I. ENVIRONMENTAL BIOTECHNOLOGY

BIOREMOVAL OF COPPER(II) VIA HYDROGEN FERMENTATION OF ECOLOGICALLY HAZARDOUS MULTICOMPONENT FOOD WASTE

Olesia Havryliuk, Vira Hovorukha, Galina Gladka, Oleksandr Tashyrev

Abstract: The environmental pollution by copper and the increasing amount of environmentally hazardous organic waste destroy natural ecosystems and have negative and even lethal effect on living organisms. The chemical techniques of metal containing waste detoxification are expensive and hazardous being the advanced problem today. The aim was to justify theoretically and confirm experimentally the possibility of toxic Cu^{2+} removal by hydrogen producing microbiome (HPM) via dark hydrogen fermentation of solid multicomponent food waste (MFW). Colorimetric and potentiometric methods were used for pH and redox potential measurement. Volumetric and chromatographic methods were applied to control volume and composition of synthesized gas. Fermentation parameters were calculated with the use of mathematical and statistical ones. The high effectiveness of solid waste destruction and Cu^{2+} removal was shown by spore forming HPM. The MFW were fastly and effectively digested by the microbiome at the absence of Cu^{2+} . The weight of MFW was 90 times decreased ($K_d = 90$). The maximum concentration of H_2 was 35% and biohydrogen yield was 76 L/kg of MFW counting on absolutely dry weight (ADW). The fermentation process was inhibited by Cu^{2+} in the form of citrate complex. The biohydrogen yield and efficiency of waste destruction were decreased on 41% (45 L/kg of waste) and 37% ($K_d = 57$) consequently after addition of 50 ppm Cu^{2+} to the culture liquid of the bioreactor during the beginning of final phase (50 hours) of MFW fermentation. The effect of complete inhibition of H_2 synthesis was obtained in the case of adding 100 ppm Cu^{2+} to the culture liquid sampled from bioreactor during the final phase (80 hours) of fermentation. Nonetheless, the Cu^{2+} was bioremoved by HPM with high efficiency up to 99.0 % and 99.5% after 5 hours and 30 hours of fermentation where initially the concentrations of Cu^{2+} were consequently 50 and 100 ppm. The synthesis of gas was not significantly restored after the addition of Cu^{2+} in both variants of the experiment. Obtained patterns will be used as a basis for the development of novel universal biotechnologies of metal-containing sewage purification with simultaneous destruction of MFW.

Keywords: environmental biotechnologies, biohydrogen fermentation, toxic copper (II) compounds, multicomponent food waste, metal-containing sewage purification

INTRODUCTION

The state of the environment continuously deteriorates due to the accumulation of heavy metals in ecosystems. Environmental pollution by toxic metals causes the destruction of ecosystems and poses a significant danger to human health [1].

Copper(II) containing compounds are one of the most hazardous toxicants of water and soil environment [2]. A number of polluted sites have been discovered, including soils of urbanized area of almost all of central and south-eastern Ukraine [3]. Significant environmental pollution by metals has also been observed due to the industrial and agricultural activities of man. The application of fertilizers, pesticides and fungicides to control bacterial and fungal diseases of fruits, vegetables, nuts and field crops is one of the prevalent sources of contamination by copper(II) [4]. Also, Cu(II) at high concentration is present in wastewater of enterprises of industrial processing of metals. For example, the soluble copper in chemical-mechanical polishing of wastewater can reach the total content 100 ppm ($\text{mg} \cdot \text{L}^{-1}$) [5]. Therefore, the development of effective methods of Cu-containing wastewater

treatment and ecosystems bioremediation from Cu(II) compounds is one of the priority issues today. Modern physico-chemical methods of metals extraction, such as adsorption on new adsorbents, ion exchange, membrane filtration, electrodialysis, reverse osmosis, ultrafiltration and photocatalysis are high-cost and often environmentally hazardous [6]. Therefore, the novel biotechnological methods being ecologically-friendly, low-cost are of considerable interest to science and industry.

