FEASIBILITY TO PRODUCING SECOND GENERATION BIOETHANOL IN BOLIVIA

M. BELTRAN-SIÑANI and A. GIL

INAMAT^2-Science Department, Public University of Navarra,
Los Acebos Building, Campus of Arrosadia, 31006 – Pamplona, Spain
magicbeltran@hotmail.com; andoni@unavarra.es

***Abstract*— Bioethanol produced worldwide is mostly obtained from agricultural crops such as sugarcane and corn. However, it has negative environmental effects, so opportunity for producing bioethanol from agricultural waste arises. This study evaluates the feasibility to produce second generation bioethanol from orange waste (peel and bagasse) generated in the province of *Chapare*, Bolivia. Bioethanol production yield estimation is carried out by theoretical and experimental ways, DNS and HPLC methods are used for the quantification of reducing sugars, produced by acidic and enzymatic hydrolysis of the waste. Regarding the results obtained, the best alternative in terms of bioethanol production is the enzymatic hydrolysis. An economic and environmental impact evaluation are also included considering the production of bioethanol from real orange residues. The determined price of bioethanol production is USD 0.78 per liter.**

***Keywords*— Lignocellulosic biomass, second generation bioethanol, enzymatic hydrolysis, acid hydrolysis, orange waste, GHG emissions.**

I. INTRODUCTION

Nowadays, global warming and its effects have become one of the principal issues worldwide. Mitigation strategies to climate change are supported as a way to reduce global warming. In this context generation of clean fuels instead of fossil fuels, including bioethanol production is one of the most socially and scientifically accepted option (Wyman, 1996).

Bioethanol production is mostly from agricultural crops such as sugarcane and corn (First Generation, 1G). But its possible negative impacts on food sovereignty, land and water use; it is not an attractive alternative to mitigate climate change. However, bioethanol production from biomass (Second Generation, 2G) as orange peel is a friendly with environment alternative, in addition, would reduce the amount of organic waste that produce GreenHouse Gases (GHG) emissions without treatment.

Bioethanol industry has contributed significantly to fossil fuels replacement despite its high production cost compared to gasoline, through programs for the complementary use of ethanol in gasoline. This fact, promoted the bioethanol 1G production, reaching worldwide production of 27.050 million gallons in 2017, being the main producer United States with 58% representativeness,

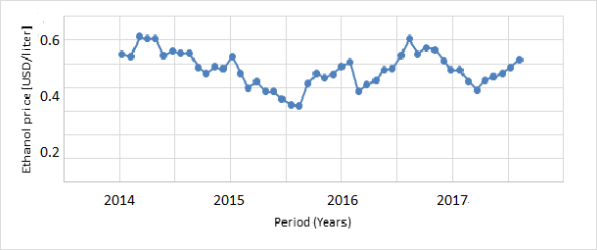
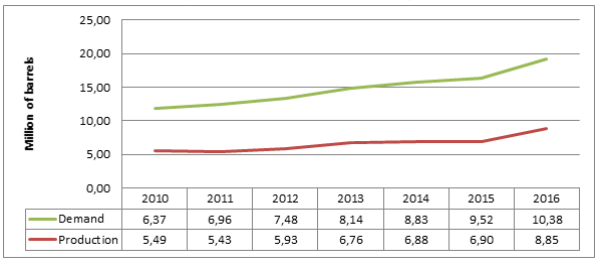


Figure 1: Ethanol price behavior expressed in dollars per liter (Business Insider, 2018).



	2010	2011	2012	2013	2014	2015	2016
Demand	6,37	6,96	7,48	8,14	8,83	9,52	10,38
Production	5,49	5,43	5,93	6,76	6,88	6,90	8,85

Figure 2: Orange production in Bolivia and Cochabamba (INE, 2018).

followed by Brazil and the European Union (Renovables, 2017), of this production, 73% is used as fuel (Sánchez and Cardona, 2008). The commercial ethanol price is a commodity that varies seasonally (Business Insider, 2018), since it is first generation it depends mainly on corn and sugarcane prices. The average ethanol price to the final consumer is USD 0.55 per liter (see Fig. 1).

