Research Article

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Metal distribution in the process of lignocellulosic ethanol production from heavy metal contaminated sorghum biomass

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Abstract

BACKGROUND: The objective of this work was to process the sorghum biomass obtained from crops cultivated on heavy metals polluted soil, for the production of lignocellulosic ethanol and to determine the amount of toxic metals in the intermediary, byproducts, and final products.

RESULTS: In the process of cellulolytic enzymes production by fungi using sorghum bagasse as substrate, a small amount of heavy metals passed into the liquid phase. During pretreatment by steam and alkali, the concentration of metals increased in the solid phase, while in the liquid phase the metals were not detected. After hydrolysis and fermentation of the pretreated biomass, the metals concentration in the solid fraction of the liquid increased, due to dissolution of the main fraction of the organic solids, although a fraction of the metals was found in the liquid fraction of the fermentation medium. During the distillation, some Pb and Cu were extracted into the distillate. Zn and Cd accumulated in the distillation residue.

CONCLUSION: The highest amounts of heavy metals accumulate in the solid residue, a small part is retained in the distillation residue, with traces of Pb and Cu potentially occurring in the distilled ethanol.
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Keywords: bioethanol; biomass; lignocellulose; heavy metals; sorghum

INTRODUCTION

Numerous studies indicated high-production biomass crops as an economically and environmentally friendly alternative to remediate heavy metal polluted sites.[1−3] Applying crops production for the phytoremediation of contaminated lands and using plants as phytoextraction tools have many advantages, such as covering polluted areas with organic matter which will lead to reconstruction of soil, reducing wind-driven particle, CO_2 fixation and O_2 production, and obtaining economic return by valorization of the biomass. Sweet sorghum (*Sorghum bicolor*) has been applied in several phytoextraction experiments in field conditions.[4−6] It is a versatile C4 plant that can be used for forage, seed production, manufacturing, but the most important and promising recent application is for energy and biorefinery. Sorghum sweet juice is an excellent fermentation medium for the production of ethanol and other biochemicals (such as lactic acid as precursor for poly (L)-lactide, or butanol) and the residual biomass bagasse can be further processed for lignocellulosic ethanol or biogas production, or as direct energy carrier, producing caloric energy by incineration.[7−9] The main drawback related to the biomass resulting from the phytoremediation of polluted land is the disposal of waste generated after processing polluted biomass. For example, in the process of pyrolysis/combustion of metal polluted biomass, almost all metals accumulate in the char/ash residue,[10,11]

raising questions regarding disposal of the heavy metals contaminated ash. The overall idea of our work is to process the sorghum biomass obtained on heavy metal-polluted soils to produce sugars, which can be converted to a wide range of biochemicals, to digest the residues for biogas production and to obtain digestate, which can be returned to the same polluted soil as a fertilizer. Therefore, heavy metals can be confined in the polluted area since there will be no wastes to be disposed of. The main objective of the present work was to assess at laboratory scale the biorefinery production path of sorghum biomass obtained from crops produced on polluted soils with emphasis on metals distribution

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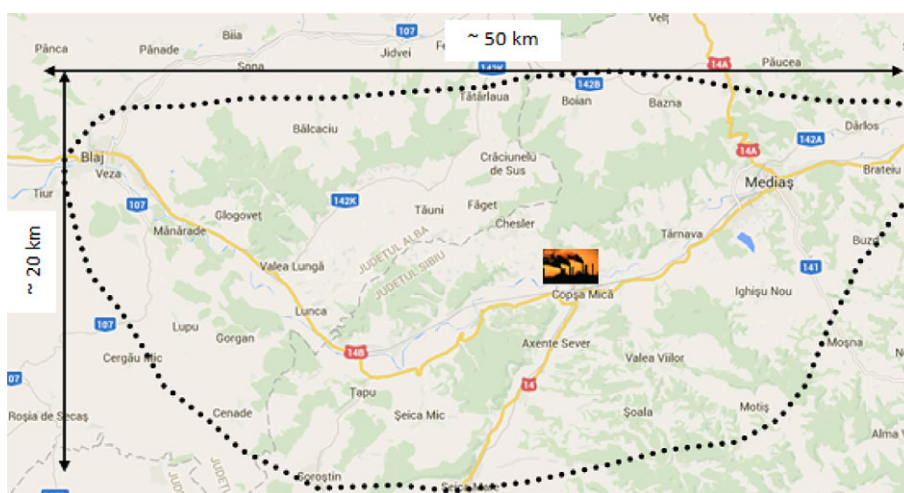d Department of Biotechnology, University of Szeged, Hungary

Figure 1. Polluted area of Copsa Mica.[12,14−17]

Table 1.	Metals content in the soil at the experimental site	
Element	Intervention threshold, according to Romanian Ministry Order 184/1997[18], mg kg^{-1}	Average*, mg kg^{-1}
Cd	5	10.10
Cu	200	24.50
Pb	100	460.00
Zn	600	530.00
*Source: Ref 14		

in the intermediary, byproducts and final products of the biorefinery.