Microbial treatment of industrial metal-containing sewage is promising research area. It is based on divalent copper accumulation, precipitation or reduction to insoluble monovalent copper compounds by microorganisms [7].

There is no doubt that copper (II) is very toxic metals-oxidizer and drastically inhibits microbial metabolism. Copper(II) is the metal of combined negative effect on microorganisms. It irreversibly oxidizes components of microbial cell and blocks its electron transporting chain [8].

Despite this, a wide range of microorganisms of different taxonomic and physiological groups are applied for the bioremediation of contaminated

environments from Cu(II) soluble compounds. However, modern biological methods of purification have certain disadvantages. Firstly, they have low efficiency of copper(II) removal. Secondly, they are empirical and don't have a theoretically grounded approach which allows developing novel effective bioremediation method. Finally, the most microorganisms are not resistant to copper(II) and die at the concentrations present in contaminated sewage.

The rapid accumulation of huge amounts of solid multicomponent food waste is another global environmental problem today [9]. The uncontrolled spontaneous decay of these waste causes contamination of environment by pathogenic microorganisms and the formation of toxic liquid and gaseous products (fatty acids, alcohols, ammonia, hydrogen sulfide, mercaptans, etc.) [10]. The outdated technologies of MFW detoxification, such as disposal and vermicomposting are still applied. Their main disadvantages are high cost and requirement of a large area for waste utilization as well as low waste recycling rate [11].

Herein, we propose the novel combined approach for fast and effective simultaneous reductive pathway of metals-oxidizers detoxification and anaerobic destruction of MFW with hydrogen gas obtaining.

Thus, the purpose of our study was to investigate the patterns of toxic copper(II) removal in the form of soluble citrate complex and simultaneously hydrogen fermentation of multicomponent food waste.

MATERIALS AND METHODS

Thermodynamic prediction as the universal method to predict possibility of microbial interaction with metals

Thermodynamic prediction was applied as the theoretical background to predict the possibility of citrate complex of Cu^{2+} removal from sewage by hydrogen-producing anaerobic microbiome. The thermodynamic prediction allows theoretically substantiating the most effective mechanisms of soluble toxic copper(II) ions detoxification by microbial reduction to insoluble copper(I) in the form of Cu_2O or precipitation of Cu^{2+} in the forms of $\text{Cu}(\text{OH})_2$, CuCO_3 etc. The Pourbaix diagram (the diagram of stability of the elements as aqueous electrochemical system in "pH-Eh" coordinates) was used as the basis for thermodynamic prediction of microbial interaction with copper(II) compounds [12].

Bioremoval of Cu(II) via fermentation of model multicomponent food waste

The effectiveness of copper(II) removal via hydrogen fermentation of multicomponent food waste was determined using the glass bioreactor. Its volume was 0.5 L. The community of spore-forming anaerobic microorganisms was used as the low-potential donor system. Pasteurized soil was used as inoculum and source of spore-forming hydrogen-producing microorganisms. For this purpose, solid multicomponent food waste (MFW) were prepared: 25 g of potato peels, 5 g of tomato tops, 5 g of apple peels, 10 g of rotten apricots, 5 g of meat (Fig. 1) waste and 10 g of pasteurized black soil (chernozem) were mixed in the sterile bioreactor (0.5 L).

Fig. 1. Mixture of solid model multicomponent food waste for hydrogen fermentation

All components of model waste were also pasteurized for 20 minutes in the water bath at 90 °C to eliminate the non-spore forming microorganisms and mixed with 250 ml of boiled tap water. The bioreactors were sealed with rubber stoppers with metal securers. The cultivation took place during four

days at 30 °C. The gas synthesized in the fermentation process was collected in a gas-holder. For this purpose, we used the gas-holder with rubber pipe and a needle. The rubber stopper of the bioreactor was pierced by the needle and the gas was removed to the gas-holder through the pipe, connected to the needle (Fig. 2).

Fig. 2. The process of gas volume measurement: A – the bioreactor, B – the gas-holder, C – the water seal

Air was the initial gas phase in the bioreactors. Saturated solution of NaHCO_3 was added to the reactor to provide optimal values of pH (close to pH=7.0) for optimal pathway of hydrogen fermentation of MFW.