In this approach, 2G bioethanol production is studied, considering orange waste in a fruit processing plant from Chapare in Cochabamba Department of Bolivia; orange waste composition are 25% peel, 22.5% bagasse and 0.2% seed. Orange weight varies proportionally to its size from 100 to 130 g, with approximate 9 cm diameter; and its fiber is made up of 18% hemicellulose, 12% cellulose and 1.7% lignine (Galindo, 2017). Bolivian orange production has increasing trend, reaching 180,021 tons (see Fig. 2) with use of 22,857 hectares in 2017 (INE, 2018), 66.3% (83,642 tons) of it was produced in Chapare followed by the *Chiquitanía* and *Pantanal* region with 25.7%, orange waste generated was 40,000 tons.

In Bolivia, ethanol market as fuel has not been promoted in order to guarantee food sovereignty. However, in 2018 its production was formalized and its consumption as a gasoline additive was promoted. This, for the sugar cane overproduction that guaranties its internal supply and gasoline imports reduction need (national

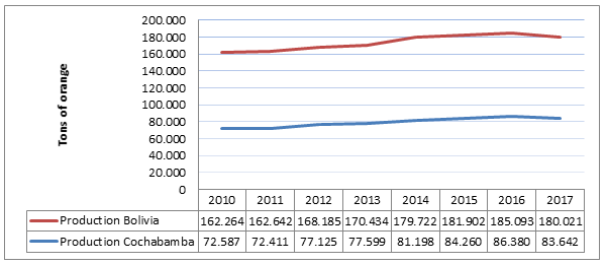


Figure 3: Gasoline production and demand in Bolivia 2010 – 2016 (INE, 2018).

production supplies 90% of the demand). Gasoline price is frozen and subsidized, being its final consumer price USD 0.65 per liter, and USD 0.72 per liter for the producer. Given the continuous automotive fleet growth, it increases the expenditure for the subsidy to the gasoline. In 2016, the gasoline demand as is shown in Fig. 3 was 10.38 million barrels and its production 8.85 million barrels, being the national import 1.53 million barrels.

During last 26 years, three sugar mills Guabirá, Unagro and Aguaí located in the Santa Cruz Department have been ethanol producers (IBCE, 2017). Bolivian Institute of Foreign Trade (IBCE, 2017) data shows ethanol exports declination in recent years, this behavior maybe appears for ethanol price fall in destination countries such as Peru, Chile and Argentine; and granting of export permits delayed by Bolivian authorities, since the government restricted sugarcane and its derivatives exports in 2013 to guaranteeing its supply (IBCE, 2017). Ethanol imported use is mostly industrial, being the main suppliers Germany, Argentine and Brazil.

Now, regulatory framework for bioethanol use is important, promoting the import and production of flex vehicles (flexible fuel), taking advantage of the availability of bioethanol and reducing greenhouse gas emissions by this sector.

II. METHODS

In order to have theoretical and experimental results about the ethanol production yields, sugars and ethanol measurements are carried out during the process. 2G bioethanol production theoretical estimation is based on reducing sugars (DNS method) and individual sugars (HPLC method) quantification of acid and enzymatic hydrolyzed samples. Subsequently, experimental performance is based on ethanol quantification in the fermented samples by HPLC technique.

The experimental process for bioethanol production from orange waste (peel and bagasse) is detailed in Fig. 4, for it all analyzes were performed in triplicate. Initially 50 g of orange residues were weighed, which underwent a mechanical pretreatment in two phases. In the first phase, raw material was cut into 2 cm pieces approximately, in the second phase, results were crushed into a power crusher for 15 min (Galindo, 2017), with 850 µm particle size in 64% (see Fig. 5). The second stage, lignin structures are break in order to obtain major amount of sugar from the samples that were immersed in 100 mL of 0.1 M NaOH for 24 h (Giovanni *et al.*, 2013). Subsequently, 0.8 g of $CaSO_4$ was added to let it stand for 3 h

