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# BIOMASS AND BIOHYDROGEN PRODUCTION BY *ESCHERICHIA COLI* UPON CONSUMPTION OF MEAT INDUSTRY AND LIGNOCELLULOSIC CORN WASTES MIXTURE

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Physicochemical pretreatment of lignocellulosic corn fruit wastes (husk, shank, stalk) has been done. *E. coli* K12 growth, ORP kinetics, and $H_2$ production were followed upon utilization of corn waste hydrolysates (CWH) separately and their mixture. During the bacterial log growth phase with the decrease of medium pH the readings of Pt electrode from positive to low negative values (~ –450±10 *mV*) were observed, with the yield ~ 0.75 *mmol* $H_2$ /*L* and 0.29 bacterial cell dry weight *g*/*L* biomass in CWH based medium. Meat (chicken wastes) broth supplementation ~2 fold enhanced both *E. coli* biomass formation and $H_2$ production on CWH mixture.

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***Keywords*:** biomass, biohydrogen, meat and lignocellulose waste materials, *Escherichia coli*.

**Introduction.** Finding renewable energy sources requires a global effort to reduce the harmful effects of global climate change, as well as to meet future energy needs. Molecular hydrogen ($H_2$) is an efficient, valuable, and environmentally friendly fuel of the future. It can be obtained upon microbial growth on different waste materials [1, 2]. Currently, the management of waste or organic by-products is a major dilemma for many countries, moreover, the global rate of solid waste generation is expected to triple by 2100 [3]. In Armenia this situation is even worse: Armenia is underdeveloped in its waste management and recycling activities. According to the Statistical Committee of Armenia, 55.2 million metric tons of waste were produced in 2016 (www.armstat.am, retrieved 2018.02.18). There are up to 16 landfills in Armenia, however, no waste sorting, recycling, or reuse takes place at any of them. Recently, there have been several attempts initiated by public activists to face this problem.

*Escherichia coli* can ferment many natural sugars to form biomass, $H_2$, or other valuable bio-products. $H_2$ is produced upon glucose or xylose or arabinose fermentation during formate decomposition by formate dehydrogenase H (FDH-H) and hydrogenase (Hyd) enzymes [4–6]. In *E. coli* four membrane-bound [NiFe] Hyd reversible enzymes participate in $H_2$ metabolism and have an important role in cell

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energetics [4, 7]. Hyd enzymes' relationship to proton pumping $F_0F_1$ ATPase was shown [8].

Lignocellulosic biomass consists of cellulose, hemicellulose and lignin as the main components [2, 9]. Both cellulose and hemicellulose fractions are polymers of sugars and, therefore, a potential source of fermentable carbohydrates [10]. There are different approaches for lignocellulosic material pretreatment including physico-chemical, ionic liquid pretreatment, enzymatic, and others [2, 9]. However, the optimization of nutritional parameters and the environment (fermentation conditions) is of importance for the development of biological processes. Glucose, xylose and arabinose are the three most common sugars obtained by splitting lignocellulosic biomass and occupying up to 60–70 and 30–40% of their hydrolysates, respectively.

Lignocellulosic biomass is a potential source of fermentable carbohydrates (carbon source), whereas waste materials of the meat industry can be a source of nitrogen [11]. With the summer and beginning of autumn, corn and chicken wastes are highly available in Armenia (Fig. 1). Corn fruits are only 30% and the remaining 70% are corn husk, shank and stalk. Although corn waste has many applications, in Armenia 70% of corn is just wasted. According to the Statistical Committee of Armenia, the total production capacity is about 50 000 tons of meat a year. There are about 72 meat processing enterprises, 15 of which are relatively large. There are 20 small slaughterhouses. The total production capacity is about 5 000 *t* of fish, 7–8 thousand tons of poultry meat, 19 000 *t* of lamb products, and 5 000 *t* of total harvested corn annually.

