Romanian Biotechnological Lette *Vol. 16, No. 5, 2011*

ORIGINAL PAPER

Comparing the use of common bean extracted natural coagulants with centrifugation in the treatment of distillery wastewaters

Received for publication, January 22, 2011
Accepted, October 11, 2011

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Abstract

Distillery spent washes are highly polluted wastewaters. Pollution caused by them is one of the most critical environmental issue. In this study, the coagulation efficiency of common bean extract in treatment of different distillery wastewaters was investigated. Active components were extracted from 10 g/l of ground sample with 0.5 mol/l NaCl. Obtained coagulant was used for organic matter removal from wastewaters remained after bioethanol production on intermediate products of sugar production-extraction juice, thin juice and thick juice, and by-product-molasses, as nutrient mediums. The coagulation efficiency was assessed by jar test, and organic matter decrease was calculated based on COD measurements. The experiments confirmed that common bean extracted natural coagulant could be used successfully in distillery wastewater treatment. The best achieved efficiencies of organic matter removal were 68.8% for extraction juice wastewater, at pH 8.50 with coagulant dose of 5 ml/l, and 60% for molasses wastewater, at the original pH of this stillage (5.40) with the same dose. Better organic matter removal from thin and thick juice wastewaters were achieved by centrifugation.

Keywords: Natural coagulants, common bean, bioethanol, distillery spent wash, wastewater treatment

Introduction

Bioethanol production from different agricultural feedstocks for use as an alternative fuel has been attracting worldwide attention of scientists and industrialists because of the increasing demand for limited non-renewable energy resources on one hand, and significant fluctuation of natural gas and petroleum prices on the other hand. In many countries this demand is projected to go up because of a low for mixing some volume of ethanol with petrol. Besides, the other common usages of ethanol are in the form of industrial solvent and beverages.

Distillery spent wash is the residual liquid waste generated during bioethanol production. The production and characteristics of spent wash are highly variable and dependent on feedstocks and various aspects of the bioethanol production process. Up to 20 liters of stillage may be generated for each liter of ethanol produced [1]. Distillery wastewaters have extremelly high pollution potential: high biological oxygen demand (BOD), chemical oxygen demand (COD) and high BOD/COD ratio. They also contain inorganic substances such as potassium, phosphates, nitrogen, calcium and sulphates.

In comparing with other distillery wastewaters, molasses spent wash is more polluted. It contains dark brown polymers, called melanoidins, which are formed in Maillard reaction of sugars with proteins. These compounds are hardly degraded by the conventional treatments, and finally enter into the environment. They have antioxidant properties, which render them toxic to aquatic organisms [2] and many microorganisms such as those tipically present in wastewater treatment processes [3]. Other components that contribute to the colour of the effluent are phenolics (tannic and humic acids), caramels from overheated sugars and furfurals from acid hydrolysis [4]. The highly colored compounds of the distillery wastewater block out sunlight penetration in rivers, lakes or lagoons, hence decreasing both photosynthetic activity and dissolved oxygen concentration and affecting aquatic life. High COD and high nutrients content of the effluent may result in eutrophication of natural waters [3]. The presence of organics like skatole, indole, and other sulphur compounds gives unpleasant odor to the effluent [5].

Undiluted effluent has toxic effect on fishes such as *Cyprinus carpio* [6] and other aquatic organisms. Spent wash also leads to significant levels of soil pollution and acidification (because of low pH of wastewaters) in the cases of inappropriate land disposal. It is reported to inhibit seed germination, reduce soil alkalinity and manganese availability and damage agricultural crops [7, 8]. Also, inappropriate disposal of effluent on soil affects the groundwater quality by altering its physico-chemical properties [9].

Different biological treatment approaches have been explored for the treatment of distillery wastewater. Biological treatment is in most cases a combination of anaerobic and aerobic process. Among various biological treatments, in recent years many studies have been carried out concerning fungal, bacterial and algal treatment of distillery wastewaters. Francisca Kalavathi et al. used cyanobacterium *Oscillatoria boryana* for distillery effluent decolorization [10]. Decolorization of a spent wash using fungi *Flavodon flavus* [11] and *Phanerochaete chrysosporium* [12] was investigated, too.

Various physico-chemical methods, such as adsorption, coagulation and flocculation and oxidation processes, like Fenton's oxidation, ozonation, electrochemical oxidation using various electrodes and electrolytes, nanofiltration, reverse osmosis, ultrasound and different combinations of these methods have also been practiced for the treatment of distillery effluent [13-19].