Copper(II) solution was added to the glass bioreactor when microorganisms reached the final stage of metabolic activity. It was estimated by the decrease of hydrogen yield (concentration of H_2 in the gas phase of bioreactor decreased from 31% to 19%) and the RedOx potential began to rise. After that copper solution was added to the bioreactor to the final concentration 50 ppm Cu^{2+} . The experiment was carried out in triplicate. Statistical analysis was carried out using Exel and Origin 8.5.1. software.

Bioremoval of Cu(II) from liquid phase during final stage of fermentation of MFW

The fermented mixture of anaerobic batch bioreactor (240 L volume) sampled in the final phase (80 hours) of MFW fermentation was used to investigate the capability of microorganisms to remove 100 ppm Cu^{2+} (in complex with citrate) from liquid phase. The fermentation process was previously described [13].

For this purpose, 300 mL of culture liquid with low metabolic activity (the *Eh* increased from -360

to -150 mV, the H_2 concentration in the gas phase of bioreactor decreased) was sampled from the bioreactor (240 L) and transferred to the glass bioreactor (0.5 L). The gas phase (0.2 L) of the bioreactor was replaced by 2 L of argon. The flow rate of argon was 0.5 L/min. The bioreactor was hermetically closed. The cultivation took place during 12 hours at the 30 °C to provide strict anaerobic conditions for the optimal growth of hydrogen producing microorganisms. After that, copper solution was added to the cultivation system to the final concentration 100 ppm Cu^{2+} .

The preparation of copper solution and determination of Cu(II) concentration

The solution of 40000 ppm Cu(II) was used as the stock. It was prepared by $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$ dissolving in distilled water in a volumetric flask. The Cu(II) concentration was determined by a colorimetric method with 4-(2-pyridylazo)resorcinol (PAR) as well as the method of the substituent titration with PAR and ethylenediaminetetraacetic acid (EDTA) in the range of 0.5-7.0 ppm and 25.0-3000.0 ppm Cu^{2+} consequently. The methods are based on the ability of PAR to form colored red complexes with cations of bivalent metals including Cu(II) [14].

The control of metabolic parameters of the fermentation process

The concentration of hydrogen in the gas phase was analyzed by the standard gas chromatography method [15]. The chromatograph is equipped with two steel columns - one (I) for the analysis of N_2 , O_2 , N_2 and CH_4 , the second (II) - analysis of CO_2 . Parameters column: I - $l = 3$ m, $d = 3$ mm, molecular sieve 13X (NaX); II - $l = 2$ m, $d = 3$ mm, with carrier Porapak-Q; column temperature - $60^\circ C$, the temperature of evaporator - $+ 75^\circ C$, detector (katharometer) - $+ 60^\circ C$, the current detector - 50 mA. Carrier gas - argon, gas duct velocity - $30 \text{ cm}^3/\text{min}$. Volume of gas samples was: the first column - 2.5 cm^3 , the second - 1 cm^3 .

The redox potential (Eh) and pH of the medium were measured potentiometrically. For this purpose, determination of pH and Eh was performed using the EZODO MP-103 universal ionomer with the remote electrodes and temperature sensor. To measure pH and Eh, we used the combined Ezodo ceramic chloride electrodes with BNC connectors - PY41 and PO50 models, respectively. Before the measurement the electrodes were tested by convention standard buffer solutions. For pH check conventional pH-buffer solutions were used: solution of $KHC_2O_4 \cdot H_2C_2O_4 \cdot 2H_2O$ (pH=1.68); mixture of NaH_2PO_4 and K_2HPO_4 (pH = 6.86); $Na_2B_4O_7 \cdot 10H_2O$ (pH = 9.18). Standard pH-buffers were prepared according to the producer's manual (OJSC "Kiev plant RIAP"). For the validation of Eh measurement three buffer solutions were used. First - ferricyanide, with Eh = $+ 273 \text{ mV}$ ($13.5 \text{ g/L } K_3[Fe(CN)_6]$ and $3.8 \text{ g/L } K_4[Fe(CN)_6] \cdot 3H_2O$), second - Fe(II) citrate (10.0 g/L with Eh = $- 150 \text{ mV}$) and third - Ti(III) citrate (15.0 g/L , with Eh = $- 440 \text{ mV}$) [16].