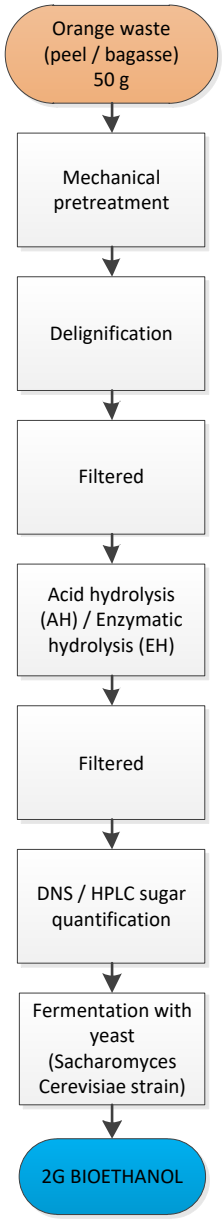


Figure 4: 2G Bioethanol production process.

(Díaz, 2015), samples were stored between 2 to 6 ºC into a refrigerator for its subsequent vacuum filtration for 20 min, where solid fraction was retained for the next stage. In the fourth stage, hydrolysis was carried out in two ways: Acid Hydrolysis (AH) and Enzymatic Hydrolysis (EH).

For AH, 50 g filtrated sample is submerged in 50 mL H_2SO_4 5% (v/v) for 1 h with ambient temperature; And for EH, 5 g filtrated sample is immersed in 35 mL water, pH is adjusted into 5.5 with 1 M HCl or NaOH. For EH Amyloglucosidase from *Aspergillus niger* (Sigma Aldrich) and α-Amylase from *Aspergillus oryzae* (Sigma Aldrich) enzymes were used; according to enzyme supplier instructions, EH was performed into a incubator with 50 rpm agitation for 3 h and 60 ºC. Once time hydrolysis is carried out, samples are filtered and the liquid fraction is recovered as follows: 4 mL is frozen until

Figure 5: Raw material from orange residues.

sugar quantification analysis, and the rest is used for fermentation phase. The product obtained in both hydrolysis is subjected to reducing sugars analysis by DNS and HPLC methods in order to determine the sugars obtained, which allows estimation of theoretical yield for 2G bioethanol production.

Fermentation is carried out in 250 mL frosted flasks previously autoclaved used as bioreactors. The liquid sample from the respective hydrolysis process is supplemented with distilled water in order to obtain 35 mL of volume, and inoculated with 0.35 g of *Saccharomyces cerevisiae* yeast directly to the cultivation medium. At the same time, fermentation was carried out in groups of three samples in an anaerobic environment, with N_2 atmosphere for its favorable reproduction factor (Mas *et al*., 2013) for 20 h with 35 - 40 ºC and 50 rpm (Galindo, 2017), pH was adjusted to 5.5 with solutions 1 M of HCl or NaOH. After fermentation, samples were centrifuged with 6,000 rpm during 10 min, obtaining supernatant liquid for its refrigeration with -20 ºC until ethanol quantification time by HPLC method for the experimental 2G bioethanol production yield determination.

Sugars quantification by DNS method is based on the spectrophotometric determination of reducing sugars (glucose) amount present in an aqueous solution. HPLC quantification (Merck-Hitachi LaChrom with UV detector, Aminex HPX-87P column (300x7.8 mm)) allowed determining the individual sugars: cellobiose, glucose and arabinose. Samples were diluted 10 times in deionized water and deposited in HPLC 2 mL vials being previously filtered through 0.45 µm filters to retain suspended solids.

III. RESULTS AND DISCUSSION

Theoretical and experimental estimation of bioethanol production yield is carried out by sugar quantification methods from hydrolyzed samples (Acid and Enzymatic) that are presented in Table 1. Theoretical estimation was determined by DNS and HPLC methods in which ethanol production was detected without fermentation which is considered in the production yield calculation. Experimental estimation was based in sugar and ethanol presence in the fermented samples by HPLC analysis.

