisiae* CNMN-Y-20.

It was established the stimulatory effect (by 18% compared to the reference sample) for carbohydrate biosynthesis in the biomass of *Saccharomyces cerevisiae* CNMN-Y-20 in the case of utilization of compound $[Mn(Gly)_2]Cl_2$ in concentration of 10.0 mg/l. The administration of zinc acetate $(CH_3COO)_2Zn$ in the optimal concentration of 10.0 mg/l contribute to the increasing of carbohydrate content in the biomass of *Saccharomyces cerevisiae* CNMN-Y-20 with 26%, compared to the reference sample**.** The coordinative compound ZnLP-2, added to the medium in concentration of 5.0 – 10.0 mg/l, had a maximum effect on the process of accumulation of carbohydrates (by 18-25% compared to the blank) in the yeast biomass.

Stimulatory properties of studied compounds of Mn^{2+} and Zn^{2+} on carbohydrate biosynthesis in biomass of *Saccharomyces cerevisiae* CNMN-Y-20 can be explained both by the action of metal and by the nature of the ligand. In the metabolism of *Saccharomyces cerevisiae* manganese and zinc has also an important role because it is incorporated in some enzymes, such as pyruvate carboxylase, glutamine synthetase, arginase, carboanhydrase and it is essential for the growth of yeasts [4].

In conclusion, the study of the effects of Mn^{2+} and Zn^{2+} in the form of sulphates, acetates or coordination compounds has been revealed that compounds $MnSO_4 \bullet 4H_2O$, [$Mn_2Ac(2PyTCH)$-1,5-Bis(piridin)tiocarbohidrazid-dimanganese-acetate] and ZnLP-2 in optimal concentrations can be recommended as effective regulators of the yeast *Saccharomyces cerevisiae* CNMN-Y-20 multiplications, as well as compounds $[Mn(Gly)_2]Cl_2$, $(CH_3COO)_2Zn$ and ZnLP-2 in established concentrations - of the carbohydrates biosynthesis in the biomass of studied wine yeast strain. The obtained biomass of wine yeast strain with high content of carbohydrates can be utilized for the obtaining of new bioproducts with the following application in the field of medicine and cosmetology.

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