MATERIALS AND METHODS

The biorefinery scheme of sweet sorghum in our work included the following steps: harvesting plants, removing leaves and panicles, pressing stems to extract sweet juice and produce bagasse, processing bagasse to obtain lignocellulosic ethanol, and anaerobic digestion of fermentation residues to produce biogas and organic fertilizer.

Sweet sorghum [*Sorghum bicolor* (L) Moench] cv. Sugargraze, was cultivated in the Copsa Mica area, Romania. In this area, the soil is polluted by a smelter that was used for the processing of metal sulfides between 1938 and 2009, and heavy metals have been reported to occur in soils especially 50 km along the Târnava Mare river (Fig. 1).[12−17]

Soil loading with heavy metals, at the level 0−20 cm, was previously determined by Iordache *et al.*[14] The values regarding concentrations of four elements found in the soil samples collected in the area of the experimental site are presented in Table 1.

Sorghum biomass was obtained from a plot of 5000 m^2, located at approximately 7 km distance from the smelter (40^{0}42'46''N, 74^{0}21''W, plot L 7.4) as presented by Iordache *et al.*[14] From the plot, 45 plants were harvested after 140 days of cultivation (89 BBCH) and transported for analysis in Timisoara, Romania. The BBCH is a scale used to identify the phenological development stages of a plant, and officially stands for 'Biologische Bundesanstalt, Bundessortenamt und Chemische Industrie'. After

measuring the plant height and weight, the stems were chopped to pieces of an average size of 2 cm using a laboratory mill with a six-discs rotor and 2 cm mesh (Retsch SM 100 - Germany). The juice was extracted from sorghum biomass using a stainless steel press (5.5 Litre Cross Beam Press – Ferrarigroup, Italy). The resulting bagasse was further processed to obtain lignocellulosic ethanol as follows.

Pretreatment of sorghum bagasse

Two pretreatment methods were applied: (i) mechanical pretreatment by milling biomass to obtain particles <1 mm; and (ii) thermo-chemical pretreatment, previously tested and indicated to be optimal for this type of lignocellulosic biomass.[19,20] The thermo-chemical pretreatment was carried out on biomass fragments of about 1 cm in size. The biomass was soaked in 2% (w/w) NaOH and then autoclaved at 1 bar (121°C) for 30 min. The biomass was then washed with 10% (w/w) H_2SO_4 until pH 6.5 when the samples were rinsed with 12 equivalent volumes of water in order to remove the inhibitors released during pretreatments. Both pretreatments were performed to increase accessibility of hydrolytic enzymes to cellulose and hemicelluloses from the lignocellulosic complex.

Production of cellulolytic enzymes

Trichoderma longibrachiatum DSM 769 and *Aspergillus niger* CMIT3.8 (local strain isolated from moldy pine sawdust) were cultivated using sorghum bagasse as substrate, to produce cellulolytic enzymes. The bagasse was pretreated as previously described to increase the rate of growth of the cellulolytic fungi and subsequently increase cellulase yields. The submerged cultures were obtained by inoculation with 10% (v/v) spores suspension (10^5 spores mL^{-1}) in 300 mL flasks containing 50 mL of Mandels basal medium[21] with 4% (w/w) milled pretreated sorghum bagasse (containing approximately 50% cellulose) as carbon source and substrate for cellulase production. With the culture conditions applied in this study (temperature of incubation 28°C; pH 4.8, agitation 150 rpm), the highest concentration of cellulolytic enzymes was obtained after 4 days of incubation. The activity of cellulolytic enzymes was evaluated according to the method described by Ghose.[22] According to this method, the total cellulases activity (expressed in filter paper units, or FPU) is established

when filter paper is used as substrate in the enzyme assay, while the activity of endoglucanase (CMCase activity) is established using carboxymethylcellulose as substrate in the enzyme assay and is expressed in U mL^{-1}. The liquid phase of the fermentation broth obtained after fungal growth contained a cocktail of cellulolytic enzymes which could be used to hydrolyze lignocellulosic biomass for ethanol production.