Coagulation and flocculation are commonly used methods of removing particulates and organic matter from wastewaters [20 - 22], and are usually conducted by adding chemicals such as salts of aluminium and iron and polyelectrolytes. The first investigations about harmful influence of these chemicals on human health were published in the 60's of the 20th century. Those and later publications showed possible link between the residues of aluminium salts in the water and adverse neurological effects, such as Alzheimer's disease [23 - 25]. Also, there are studies that indicate that some of synthetic organic polymers, such as acrylamide, have strong neurotoxic and carcinogenic effect [26]. Moreover, the sludge remained after coagulation and flocculation can not be used as an addition to fertilizers or feed, since it contains high concentrations of coagulants and flocculants.

Intensive investigations of natural coagulants have been conducted in the last years in order to replace chemical coagulants in water and wastewater treatment. It is believed that natural coagulants, that can originate from plants, animals or microorganisms, are not harmful, and besides, the resulting biodegradable sludge can be disposed in the nature without any adverse influence.

The idea of water clarification by natural coagulants is many centuries old. There are written documents from India in which the seed of nirmali tree (*Strychnos potatorum*) was mentioned as water clarifier [27]. In the XVI and XVII century, militaries of Peru used roasted and ground corn beans (*Zea mays*) for this purpose. Recently, the most investigated plant is *Moringa oleifera,* whose ground seeds are used for water clarification by women in rural areas of Sudan [28, 29]. Results of these investigations confirmed that *Moringa oleifera* seed extract is very efficient for water treatment [30-34].

Considering the fact that *M. oleifera* is a plant that originates from tropical areas, we investigated the possibility of preparing natural coagulants from sources that are cheap and easily available in the region of Balkan. Our previous investigations confirmed the fact that extracts of various strains of Leguminose could be used as natural coagulants [35]. In other study different strains of common bean were proven as good sources of natural coagulants [36]. The aim of this study was to investigate the efficiency of use of natural coagulants extracted from common bean for treatment of wastewaters remained after bioethanol production on different nutrient mediums. Obtained results will be compared with results of wastewaters treatment by centrifugation.

Materials and methods

Fermentation and wastewaters

The nutrient mediums for bioethanol production were prepared from intermediate products of sugar production-extraction juice, thin juice and thick juice and by-product-molasses (provided from sugar factories in Crvenka and Senta, Serbia, during the harvest season 2007/2008). These intermediate products were diluted by distilled water to give a total sugar mass fraction of a 13%. pH of the fermentation broths was adjusted to 5 by adding 10% (v/v) H_2SO_4. Fermentations were carried out in a 2-litre bench-scale bioreactor with fermentation medium of 1.5 l. The bench-scale bioreactor with the substrate was sterilized in an autoclave, at temperature 121ºC and pressure 2.2 bar during 30 minutes. *Saccharomyces cerevisiae* (commercial fresh baker's yeast, Alltech, Senta, Serbia) was used as a production microorganism. The sterile mediums were inoculated to give the initial yeast cell concentration of 10^8 (cells/ml) (approximately 10 g of fresh baker yeast per 1 l of medium). A fermentation process was carried out under anaerobic conditions, at the temperature of 30ºC, and agitation rate of 200 rpm. The duration of a fermentation was optimized in the earlier investigation for an extraction juice used as a substrate [37], when 38 h was determined as optimum time for fermentation including the time needed for making the suspension. To make certain that results are comparable, fermentations on the other three substrates were also stopped after 38 h. After that, bioethanol and other evaporative components were isolated from the fermented mash by distillation. The distillation was stopped when 10% of the volume of the fermented mash vaporized and condensed. The residue of the fermented mash which comes out as liquid waste is termed as spent wash or distillery wastewater. Distillery wastewaters were stored in a refrigerator on +4ºC, and experiments were conducted in the next two days approximately. In the following text next abbreviated forms are used: extraction juice wastewater (EJW) – wastewater remained after bioethanol production on an extraction juice as a substrate, thin juice wastewater (TNJW) - wastewater remained after bioethanol production on a thin juice as a substrate, thick juice wastewater (TKJW) - wastewater remained after bioethanol production on a thick juice as a substrate and molasses wastewater (MW) - wastewater remained after bioethanol production on a molasses as a substrate.

Natural coagulant

Natural coagulant was obtained in the following way: common bean seeds were ground and passed through a sieve with pore size of 0.4 mm. An amount of a 10 g/l of the smaller fraction was suspended in 0.5 mol/l NaCl. This suspension was stirred 10 minutes on a magnetic stirrer in order to extract active coagulant. After that, the suspension was filtered through filter paper Macherey-Nagel MN 651/120. Obtained filtrate, called crude extract, was stored in a refrigerator at +4ºC.