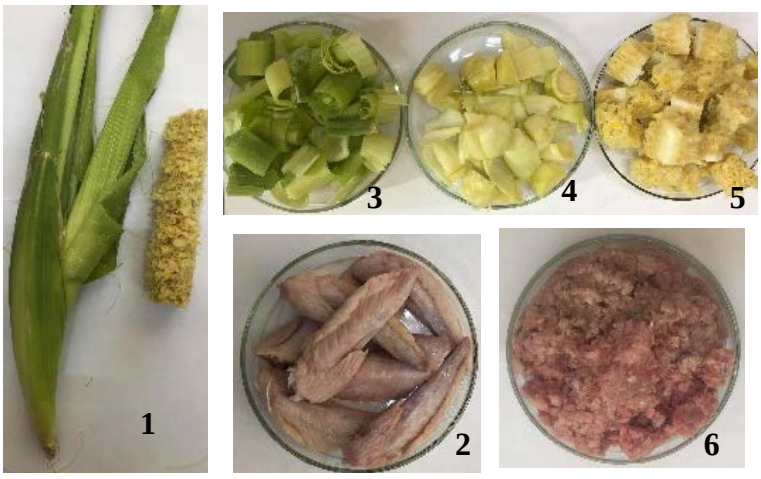


Fig. 1. Corn (1) and chicken wastes (2), corn husk (leafs) (3), corn shank (4) and corn stalk (5) and chicken milled wing tips (6).

In the present study, the pretreatment of waste materials has been done: lignocellulose-based corn wastes are used as a carbon source for bacterial cultivation, whereas meat industry waste (chicken wings tips) is considered as a source of nitrogen.

Enhanced biomass and $H_2$ production were identified upon *E. coli* K12 growth on a mixture of wastes.

**Materials and Methods.**

***Growth and Cultivation Conditions of Bacteria, $H_2$ Determination and Waste Treatment.*** Bacterial pre cultures (inoculums) were grown under fermentative conditions at pH 7.5, 37°C in peptone medium, composed of 20 $g \cdot L^{-1}$ peptone, 2 $g \cdot L^{-1}$ $K_2HPO_4$, 5 $g \cdot L^{-1}$ NaCl. pH was measured by selective to pH electrode, HJ1131B ("Hanna Instruments", Portugal) pH-meter. pH regulation was done by 0.1 *M* NaOH or 0.1 *N* HCl. Bacterial cell formation was estimated using Spectro UV-VIS Auto spectrophotometer ("Labomed", Los Angeles CA, USA), by measuring the optical density (OD) values of the bacterial culture at 600 *nm* [12].

The platinum (Pt) (EPB-1, GSEEE; or PT42BNC, "HANNA Instruments", Portugal) and titanium-silicate (Ti-Si) (EO-02, "GSEEE", Belarus) redox electrodes were used to determine oxidation-reduction potential (ORP) of *E. coli* K12 culture [13]. In contrast to Ti-Si, data obtained by Pt electrode indicate the presence of $H_2$. The electrochemical method of $H_2$ determination gives accurate data for cumulative $H_2$ yield in liquids. The $H_2$ production yield was calculated by expressing in *mmol* $H_2 \cdot L^{-1}$.

Corn wastes (husk, shank, and stalk) (Fig. 1) hydrolysates (CWH) were obtained by the dilute acid hydrolysis method in a steam sterilizer, 1 *h*, 121°C [9, 12].

Sugar concentration was measured by "One touch select glucose meter" ("Life Scan", Inc. Milpitas, CA 95035, China).

***Data Processing.*** Glucose, peptone ("Carl Roths GmbH", Germany) and other reagents used were of analytical grade. For data processing, Microsoft Excel 2016 was used. The average data were expressions of 3 independent experiments; the standard errors and Student criteria (p) were used to confirm the difference in average data between different series of experiments. Thus, the difference was applicable when $p<0.05$.

**Results and Discussion.**

***Determination of Biomass and $H_2$ Yield Upon E. coli Growth on CWH and MW.*** Preliminary treatment of lignocellulosic corn wastes was carried out to destroy the solid lignocellulosic structure. Wastes were treated by mechanical and physico-chemical methods: 8% of crushed corn wastes were treated with 0.75% $H_2SO_4$, and the hydrolysate was obtained 1 *h* after autoclaving. 150 *g* of meat waste (chicken wings tips) was crushed and boiled in 500 *mL* of water. Different amounts of broth (MW) were used in the experiments. After filtering the hydrolysates, the pH was adjusted to 7.5 with 0.1 *M* KOH, then centrifuged at 6000 *rpm* at 10°C for 4°C with a Rotina 420R ("Hettich", Germany) centrifuge. In this way, favorable conditions for the growth of bacteria and $H_2$ production were developed.