Enzymatic hydrolysis

In this experiment, pretreated bagasse was hydrolyzed using commercial cellulases, NS22086 cellulase complex, part of a *Novozymes* cellulosic ethanol enzyme kit. NS22086 contains cellulase and xylanase (endo -1,4-) and catalyzes the breakdown of cellulose into glucose, cellobiose, and glucose oligomers. The specific activity of the product was 80 FPU mL^{-1}. 1 unit of cellulase is defined as the amount of enzyme that releases 1 mg of glucose per min at 50°C and pH 4.8. The hydrolysis was carried out in 500 mL flasks with baffles and threaded openings containing 300 mL hydrolysis medium consisting of pretreated sorghum bagasse 50 g L^{-1} (D.M.), peptone 20 g L^{-1}, yeast extract 10 g L^{-1}, all suspended/dissolved in citrate buffer 0.05 mol L^{-1}, pH 5. Peptone and yeast extract were added to be present in the next phase – fermentation of hydrolyzate. The enzymes were added at the concentration of 15 FPU per gram of cellulose after sterilization of the hydrolysis medium. The flasks closed with screw caps containing the aforementioned components were incubated at 50°C and 150 rpm for 24 h.

Fermentation

After 24 h of hydrolysis, the temperature was decreased to 35°C and the broth was inoculated with dry *Saccharomyces cerevisiae* (provided by Protect Consult Bucharest, Romania) at a ratio of 1 g per 100 g of hydrolysis medium. The enzymatic activity of cellulases decreased at 35°C. This phase of alcoholic fermentation can be considered as simultaneous hydrolysis and fermentation. The screw caps of the flasks were replaced with NIR sensors (BlueSens, Germany) to measure and display in real time the concentration of CO_2 in the head space and dissolved ethanol during fermentation. The released gas was collected and measured with gas counters (BlueSens, Germany). The fermentation was terminated when the concentration of ethanol was constant and gas production ceased.

Distillation

The fermented broth was centrifuged to separate solids, and the supernatant was distilled using a conventional installation comprising a boiling flask, a condenser and a collecting flask. Four consecutive fractions of distillate (50 mL each) were collected from 500 mL fermented broth and noted as the distillation fraction 1, 2, 3 and 4. The rest of the broth was retained as vinasse.

Anaerobic digestion

The solids recovered after alcoholic fermentation were collected and transferred in 500 mL flasks for anaerobic digestion and inoculated with slurry originating from a UASB biogas reactor. The inoculation ratio was 1.2 parts inoculum to 1 part substrate, estimated on organic dry matter basis. The anaerobic digestion experiment was conducted according to the standard method VDI 4630.[23]

Analysis

The concentrations of micro- and toxic elements were measured by using inductively coupled plasma mass spectrometry (ICP-MS;

Thermo Scientific XSeries II, Asheville, NC, USA). The given samples of shoots and leaves of sorghum plants were dried at 70°C for 72 h. After drying, 8 mL of 65% (w/v) nitric acid and 2 mL of 30% (w/v) H_2O_2 were added to the dried and ground samples (100 μg each), which were next subjected to 200°C and 1600 W for 15 min in a high pressure microwave destructor (MARS X-Press, CEM, Matthews, NC, USA). Each sample was diluted post-digestion to the final volume of 20 mL using double distilled water. The metal levels are given in mg kg^{-1} dry weight. The analysis of biomass composition (dry matter, ash, organic dry matter, cellulose, hemicellulose, acid insoluble lignin) was conducted according to the procedures recommended by NREL (National Renewable Energy Laboratory, NREL Laboratory Analytical Procedure).[24] To determine the reducing sugars content of the juice extracted from sweet sorghum, as well as the sugars released after the enzymatic hydrolysis of bagasse, the DNS method was applied.[21] An enzymatic kit based on GOD POD enzymes was used to measure glucose concentration and the absorption of the resulting red quinonic complex was measured at 500 nm. Total sugar content was determined using a RMR200 refractometer (Hanna Instruments). A C932 pH meter (Consort, Belgium) was used to determine the pH values in the pretreatment, hydrolysis, fermentation and anaerobic digestion processes. The dry matter content of the biomass, bagasse and fermentation residues was quantified by oven-drying at 105°C. Finally, the ethanol concentration was assessed by NIR methods [Near Infrared, 1150 nm – 1200 nm] using an Alcolyzer M (Anton Paar, Austria).[25]

Heavy metals concentration results, sugars yields, hydrolysis rates, composition indices of biomass reported in this paper were expressed as means of replicates ± SD. To determine the influence of pretreatment method on metal retention in the solid phase of the fermentation broth a Mann–Whitney U Test was performed for each metal analyzed to compare the corresponding ratios between the metal concentrations measured in bagasse and those found in the solid phase of the fermentation broth. Statistical analysis was conducted using Statistica 10 software package (Statsoft Inc.). A P value <0.05 was considered significant.