Coagulation test

The coagulation activity was assessed by jar test using wastewaters obtained after bioethanol production. pH value of wastewaters was adjusted by adding 33% NaOH just before performing coagulation test. Jar test was carried out by adding different amounts (0.05 ml, 0.1 ml, 0.2 ml, 0.5 ml, 1 ml and 2 ml) of extract to 100 ml of wastewaters. After fast stirring at 200 rpm for 1 minute, it was continued with slower stirring at 80 rpm for 30 minutes [38], and after that systems were left for 1 h for sedimentation. The same coagulation test was conducted with no coagulant as a blank. After sedimentation for 1 h, residual COD was determined in upper clarified liquid, and organic matter decrease was calculated, based on COD values in blank and samples after coagulation.

Analytical methods

Three types of samples were analysed: wastewaters and sludge cakes and supernatants obtained after centrifugation of wastewaters at 3000 rpm. Next parameters were determined according to Standard methods [39] (Table 1):

Table 1. Parameters determined in samples according to Standard methods

Parameter	Method Number [33]	Analysed sample:		
		Waste-waters	Sludge cakes	Superna-tants
Total Solids (mg/l)	2540 B	+	+	/
Fixed Solids (mg/l)	2540 E	+	/	/
Volatile Solids (mg/l)	2540 E	+	/	/
Total nitrogen (mg/l)	4500-N_{org} B	+	+	+
Settleable matter (ml/l)	2540 F	+	/	/
Chemical oxygen demand (COD) (mg O_2/l)	5220 B	+	/	+
Biological oxygen demand (BOD_5) (mg O_2/l)	5210 B	+	/	/
Suspended solids (mg/l)	2540 D	+	/	/

In wastewaters permanganate demand and pH were determined, too. Permanganate demand (mg $KMnO_4$/l) was assessed in an acid medium according to Kübel-Tiemann method [40] and pH was measured on Oakton® Ion 6 pH-meter.

Results and Discussions

Analysis of wastewaters

Considering the fact that four different wastewaters were obtained, depending on nutrient medium used for bioethanol production, the first step was to analyse them. Results of analysis of these wastewaters are presented in Table 2.

Table 2. Results of analysis of wastewaters obtained after bioethanol production on different substrates

	EJW[1]	TNJW[2]	TKJW[3]	MW[4]
Total Solids (mg/l)	34 543	28 258	32 132	109 078
Fixed Solids (mg/l)	5 052	3 994	4 140	26 946
Volatile Solids (mg/l)	29 491	24 264	27 992	82 132
% of organic dry matter	85.37	85.86	87.11	75.30
Total nitrogen (mg/l)	1 326	1 015	983	5 675
Settleable matter (ml/l)	31.7	8.5	2.1	n.d.*
COD (mg O_2/l)	66 850	60 730	96 960	126 170
BOD_5 (mg O_2/l)	41 000	12 000	26 700	23 800
(BOD_5/COD)x100 (%)	61.33	19.76	27.54	18.86
Permanganate demand (mg $KMnO_4$/l)	53 190	37 800	51 510	92 900
pH	4.23	4.40	4.24	5.40
Suspended solids (mg/l)	12 550	8 698	9 215	10 164

* not determined
[1] EJW – extraction juice wastewater
[2] TNJW – thin juice wastewater
[3] TKJW – thick juice wastewater
[4] MW – molasses wastewater

As can be seen from these results, organic load was very high in all of four wastewaters, as well as suspended solids and total nitrogen contents. In comparing with other three wastewaters, the MW differed significantly – dry matter, COD, and all other parameters were higher in it. An explanation for this is different composition of molasses.

The EJW was more polluted than TNJW and TKJW. It is known in technology of sugar production, that an extraction juice is cleaned by a precipitation, giving a thin juice. Production microorganism (*Saccharomyces cerevisiae*) assimilates mostly glucose and can not assimilate many of the matters that can be find in an extraction juice (and can not be find in a thin and thick juice), which than appear as a pollution in a stillage, making its BPK_5 significantly higher than it is in other stillages. As can be seen from Table 2, amount of settleable matter was significantly higher in EJW than in other wastewaters. These settleable matters are mostly formed of sand and silt – particles that contribute to the coagulation. Suspended solids were also higher in EJW. They are comprised from coloidal nonsugar compounds that are easily removed by coagulation and flocculation.