The following metabolic parameters of MFW fermentation were controlled: 1) the duration of fermentation period (T , days); 2) biohydrogen yield (L/kg of waste); 3) the coefficient of waste destruction (Kd^1) [17].

RESULTS AND DISCUSSION

The following theoretical position is the basis of our approach. All metabolic oxidation-reduction processes are carried out by microorganisms only in the zone of thermodynamic stability of water. In neutral conditions (pH=7.0) water is stable in the

range of standard values of the redox potential E_o' from $- 414$ to $+814 \text{ mV}$. The upper limit of water stability is determined by the reversible oxidation reaction from water oxygen to molecular oxygen: $2H_2O = O_2 + 4H^+ + 4e^-$; $E_o' = +814 \text{ mV}$. The lower limit of water stability is determined by the reversible reaction of proton reduction to molecular hydrogen: $2H^+ + 2e^- = H_2$; $E_o' = - 414 \text{ mV}$. In the case when the redox potential of the aqueous solution is higher than $+ 814 \text{ mV}$, the oxygen of water acts as the reducing agent, and is oxidized to O_2 . As the result, the Eh returns to the initial value $+ 814 \text{ mV}$. In the case if the potential is lower than $- 414 \text{ mV}$, the proton of water acts as the oxidizing agent and is reduced to H_2 . The Eh also returns to the initial value, $E_o' = - 414$. It follows that water has the properties of a binary redox buffer that is stable in the range of the standard redox potentials from -414 to $+814 \text{ mV}$ [18][12]. In accordance with our hypothesis microorganisms' growth in the presence of the oxidized forms of toxic metals at high concentrations and interaction with them are theoretically permissible if the redox potential of the system formed by the metal and its reduced form is within in the zone of thermodynamic stability of water [18]. The redox potential of copper(II) reduction is equal to $+520 \text{ mV}$ (Fig. 3) [18]:

$$E_{o(pH=5.0)} = +520 \text{ mV} \quad (1)$$

So, the redox potentials of the reaction underlying the microbial removal of copper are inside of this zone.

We consider metal reduction by microorganisms as a binary redox reaction. In this reaction, metabolically active microorganisms are the donor system, and high-potential compounds of copper(II) are the acceptor system. It is obviously that the efficiency of metal reduction is proportional to the potential difference between the acceptor and donor systems. It follows that microorganisms with the lowest values of the redox potential reduce copper(II) with maximum efficiency. The hydrogen-producing anaerobic bacteria are known to create the lowest redox potential ($E_o' = - 414 \text{ mV}$):

It is obviously that the potential difference in 934 mV between the donor and acceptor systems will ensure the fastest possible reduction of copper(II) compounds.

From this follows that only strict anaerobes including hydrogen producing microbial community are the most effective to remove copper compounds from solutions.

¹ Kd - coefficient of destruction of MFW. $Kd = m_1 : m_2$, where m_1 and m_2 are the initial and final weight of MFW, respectively.

Fig. 3. The Pourbaix diagram showing thermodynamically stable forms of copper compounds: the zone of thermodynamic stability is limited by reactions **a** ($\text{H}_2\text{O} = \text{O}_2 + 4\text{H}^+ + 4\text{e}^-$, $E'_0 = +814$ mV) and **b** ($\text{H}_2 = 2\text{H}^+ + 2\text{e}^-$, $E'_0 = -414$ mV). Point **c** determines the precipitation of the Cu^{2+} -cation in hydroxide $\text{Cu}(\text{OH})_2\downarrow$.

The equations of the reduction reactions of Cu^{2+} to Cu^+ and Cu^0 in “pH-Eh” coordinates are shown on Fig. 3.