RESULTS AND DISCUSSION

After milling and pressing sorghum biomass, the productivity of extracted juice was between 0.16 and 0.27 g g^{-1} reported to biomass fresh mater. Data in Table 2 indicate good productivity regarding sugar content of the sorghum juice. Regarding the content of heavy metals in juice, it is important to highlight the effect of the heavy metals on fermentation properties of the sweet juice, as the main reason to harvest sweet juice from sorghum is for ethanol production. Previous research indicates general toxicity level of heavy metals in *S. cerevisiae* starting from 200 μmol L^{-1} (16.4 mg kg^{-1} for Pb, 9.6 mg kg^{-1} for Cd).[26] Results in Table 2 indicate that the presence of Cd was not detected in juice extracted from sorghum biomass produced on heavy metals polluted soil and the concentrations of Pb and Zn are under toxicity levels. These findings recommend sorghum as a crop able to grow on polluted soil and to provide readily fermentable sugars by juice extraction and more than that, by hydrolysis of lignocellulose additional fermentable sugars can be obtained. In the next section we evaluate the production of fermentable sugars from sorghum biomass and the distribution of heavy metals will be followed.

The solid part of sorghum biomass retained after milling and pressing (i.e. bagasse) was submitted to pretreatment. The two

Table 2. Sugars content and metals concentration in sorghum juice

	Glucose (g L^{-1})	Reducing sugars (g L^{-1})	Total sugars (Brix)	Cd (mg kg^{-1})	Cu (mg kg^{-1})	Pb (mg kg^{-1})	Zn (mg kg^{-1})
Average	15.67	67.47	14.27	n.d.*	n.d.	0.70	50.78
Variation interval	14.35–16.42	64.58–84.52	11.6–15.8	n.d.	n.d.	0.5–1.0	22.7–86.2

*n.d. = not detected

Table 3. Main composition of mechanically and thermo-chemically pretreated bagasse

Type of biomass	Dry matter (%)	Ash (%)	ODM *(%) (reported to fresh matter)	ODM *(%) (reported to dry matter)	Cellulose (%)	Acid insoluble lignin (%)	Hemicellulose (%)
1	2	3	4	5	6	7	8
Milled bagasse	91.01 ± 0.43	9.05 ± 0.66	82.78 ± 0.94	90.95 ± 0.66	31.27 ± 2.17	17.43 ± 1.03	22.24 ± 0.72
NaOH/steam pretreated bagasse	19.58 ± 1.60	2.61 ± 0.51	18.94 ± 1.69	96.72 ± 1.60	51.46 ± 2.81	9.72 ± 0.98	27.63 ± 0.35

*Organic dry matter

Table 4. Content of heavy metals in original and pretreated sorghum bagasse

	Original and milled bagasse		Thermo-chemically pretreated bagasse	
	Average	Variation interval	Average	Variation interval
Cd (mg kg^{-1})	3.60	0.90 -5.36	4.03	2.5-4.03
Cu (mg kg^{-1})	15.57	13.53-18.12	25.46	10.50-38.90
Pb (mg kg^{-1})	11.24	3.14-18.52	19.38	9.90-34.00
Zn (mg kg^{-1})	123.50	71.31-162.60	134.19	83.10-192.30

methods of pretreatment applied in this research provided two different types of raw material to be used in the hydrolysis phase. The milled biomass was a powdery material, where the access of cellulases to cellulose was facilitated by destructuring the lignocellulosic complex. In the case of thermo-chemical pretreatment, the biomass was soaked in NaOH, autoclaved, treated with acid and water to lower the pH and the resulting wet material became amorphous compared with the fibrous structure of the biomass before pretreatment. The thermo-chemically pretreated biomass absorbed water, its volume increased and was immediately processed by enzymatic hydrolysis to prevent pore collapse into the biomass micro-structure. The irreversible pore collapse can occur during drying, or sublimation in the case of prolonged freezing. The treatment of bagasse with NaOH under pressure and steam led to the absorption of liquids, and consequently, the dry matter content decreased from >90% in the dry bagasse to <20% (Table 3). Cao *et al.* reported that a similar pretreatment applied to sorghum bagasse caused swelling that increased the biomass internal surface area, and moreover, partially disrupted the lignin structure.[27] This was confirmed by our results (Table 3, column 7), which disclose a 55.77% decrease in lignin content after the NaOH/steam pretreatment. Silverstein *et al.* reported 65.63% delignification of biomass applying similar combination of NaOH solution and steam pressure at 121°C for 90 min.[28] Another effect of thermo-chemical pretreatment is the increase of cellulose/lignin and cellulose/hemicellulose ratios