The concentration of sugar in the waste hydrolysates of different parts of corn was estimated: 518, 368 and 450 *mg*/*dL* glucose were identified in corn stalk, husk and shank hydrolysates, respectively. For comparison, the concentration of sugar in beer waste hydrolysate was 114 *mg*/*dL* [12]. *E. coli* K12 growth, ORP kinetics, and $H_2$ production were followed upon utilization of corn waste hydrolysates (CWH): Bacterial growth and hydrogen production has been studied both in the hydrolysates of corn shank, stalk, and husk separately, and in the mixture hydrolysates with different ratios to meat waste (MW). 3% of bacterial pre-culture was introduced into the growth mediums, at pH 7.5.

The growth of bacteria was followed during 24 *h*: 50% of MW was mixed with 50% of corn husk, stalk and shank hydrolysates. A decrease in pH from 7.5 up to 5.5 was observed along with bacterial growth and $H_2$ production, which is presumably due to the formation of organic acids during the assimilation of sugar in hydrolysates (Fig. 2).

Compared to control (peptone medium with 0.2% glucose) and samples with MW supplementations more acidification was observed when CWH was applied for bacterial cultivation. Besides bacterial growth and organic acid formation, buffering capacity of the solutions (peptone medium or CWH or CWH with MW) might influence pH value, which is probably low in CWH.

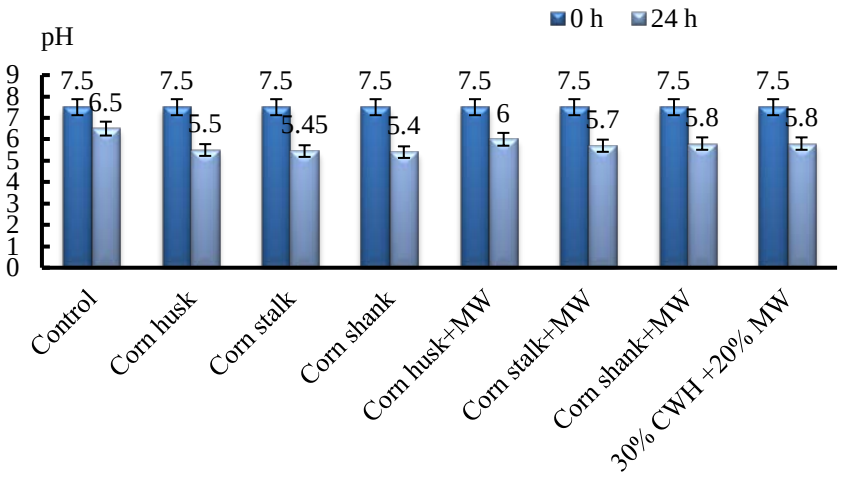


Fig. 2. The change in pH after 24 *h* of *E. coli* K12 growth at pH 7.5. Bacteria were grown anaerobically. For other details, see the legend to Fig. 1.

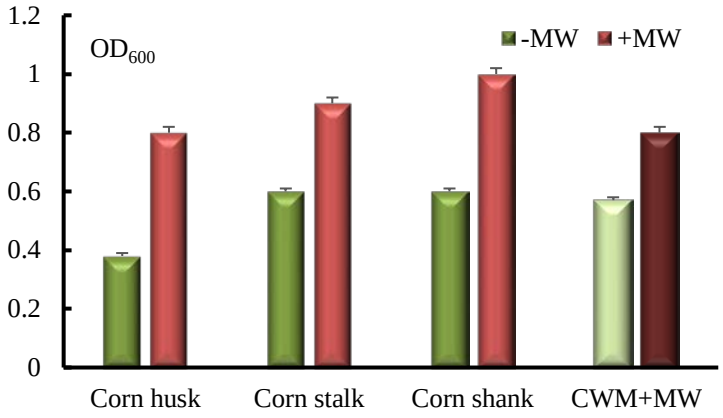


Fig. 3. Growth of *E. coli* K12 (OD) after 24 *h*, pH 7.5. Bacteria were grown anaerobically on corn waste hydrolyzates alone and mixed with MW: 50% husk, stalk and shank hydrolysate and 50% MW, and 30% CWH with 20% MW were applied.