The reduction of $\text{Cu}(\text{II})$ to $\text{Cu}(\text{I})$ compounds is described by reactions 3, 5 and 6, and the reduction of $\text{Cu}(\text{II})$ to Cu^0 is described by reactions 1 and 7. Reactions 2 and 4 describe the reduction of $\text{Cu}(\text{I})$ compounds to Cu^0 (Fig. 3). The redox potentials of mentioned reactions are located inside the zone of thermodynamic stability of water (from -414 to $+814$ mV). Hence, all these reactions are theoretically premised to be carried out by microorganisms. However, the reaction of Cu^{2+} to $\text{Cu}_2\text{O}\downarrow$ reduction (5) is the most probable and available for microorganisms. Thus, the Cu^{2+} is highly soluble and available for the biochemical reaction of its reduction. Moreover, the significant redox potential difference (934 mV) between the donor and acceptor systems (dehydrogenases and Cu^{2+} respectively) will ensure the inevitable and fast reduction of Cu^{2+} to insoluble $\text{Cu}_2\text{O}\downarrow$. It is unlikely that microorganisms will be able to carry out the other redox reactions. A strongly acidic and unsuitable for microorganisms conditions (pH not higher than 1.0) are required for reactions 2 and 3. The standard redox potential of reaction 1 (+330 mV) provides the possibility of microbial reduction of Cu^{2+} to Cu^0 . However, it must

be kept in the mind that this reaction can be blocked by an intermediate compound insoluble copper(I) oxide (reaction 5):

The reactions 4, 6 and 7 (Fig. 3) cannot be carried out by microorganisms, because insoluble copper compounds ($\text{Cu}(\text{OH})_2\downarrow$, $\text{Cu}_2\text{O}\downarrow$) are not available for microorganisms.

Thus, the reduction of the well soluble Cu^{2+} cation to insoluble copper(I) oxide ($\text{Cu}_2\text{O}\downarrow$) is most likely to be carried out by microorganisms (Fig. 3, reaction 5). It follows that this reaction can provide effective purification of copper-containing sewage.

The rate and effectiveness of the redox reaction is proportional to the redox potential difference between the donor and acceptor systems. Among all microorganisms the lowest redox potential is created by anaerobic hydrogen- synthesizing bacteria ($2\text{H}^+ + 2\text{e}^- = \text{H}_2$, $E'_0 = -414$ mV). Therefore, it is obvious that exactly these strict anaerobic microorganisms are the most suitable for the purification of copper-containing sewage.

Microbial removal of Cu^{2+} -compounds from solution is possible not only due to the reduction reaction. Firstly, non-toxic insoluble copper hydroxide $\text{Cu}(\text{OH})_2\downarrow$ can precipitate owing to the biologically mediated increase of the medium pH up to 4.5-5.0

(Fig. 3, c). Secondly, Cu^{2+} cation can interact with CO_3^{2-} anion and form insoluble copper carbonate $\text{CuCO}_3\downarrow$ in neutral and slightly alkaline conditions:

Thus, all metabolic pathways of microorganisms' interaction with copper(II) can be theoretically justified by thermodynamic prediction method to provide environmental effect of the purification of contaminated solutions and industrial sewage from soluble toxic Cu(II) compounds.

Different mechanisms and conditions of copper removal by microorganisms have already been investigated. The efficiency of soluble copper(II) compounds biosorption depends on the pH values. For example, the biosorption and bioaccumulation of Cu^{2+} by the strain *Cupriavidus gilardii* CR3 was highly pH-dependent, and the optimum pH value was 5.0. Under these optimal conditions, the efficiency of copper(II) extraction was 27% [19]. The strain *Pseudomonas stutzeri* N5a showed maximal bioremoval efficiency of 82% in 20 ppm of Cu^{2+} -containing medium at pH 6.8 and 30 °C [20].

Shahin et al. showed the removal efficiency by calcined sludge depended on the concentration of Cu^{2+} . It was decreased from 100% to 88.6% with the increase of the concentration from 50 to 1000 ppm Cu^{2+} [21]. Copper biosorption efficiency by *Pseudomonas* sp. strain NA isolated from the copper contaminated vineyard soil increased with biomass growth. After 12 hours 23.3% of Cu(II) was bioremoved [22].