in the biomass. These ratios increased from 1.79:1 cellulose/lignin and 1.41:1 cellulose/hemicellulose in mechanical treated bagasse to 5.29:1 cellulose/lignin and 1.86:1 cellulose/hemicellulose in thermo-chemical treated bagasse. As a result, it can be concluded that the thermo-chemical pretreatment improved the glucose yields in the hydrolysis phase by removing lignin and hemicelluloses and increasing cellulose accessibility.

In addition, the samples from the original and milled bagasse, as well as those from the thermo-chemically pretreated bagasse were analyzed to assess their heavy metal content (Table 4). The average levels found in the thermo-chemically pretreated biomass were higher than those measured in the milled biomass. This may result from the increasing availability of free metals after efficient decomposition of the lignocellulosic complex by the combined action of heat and alkalinity. The partial loss of insoluble compounds from the biomass (e.g. hemicellulose) by degradation into soluble smaller molecules (e.g. arabinose, xylose, glucose) during pretreatment may also cause this composition change in solid biomass after pretreatment.

A fraction of milled bagasse was used as a substrate in submerged cultures of *T. longibrachiatum* and *A. niger* for biosynthesis of cellulolytic enzymes. The activity of endoglucanase in the samples harvested from the liquid phase of fungal cultures reached a maximal level of 2.25 U mL^{-1} (CMCase activity) in the first 24 h of cultivation. Regarding the total cellulases activity, the maximum of activity of 1.2 FPU mL^{-1} was observed after 96 h of culture. Enzyme production ceased after 120 h. The solid and liquid phases of fungal culture broth were separated by sedimentation and the metal contents in both phases were analysed. The results are presented in Table 5.

The liquid phase of fungal culture broth contained cellulolytic enzymes that could be used in the hydrolysis of pretreated sorghum biomass. The present data (Table 4) reveal that the liquid collected from fungal cultures obtained in media containing sorghum bagasse have a low metal content and the major part of the metals are bound to the solid fraction. Thus, the heavy metal-contaminated sorghum biomass has the potential to be used as a substrate for cellulase production and the polluting metals will not be found in high concentrations in the enzyme preparation.

Table 5. Content of heavy metals in solid and liquid phases of fungal culture broth

| Element | Concentration | | | |
| | in solid phase, mg·kg⁻¹ | | in liquid phase, mg·l⁻¹ | |
	Variation interval	Average	Variation interval	Average
Cd	1.82-2.85	2.335	0.74-0.76	0.75
Cu	12.75-14.01	13.38	0.03-0.09	0.06
Pb	11.40-13.27	12.34	2.49-2.58	2.54
Zn	68.5-122.8	95.65	12.5-19.6	16.05