Compared to control (peptone medium with 0.2% glucose, OD 1.3) ~3.4, 2.1 and 2 fold less biomass formation was observed when bacteria were grown in 100% corn husk, stalk and shank hydrolysates, respectively (Fig. 3). However, 50% MW supplementations stimulated bacterial growth on 50% corn husk, stalk and shank hydrolysates ~2, 1.5 and 1.7 fold, respectively. When 30% CWH was applied with 20% MW the growth stimulation was ~1.4 fold (Fig. 3).

The kinetics of ORP and $H_2$ production in wild-type *E. coli* under conditions of uptake of various hydrolysates was studied (see Fig. 4 and Table). The observation was made from the very start: from the beginning log phase of the growth, the Pt electrode reading decreased from positive to negative values: –460±5 *mV* and –440±10 *mV* decrease in ORP was observed in the corn stalk hydrolysate and control, and in the husk hydrolysate, respectively (Fig. 4). Later, in the 5$^{th}$ hour of growth, a drop in ORP up to –490 *mV* was observed in shank hydrolysate solution (Fig. 4).

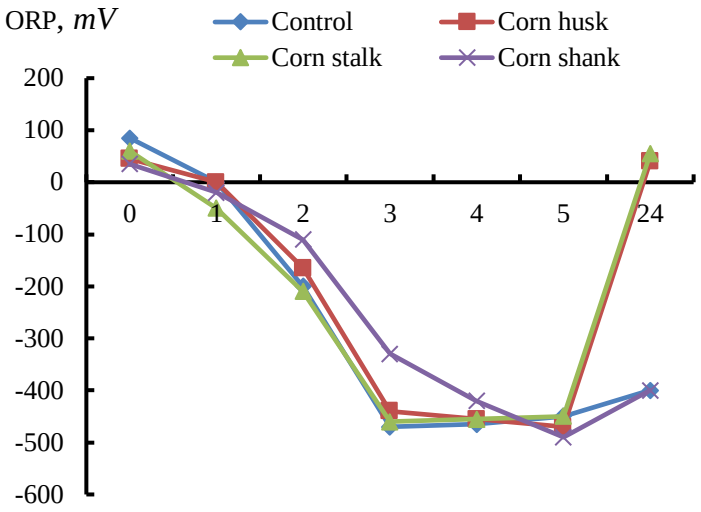


Fig. 4. The ORP kinetics by *E. coli* K12 during corn waste hydrolysates utilization, pH 7.5. Bacterial were grown anaerobically, ORP measured by Pt electrode was expressed in *mV* (vs Ag/AgCl, saturated by KCl). Control is bacterial growth on peptone medium with 0.2% glucose supplementation.

Bacterial growth and $H_2$ production were studied under conditions of assimilation of mixtures with different waste ratios. In 30% CWH+0% MW medium with the growth of bacteria, the yield of 1.35 *mmol/L* $H_2$ and 60% CWH+40% MW 1.45 *mmol/L* $H_2$ was stated (see Table).

As shown in Table, compared to control, in corn shank hydrolysate $H_2$ yield is stimulated ~1.7 fold during the bacterial log growth phase. Moreover, MW supplementation ~2 fold increased the production of $H_2$ in various hydrolysates.

$H_2$ production was detected after 24 *h* of bacterial growth, in the stationary growth phase in control samples, as well as, in the conditions when corn husk, stalk and shank was supplemented with 50% MW.

*The yield of $H_2$ during E. coli growth on waste hydrolysates, pH 7.5*

| Growth medium* | $H_2$ yield, *mol/L*, log growth phase | $H_2$ yield, *mol/L*, stationary growth phase |
|---|---|---|
| Control | 0.81±0.02 | 0.73±0.01 |
| Corn shank | 1.35±0.01 | – |
| Corn stalk | 0.80±0.02 | – |
| Corn husk (leafs) | 0.81±0.01 | – |
| 50 % Corn hank+50% MW | 1.45±0.01 | 0.73±0.01 |
| 50 % Corn stalk+50 MW | 1.45±0.02 | 1.44±0.02 |
| 50 % Corn husk+50 MW | 2.20±0.01 | 1.35±0.03 |
| 60% CWM+40% MW | 1.45±0.01 | 1.40±0.03 |
| 30% CWM+20% MW | 1.35±0.01 | – |

***** Control-peptone medium with 0.2% glucose.

Thus, the components of MW support prolonged $H_2$ formation during bacterial growth on corn waste hydrolysates.