At the same time, dark hydrogen fermentation of organics is the promising and eco-friendly method for destruction of rotting ecologically hazardous solid and semi-solid organic waste [22–25]. Anaerobic microorganisms of the *Clostridium* genus are the most common producers of hydrogen via dark fermentation process [26–29]. The efficiency of such anaerobic processes was high because the waste used as substrates contained the concentrate of easily digestible carbohydrates – starch [31], glucose [26], sucrose [32] etc. For example, the digestion gas contained 47.2% of H_2 under fermentation of sucrose by *Clostridium* spp. The hydrogen production rate (HPR) ranged from 1.42 to 4.52 mol- H_2 /mol sucrose and the specific hydrogen production rate ranged from 78 to 841 mmol H_2 / g VSS-day (volatile suspended solids) [32].

However, no one had previously considered the possibility to apply hydrogen fermentation to purify copper-containing sewages. Exactly the thermodynamic prediction method allowed arguing that this type of microbial metabolism is the most

effective for the removal of soluble copper(II) compounds by microorganisms.

In our investigation, anaerobic hydrogen producing microbiome has provided high efficiency of MFW fermentation in control conditions (without copper) under the regulation of microbial metabolism by adding of regulators of microbial metabolism, i.e. RMM (Fig. 4, A).

The redox potential (Eh) of the culture medium was decreased from +318 mV to – 344 mV at the first 32 hours of hydrogen fermentation of MFW. The pH value decreased from 7.1 to 5.0 during the first 24 hours of cultivation. The pH value was stabilized in the range 6.5-7.2 due to addition of RMM during the first 32 hours of fermentation to provide optimal conditions for HPM growth. The maximum concentration of H_2 in the control conditions was 35% (Fig. 4, A). The high efficiency of MFW fermentation at the absence of copper were obtained: biohydrogen yield was 76 L/kg of solid food waste, coefficient of waste destruction $K_d = 90$.

As expected, the addition of 50 ppm Cu^{2+} after 50 hours of cultivation (at the beginning of the final phase of fermentation) caused a sharp increase in the redox potential from – 265 mV to + 256 mV and inhibition of hydrogen synthesis (Fig. 4, B, Fig. 6). The pH value did not change after addition of 50 ppm of copper(II) (Fig 4, B). It may indicate the inhibition of the metabolic activity of microorganisms. The concentration of H_2 decreased from 19% to 15% during 5 hours of copper(II) bioremoval (Fig. 6) and remained at this level until the end of the fermentation process (Fig. 4, B).

Under these conditions, the H_2 yield was 45 L/kg of solid food waste, coefficient of waste destruction $K_d = 57$. Thus, the process of fermentation was inhibited by 50 ppm Cu^{2+} (Fig. 4, B). The biohydrogen yield and efficiency of waste destruction were decreased on 41% and 37% consequently.

The cessation of gas synthesis and foam formation, as well as a much lower degree of waste particles destruction (Fig. 5) compared to the control after 70 hours of fermentation, also evidenced the inhibition of the MFW fermentation by Cu^{2+} .

Despite this, the high effectiveness of 50 ppm copper(II) removal by spore forming hydrogen-producing microorganisms was shown in our study. The concentration of soluble copper was half decreased after two hours of fermentation. The efficiency Cu(II) bioremoval was 99.0 % after 5 hours of waste fermentation (Fig. 6).