The enzymatic activity of the cellulolytic preparation was rather low and the cellulases were unstable in water, therefore in the hydrolysis phase commercial cellulases were used, i.e. the NS22086 cellulase complex, part of a *Novozymes* cellulosic ethanol enzyme kit.
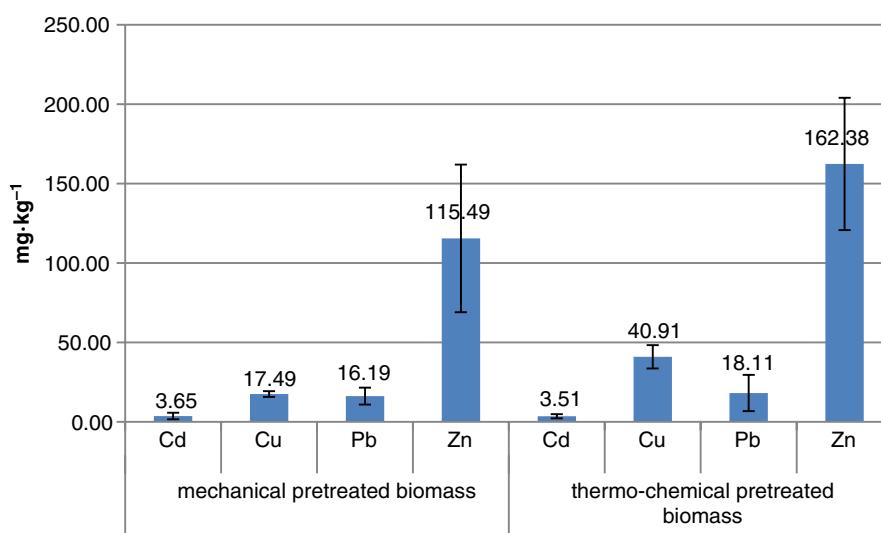
After enzymatic hydrolysis and fermentation of mechanical (milled) and thermo-chemically pretreated bagasse, the solid phase of the fermentation broth was separated by sedimentation and centrifugation. Regarding sugar yields and hydrolysis rates (dry matter loss) obtained in the two types of pretreated biomass, consistent differences were obtained. In the case of mechanically pretreated bagasse, the hydrolysis rates ranged between 7.3% and 9.0%, whereas using the thermo-chemically pretreated bagasse yielded hydrolysis of over half of the solid biomass (53.83–69.24%). In sugar yields, 6.16–7.34 mg mL⁻¹ (123–147 mg g⁻¹ reported to dry matter biomass) of reducing sugars were released by the hydrolysis of mechanically pretreated biomass and 16.24–27.16 mg mL⁻¹ (325–543 mg g⁻¹ reported to dry matter biomass) reducing sugars were liberated by the hydrolysis of thermo-chemically pretreated biomass. Lignin removal via NaOH/steam pretreatment increased enzyme activity by augmenting the access to cellulose in the lignocellulose complex. These results confirm the data reported by Cao *et al.* and Wu *et al.*, indicating higher conversion rates following thermo-chemical pretreatments of sorghum bagasse compared with those obtained after mechanical pretreatments.[27,29]

Figure 2 depicts the average metal concentrations in solid residues after sorghum bagasse hydrolysis and fermentation. Given the higher hydrolysis rates in thermo-chemically pretreated biomass, the investigated heavy metals primarily accumulated solid residues resulting after this type of pretreatment. The only exception is Cd, which was found in similar concentrations in the two types of solid fermentation residues. The balance between the four metal contents remained unchanged in the two types of residues, with Zn concentration being the highest, followed by Cu, Pb and Cd. However, it changed in the fermentation broth liquid fraction, where Pb concentration followed Zn concentration, whereas Cu and Cd were found in trace amounts (Fig. 3).

Statistical analysis showed that the pretreatment method applied can influence metal retention in the solid phase of the sorghum fermentation broth. For Cu and Zn, which both function as essential metals for all living organisms (data not presented in the manuscript), the ratio between the concentrations measured in bagasse and those found in the solid phase of the fermentation broth were significantly higher in thermo-chemical pretreated samples than in those processed by mechanical pretreatment (Mann–Whitney test, $P < 0.05$). Non-essential metals (i.e. Pb, Cd) revealed, however, a different trend. Thus, in the case of Pb, this ratio was consistently elevated in the mechanical pretreated biomass when compared with the thermo-chemical pretreated biomass, (Mann–Whitney test, $P < 0.05$). In contrast, no significant differences were observed for Cd between the two methods of pretreatment applied in this research (Mann–Whitney test, $P > 0.05$).

The metals from fermentation broth behave differently during distillation. Hence, Zn and Cd were not found in distillation fractions and in the distillation residue (vinasse). In contrast, Cu was observed to occur in approximately homogenous concentrations in all four distillation fractions, but was not detected in vinasse. The Pb concentrations were elevated in the first distillation fractions and low in vinasse.

These findings indicated that in the process of bioethanol production from heavy metals polluted biomass the heavy metals analyzed here accumulate mainly in the solid residues and only small amounts are retained in the liquid broth. In the fermentation broth these metals were also differently distributed during distillation: Zn and Cd accumulated in vinasse, whereas Cu and especially