Analysis of wastewaters after centrifugation at 3000 rpm

Although analysed stillages did not contain high amount of settleable matter, they still contained significant amount of suspended solids. In order to determine a contribution of suspended solids to COD, stillages were centrifuged at 3000 rpm. Obtained sludge cakes and supernatants were analysed, and results are shown in Table 3 and Table 4.

Table 3. Results of analysis of sludge cakes obtained after centrifugation of wastewaters at 3000 rpm

	Sludge cake from:			
	EJW	TNJW	TKJW	MW
Total Solids (%)	26.68	27.26	30.90	37.61
Content of nitrogen (% of dry matter)	6.23	4.42	4.46	5.15

Precipitation of a sludge cakes with high moisture and high content of nitrogen that probably originate from yeast cells, was achieved. There is a possibility of sludges usage as a fodder, but additional analyses should be conducted first.

Table 4. Results of analysis of supernatants obtained after centrifugation of wastewaters at 3000 rpm

	Supernatants from:			
	EJW	TNJW	TKJW	MW
COD (mg O_2/l)	50 298	45 046	64 590	98 112
COD decrease (%)	24.76	25.83	33.38	22.24
Content of nitrogen (mg/l)	563	680	589	4 933
Nitrogen content decrease (%)	57.55	33.00	40.10	13.07

Content of organic matter and especially content of nitrogen in supernatants were significantly decreased by centrifugation. Just in case of MW, the decrease of nitrogen content was not so good. Analysed stillages were still very contaminated after precipitation.

Wastewaters treatment by natural coagulant

In this part of the experiment, the efficiency of common bean extract as natural coagulant for organic matter removal from waste waters, was investigated. Organic matter decrease (based on COD removal) was assessed by jar test with different doses of coagulant. Coagulation tests were conducted at original pH of wastewaters. After coagulation, COD was determined in upper clarified liquid. Results are shown in Table 5.

Table 5. Influence of coagulant dose on organic matter decrease in wastewaters after coagulation

Dose of coagulant (ml/l)	Organic matter decrease (%) in:			
	EJW (original pH=4.23)	TNJW (original pH=4.40)	TKJW (original pH=4.24)	MW (original pH=5.40)
0.5	n.e.*	12.5	13.3	3.0
1	n.e.*	n.e.*	n.e.*	3.0
2	17.6	12.5	5.0	n.e.*
5	17.6	n.e.*	n.e.*	60.0
10	7.8	n.e.*	n.e.*	20.0
20	23.5	n.e.*	n.e.*	10.0

* no effect

As can be seen from Table 5, higher organic matter decrease in EJW and MW was achieved with higher doses of coagulant, and in case of TNJW and TKJW, better results were gained with lower doses of coagulant. Considering this, in the following experiments a three doses, which showed the best results, were chosen for each of stillage: 0.5 ml/l, 1 ml/l and 2 ml/l for TNJW and TKJW, and 5 ml/l, 10 ml/l and 20 ml/l for EJW and MW.

The next step was to investigate an influence of pH of wastewater on organic matter decrease, at different applied doses of coagulant. Results for EJW are presented in Table 6. Since in the previous investigation, when common bean extract was used as a natural coagulant for coagulation on model water, lower pH values showed as inadequate [35], we decided to conduct experiment at original and higher pH values of wastewaters.

Table 6. The influence of pH of EJW and coagulant dose on organic matter decrease

Dose of coagulant (ml/l)	Organic matter decrease (%) on:		
	pH=4.23 (original)	pH=6.50	pH=8.50
5	17.6	4.4	68.8
10	7.8	n.e.*	29.2
20	23.5	4.4	8.3

* no effect

Addition of coagulant at pH 6.50 did not decrease content of organic matter significantly. The best result, decrease of organic matter of 68.8%, was attained at pH 8.50 when dose of coagulant 5 ml/l was applied. This was expected since impurities were removed from extraction juice by precipitation at pH 9 even during conventional sugar production.

The influence of pH on organic matter decrease in TNJW is shown in Table 7.

Table 7. The influence of pH of TNJW and coagulant dose on organic matter decrease

Dose of coagulant (ml/l)	Organic matter decrease (%) on:		
	pH=4.40 (original)	pH=6.48	pH=8.50
0.5	12.5	15.6	2.0
1	n.e.*	n.e.*	1.6
2	12.5	6.2	8.1

* no effect

The use of natural coagulant for treatment of TNJW showed lower effect in comparing with treatment of EJW. A thin juice does not contain impurities such as extraction juice does (since they are removed in a step of purification of extraction juice), and it could be an explanation for a small contribution of natural coagulant to organic matter removal. The best result, decrease of organic matter of 15.6%, was attained at pH 6.48 when dose of coagulant 0.5 ml/l was applied. It was not significantly better than organic matter decrease achieved at original pH of the stillage with the same applied dose of coagulant, though. This means that coagulation could be conducted at original pH of this stillage.