**Conclusion.** Bioconversion of lignocellulose waste into biohydrogen is a promising strategy for both the production of less expensive energy and the simultaneous treatment of waste. The optimization of nutritional parameters and the environment (fermentation conditions) is of importance for the development of biological processes. The application of small amounts of meat industry waste significantly enhanced the heterotrophic growth of bacteria. It was shown that meat and corn wastes mixture can be a valuable source for the cultivation of *E. coli* and obtaining biomass and biohydrogen.

The results will open the new possibility of using cheap sources for microbial cultivation, corn and kitchen wastes mixture as effective substrates for obtaining diverse bacterial biomass and Hyds, which will provide not only low-priced energy generation but also solve the ecological problem of wastes utilization in Armenia.

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## ԿԵՆՍԱԶԱՆԳՎԱԾԻ ԵՎ ԿԵՆՍԱՋՐԱԾՆԻ ԱՐՏԱԴՐՈՒԹՅՈՒՆԸ *ESCHERICHIA COLI* ՄԱՆՐԷՆԵՐՈՒՄ ՄՍԻ ԱՐՏԱԴՐՈՒԹՅԱՆ ԵՎ ԵԳԻՊՏԱՑՈՐՆԻ ԼԻԳՆՈՑԵԼՅՈՒԼՈԶԱՅԻՆ ԹԱՓՈՆՆԵՐԻ ԽԱՌՆՈՒՐԴԻ ՅՈՒՐԱՑՄԱՆ ՊԱՅՄԱՆՆԵՐՈՒՄ

Իրականացվել է եգիպտացորենի լիգնոցելյուլոզային թափոնների (կեղև, կոթուն, ցողուն) ֆիզիկաքիմիական նախամշակում: Ուսումնասիրվել է *E. coli* K12 աճը, ՕՎՊ-ի կինետիկան և $H_2$-ի արտադրությունն եգիպտացորենի թափոնների հիդրոլիզատներում (ԵԹՀ) ինչպես առանձին, այնպես էլ դրանց խառնուրդներում: Աճի լոգ փուլում pH-ի նվազման հետ մեկտեղ դիտվել է Pt էլեկտրոդի ցուցմունքի անկում՝ դրականից մինչև բացասական (~ –450±10 *մՎ*), $H_2$-ի ելքը եղել է 0,75 *մմոլ* $H_2$/*լ*, իսկ կենսազանգվածը՝

0,29 ՉՔ *գ/լ*: Մսային (հավի թափոններ) արգանակի հավելումը ԵՖՀ խառնուրդին ~2 անգամ խթանել է ինչպես *E. coli* կենսազանգվածի ձևավորումը, այնպես էլ $H_2$-ի արտադրությունը:

С. С. БЛБУЛЯН, С. Н. МИРЗОЯН, К. А. ТРЧУНЯН, А. А. ПОЛАДЯН

## ПРОИЗВОДСТВО БИОМАССЫ И БИОВОДОРОДА У *ESCHERICHIA COLI* ПРИ ИСПОЛЬЗОВАНИИ СМЕСИ МЯСНЫХ И ЛИГНОЦЕЛЛЮЛОЗНЫХ ОТХОДОВ

Проведена предварительная физико-химическая обработка лигноцеллюлозных отходов кукурузы (листья, стебель, початок). Рост *E. coli* K12, кинетика ОВП и выделение $H_2$ изучены в гидролизатах отходов кукурузы (ГКО) как по отдельности, так и в смесях отходов. Во время log-фазы роста наряду с уменьшением pH наблюдалось снижение показаний Pt-электрода от положительного значения до отрицательного (~ –450 ± 10 *мВ*), выход $H_2$ составлял 0,75 *ммоль* $H_2$/*л*, а биомассы – 0,29 *г/л* сух. веса. Добавление мясного (из куриных отходов) бульона к смеси ГКО примерно в 2 раза стимулировало как продукцию биомассы *E. coli*, так и выделение $H_2$.