Figure 2. Concentration of metals found in solid residues after hydrolysis and fermentation of sorghum bagasse.
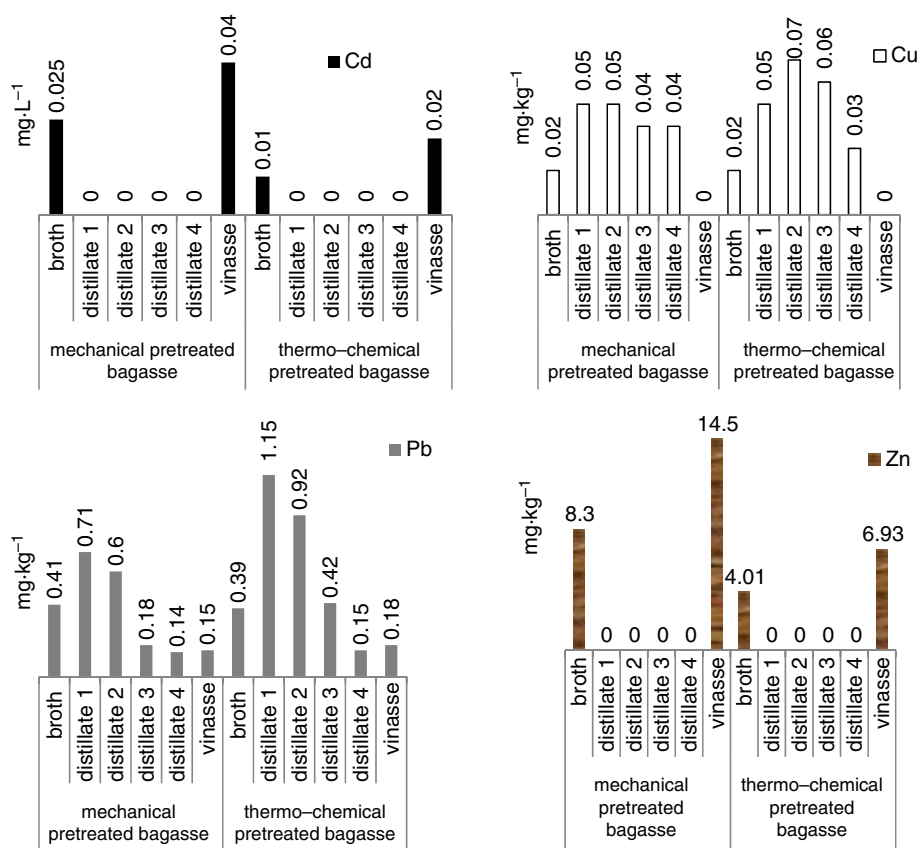
Figure 3. Distribution of Cd, Cu, Pb and Zn in fermentation broth, distillation fractions and residual vinasse after hydrolysis and fermentation of sorghum bagasse.

Table 6. Concentration of ethanol and distribution of metals by distillation of fermentation broth

	Fermentation broth	Distillate 1	Distillate 2	Distillate 3	Distillate 4	Vinasse
Volume (mL)	500	50	50	50	50	300
Fermentation batch containing NaOH/steam pretreated bagasse						
Ethanol (mg·g^{-1})	17.1	83.7	26.0	7.5	1.6	7.4
Cd (mg·kg^{-1})	0.01	0	0	0	0	0.02
Cu (mg·kg^{-1})	0.02	0.05	0.05	0.06	0.03	0
Pb (mg·kg^{-1})	0.39	1.15	0.92	0.42	0.15	0.18
Zn (mg·kg^{-1})	4.01	0	0	0	0	6.93
Fermentation batch containing mechanically pretreated bagasse						
Ethanol (mg·g^{-1})	10.3	49.8	15.6	4.5	0.9	4.4
Cd (mg·kg^{-1})	0.025	0	0	0	0	0.04
Cu (mg·kg^{-1})	0.02	0.05	0.05	0.04	0.04	0
Pb (mg·kg^{-1})	0.41	0.71	0.6	0.18	0.14	0.15
Zn (mg·kg^{-1})	8.3	0	0	0	0	14.5

Pb were extracted from the fermentation broth during distillation and found in the alcoholic solution. Regarding the ethanol yields, an average ethanol concentration of 17.1 g g^{-1} (±0.4) in fermentation broth containing 5 g (dry matter) of NaOH/steam pretreated bagasse with an average concentration of 51.46% cellulose was obtained by hydrolysis and fermentation. Table 6 shows the data obtained after distillation of the combined liquids harvested from three hydrolysis and fermentation batches containing as substrate NaOH/steam pretreated bagasse and three hydrolysis and fermentation batches containing as substrate mechanically pretreated bagasse.

The solid spent bagasse obtained after fermentation and the distillation residue can serve as excellent substrate for biogas production. Our results indicate biogas production between 610 and 650 Nl kg^{-1} ODM and methane productions between 370 and 410 Nl kg^{-1} ODM of spent sorghum bagasse after hydrolysis and ethanol fermentation. The digestate of anaerobic digestion can be used as fertilizer to maintain the fertility of heavy metal-polluted/contaminated soils and to produce more biomass used as biorefinery feedstock.