In Table 8, results for TKJW are presented. Organic matter decrease did not change with increasing of pH from original 4.24 to 6.43, and was the highest (13.3%) with the lowest applied dose of coagulant 0.5 ml/l. The subsequent increasing of pH to 8.98 led to decline of organic matter removal efficiency, since content of organic matter even enlarged after coagulation at applied doses of coagulant 0.5 ml/l and 1 ml/l. It is a consequence of organic nature of added coagulant. Considering this, it can be concluded that coagulation should be conducted at original pH of this wastewater.

Table 8. The influence of pH of TKJW and coagulant dose on organic matter decrease

Dose of coagulant (ml/l)	Organic matter decrease (%) on:		
	pH=4.24 (original)	pH=6.43	pH=8.98
0.5	13.3	13.3	n.e.*
1	n.e.*	n.e.*	n.e.*
2	5.0	5.0	4.6

* no effect

The influence of pH of MW on organic matter decrease is shown in Table 9.

Table 9. The influence of pH of MW and coagulant dose on organic matter decrease

Dose of coagulant (ml/l)	Organic matter decrease (%) on:		
	pH=5.40 (original)	pH=7.04	pH=8.99
5	60.0	12.4	15.0
10	20.0	n.e.*	10.0
20	10.0	n.e.*	n.e.*

* no effect

The pH increase in MW did not lead to better organic matter removal. The best efficiency of organic matter removal (60%) from the MW was achieved at the original pH of the stillage (5.40), with the dose of coagulant of 5 ml/l, which is significant from economical point of view due to potential applicability. In the opposite of EJW, where the best efficiency of organic matter removal was achieved at high pH value and applied coagulant dose 5 ml/l, the best result in MW was achieved with the same applied dose of coagulant, but at lower (original) pH. As can be seen from Tables 7, 8 and 9, the increase of pH did not lead to better organic matter removal in TNJW, TKJW and MW. Compounds that contribute to better coagulation, were already removed during a process of extraction juice purifying by adding $Ca(OH)_2$ at pH 9.

Conclusions

Results of analysis of wastewaters remained after bioethanol production on intermediate products of sugar production: extraction juice, thin juice and thick juice, and by-product - molasses, as nutrient mediums, could lead to the next conclusions:

- All investigated wastewaters were very contaminated, but still the most polluted was the wastewater remained after bioethanol production on molasses as a nutrient medium, that was a consequence of composition of molasses.

- A significant decrease of content of organic matter and especially content of nitrogen (except in case of molasses wastewater) was achieved by centrifugation of wastewaters.
- Obtained sludge cakes, with high content of proteins could be used as a fodder, but additional analyses should be conducted first.

Considering performed experiments and obtained results for usage of natural coagulant extracted from common bean for treatment of investigated wastewaters, next conclusions could be presented:

- An increase of pH of extraction juice wastewater influenced significantly on organic matter decrease. The highest organic matter decrease (68.8%) was achieved at pH 8.5 and the lowest applied dose of coagulant 5 ml/l.
- The use of natural coagulant for treatment of thin juice wastewater showed lower effect in comparing with treatment of extraction juice wastewater. The best result, decrease of organic matter of 15.6%, was attained at pH 6.48 when dose of coagulant 0.5 ml/l was applied.
- Coagulation in thick juice wastewater should be conducted at original pH of this wastewater, since the best result, organic matter decrease of 13.3%, was achieved at this pH and low applied dose of coagulant 0.5 ml/l. However, better organic matter removal from thin and thick juice wastewaters were achieved by centrifugation.
- An increase of pH did not lead to better organic matter removal in molasses wastewater. The best efficiency of organic matter removal (60%) from the molasses wastewater was achieved at original pH of the stillage (5.40) with the dose of coagulant 5 ml/l.
- Natural coagulants extracted from common bean can be used successfully for organic matter removal from extraction juice wastewater and molasses wastewater instead of centrifugation. Obtained sludges, that are additionally analysed, could be also used as a fodder.

Acknowledgements

This research was supported by the grant number TR 31002 from the Ministry of Science and Technological Development of the Republic of Serbia

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