Fig. 4. High efficiency of MFW fermentation in control conditions (A) and at the presence of 50 ppm Cu^{2+} (B) (RMM – the points when the regulators of microbial metabolism were added)

Fig.5. Inhibition of MFW fermentation by Cu^{2+} (70 hours of fermentation)

Fig. 6. Bioremoval of 50 ppm Cu^{2+} during final phase of hydrogen fermentation of multicomponent food waste

To investigate the possibility of Cu(II) removal during the final phase of hydrogen fermentation of solid multicomponent food waste by liquid phase (i.e. culture medium) was another important step of our work. To study this process, we sampled the liquid phase during hydrogen fermentation of waste in the bioreactor (240 L). The end parameters (Fig. 7) of metabolic activity of liquid phase testified at completion of the fermentation process. The concentration of H_2 in the gas phase of the bioreactor decreased from 35% to 15%, the value of the Eh increased from -360 mV to -150 mV, as well as the gas volume increase was completed. After that the liquid phase was transferred (after 80 hours of fermentation of MFW) into a glass bioreactor (0.5 L) and cultivated for 12 hours at 30°C to stabilize the metabolic activity of the anaerobic HPM. Due to residual metabolic activity, the microorganisms showed reductase activity and synthesized H_2 . The initial parameters before the addition of Cu^{2+} were as follows: 15% of hydrogen, $Eh = -150$ mV, as well as the absence of gas volume increase. These parameters indicated the final phase of MFW fermentation. Under such conditions, the addition of

high potential Cu(II) at the concentration of 100 ppm causes considerable stress for strict anaerobic microorganisms. The redox potential rapidly increased from -150 mV to $+320$ mV. Therefore, microorganisms required 26 hours to adapt and return to the weak metabolic activity (the slight decrease of Eh from $+320$ mV to $+100$ mV). The concentration of Cu^{2+} has been reduced from 100 ppm to 66 ppm within 20 hours after copper addition. In general, the copper removal from liquid phase of bioreactor continued 30 hours. The Cu^{2+} was bioremoved by HPM to minimum detectable concentration in culture medium such as 0.5 ppm. Effectiveness of Cu^{2+} removal was 99.5 % (Fig. 7). As soon as the soluble copper(II) was bioremoved, it led to a correlatively decrease of the Eh (from $+320$ mV to 0 mV) and a slight increase of the concentration of hydrogen (from 15% to 18%).

Despite the fact that the H_2 concentration in the gas phase of the bioreactor increased after removal of copper, the synthesis of gas volume i.e. further fermentation was not restored (Fig 7.).

Fig. 7. Bioremoval of 100 ppm Cu^{2+} during final phase of hydrogen fermentation of MFW

Obviously, the bioremoval of copper was via the mechanisms of Cu^{2+} reduction to insoluble $Cu_2O\downarrow$ (Fig. 3, reaction 5) or precipitation in the form of $CuCO_3\downarrow$ due to the interaction with CO_3^{2-} anion [18]. Thus, we have proven that anaerobic hydrogen-forming bacteria effectively removed dissolved copper(II) (Fig. 7).

Strict anaerobic microorganisms are rarely used to bioremove oxidizing metals, including copper(II). For example, there is only one experiment when the

mix culture of bacteria containing *Clostridium* sp. has the ability to remove 89-92% of toxic Cu^{2+} in the range of temperatures $20-35^\circ\text{C}$ [33]. However, the methods for the complete removal of divalent copper from liquids have not yet been developed.

CONCLUSIONS

The possibility of effective extraction of toxic copper(II) from solutions by strict anaerobic microbiome via hydrogen fermentation of

environmentally hazardous solid multicomponent food waste was thermodynamically justified and experimentally confirmed. Obtained patterns of microbial detoxification of copper(II) are promising for the development of novel combined biotechnologies of metal-containing sewage purification and hydrogen gas obtaining via fermentation of ecologically dangerous multicomponent food waste.

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Ph.D. student Olesia Havryliuk

Researcher Vira Hovorukha, PhD. Deputy Head of the Department,

Researcher Galina Gladka, PhD

Prof. Oleksand Tashyrev, Head of the Department

Zabolotny Institute of Microbiology and Virology of the NAS of Ukraine,
Department of Extremophilic Microorganism Biology, rooms 122, 224, 116
154, Zabolotny str., Kiev, 03143, Ukraine
tel.: +380445263297

e-mails: gav_olesya@ukr.net

vira-govorukha@ukr.net

gladkagv@ukr.net

tach2007@ukr.net