According to Romanian Ministry Order 184/1997, toxicity thresholds in the soil for the four metals studied in our research
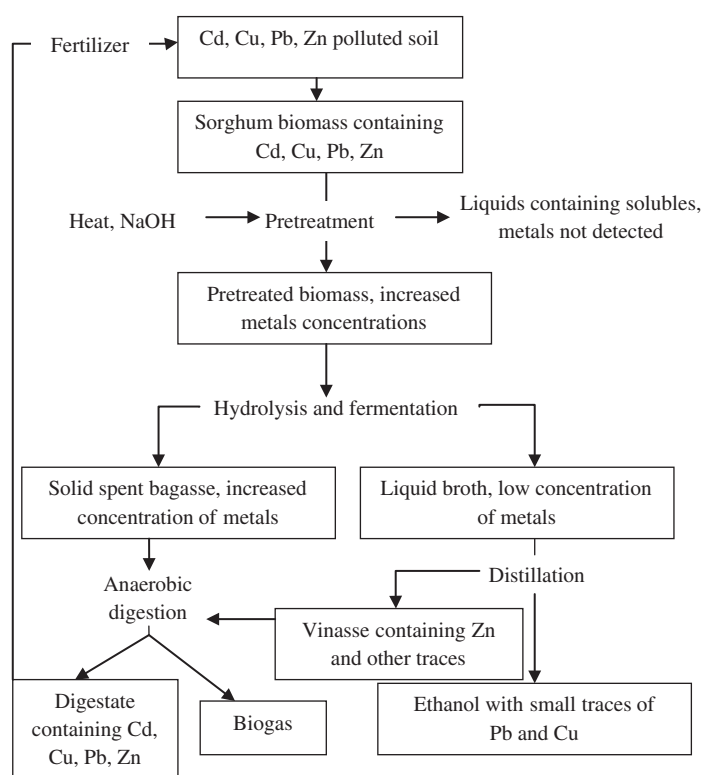
Figure 4. Scheme of the consolidated process for conversion of polluted biomass to ethanol and biogas and distribution of metals.

are shown in Table 1. The same table shows that the Cd and Pb concentrations in the soil of the studied area are above the intervention threshold. Other studies show that the main crops in Copsa-Mica area (corn, wheat, fodder beet, potatoes) are not fit for human or animal consumption due to high concentrations of Cd and Pb.[30–32] According to Romanian Ministry of Health Order 975/1999, the metal concentrations in sorghum juice obtained in our research (Table 2) are below the established maximum thresholds, therefore the sorghum juice can be used as a food.[33] In contrast, the metal concentrations in sorghum bagasse are above maximum thresholds. Therefore, sorghum obtained on these soils can be used exclusively for non-food industrial applications such as the production of renewable energy, according to the technology described in this study.

Figure 4 summarizes the entire process developed at laboratory scale and highlights the course of metals. Scaling up this process to industrial level can be a useful solution to utilize such soils for production of renewable energy instead using them for producing food or feed containing heavy metals. The conversion of biomass to energy carriers as ethanol and methane can be conducted in a biorefinery placed inside the polluted/contaminated area, and therefore, the transportation costs will be reduced. Importantly, the residue (the digestate) containing the polluting metals can be distributed to the same polluted fields, thus preventing pollutant spreading to other areas, as in the case of scattering ash resulting from burning polluted biomass.

due to the large amount of water and acid used to wash and obtain a proper pH value for the biomass.

In the process of cellulase production on sorghum bagasse, a fraction of the pollutants have passed in the liquid phase, which will be used in the hydrolysis of pretreated biomass. The main fraction of the metals remained in the solid phase, which was a residue of the process.

The pretreated biomass was hydrolyzed using cellulases and fermented to ethanol with *Saccharomyces cerevisiae*. The concentration of the metals in the solid phase of the hydrolysis/fermentation broth increased due to solubilisation of the main fraction of the organic solids, and a fraction of the metals (especially Zn) was found in the liquid phase of the fermentation medium.

During distillation of the fermented broth, part of Pb and Cu was extracted into the distillate. In the case of Pb, the main fraction of the metal content was found in the first distillation fraction and the concentration decreased in subsequent distillation fractions. Zn and Cd were not extracted from the broth by distillation and were found in vinasse (distillation residue).

Sorghum crops obtained on polluted soil can be used as substrate for lignocellulosic ethanol production. The main part of the polluting metals remained in the solid residue, a small fraction in the distillation residue, and traces of Pb and Cu were found in the distilled ethanol.

Residues were subjected to anaerobic digestion to obtain biogas and the digestate, which can be returned to the same polluted fields as fertilizer.

CONCLUSIONS

In the pretreatment process (NaOH/steam), the concentration of metals increased in the solid phase (due to a slight hydrolysis of the solids), while in the liquid phase the metals were not detected

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