Theoretical and experimental yield of bioethanol production are presented Table 2. Theoretical estimation by DNS method shows good production yield by enzymatic hydrolysis with 0.05 mL bioethanol per orange peel gram using amyloglucosidase enzyme, and 0.012 mL bioethanol per orange bagasse gram by acid hydrolysis; its estimation by HPLC analysis considers the presence of ethanol in samples without fermentation resulting 0.012 mL bioethanol per orange peel gram and 0.009 mL ethanol per orange bagasse gram by acid hydrolysis. Experimental result by HPLC analysis shows that 0.031 mL bioethanol per orange peel gram by enzymatic hydrolysis with Amyloglucosidase and α-Amylase mixed is the best production yield, and 0.028 mL bioethanol per orange bagasse gram by acid hydrolysis.

With the results above indicated, enzymatic hydrolysis with amyloglucosidase enzyme has the best theoretical production yield for orange peel process and acid hydrolysis for orange bagasse. Experimentally, the best production alternative is enzymatic hydrolysis with Amyloglucosidase and α-Amylase mixed for orange peel process and acid hydrolysis for orange bagasse. As theoretical and experimental results, orange bagasse has lower production yield than orange peel.

Results presented in this study are similar to other authors, for example, a bioethanol plant in Spain produces 0.060 mL per gram of organic waste (Sánchez and Vázquez, 2013); “Production of bioethanol from the alcoholic fermentation of glucose syrups derived from orange and pineapple peels” publication carried out in Colombia obtained a production yield of 0.011 mL Bioethanol per orange peel gram by acid hydrolysis (Tejeda *et al*., 2010); and the study "Production of bioethanol from lignocellulosic agroindustrial products" carried out in Spain shows 0.16 mL per gram of bagasse and peel as production yield (Sánchez Riaño *et al.*, 2010).

It is also important to mention that 2G bioethanol production yield from residues is much lower than 1G. Bioethanol 1G from corn generates 0.37 mL per gram (Fernández and Lucas, 2008), and 0.070 mL per gram of sugar cane (Cardona and Julián, 2005). However, 2G bioethanol production has GHG emission reduction approach that makes its production very attractive as climate protection action.

LACTEOSBOL public fruit processing plant is located in *Valle del Sacta* from Chapare that process 8 million oranges per year (800 tons), processing 380 tons orange waste (200 tons peel and 180 tons bagasse). Costs analysis considers enzymatic hydrolysis process for peel

Table 1. Sugar quantification by DNS and HPLC methods (g/L)

DNS	HPLC								Hydrolisis Type
Glucose	Cellobiose		Glucose		Arabinose		Ethanol		
t	t	e	t	e	t	e	t	e	
Peel									
8.53	0.42	25.62	5.99	0.23	6.25	0.21	0.69	5.72	
5.56	1.39	21.32	4.55	-	4.71	0.11	0.50	8.56	Acid: H_2SO_4
6.23	0.71	27.63	5.52	0.24	5.67	0.24	0.34	7.14	
2.84	-	0.31	0.23	0.26	-	-	-	0.49	Enzymatic: Amyloglucosidase
0.21	0.06	0.40	0.16	0.17	0.28	0.03	0.05	0.54	Enzymatic: α-Amylase
2.26	-	0.60	0.36	0.13	0.83	0.03	0.09	0.85	Enzymatic: Amyloglucosidase and α-Amylase mixed
Bagasse									
5.30	2.40	34.85	3.51	-	4.30	0.43	0.37	6.44	
7.82	2.14	18.69	4.15	0.09	5.57	0.18	0.37	6.03	Acid: H_2SO_4
6.08	3.04	33.07	3.23	0.28	4.36	0.52	0.47	7.90	
0.45	-	-	0.11	0.13	0.11	0.03	0.04	0.45	Enzymatic: Amyloglucosidase
0.25	-	-	0.14	0.11	0.11	0.04	-	0.23	Enzymatic: α-Amylase
0.35	-	-	0.16	-	0.08	0.04	0.18	0.33	Enzymatic: Amyloglucosidase and α-Amylase mixed

(t) Theoretical; (e) experimental

Table 2. Theoretical and experimental 2G bioethanol production yield (cm^3/g_{sample}).

Sample	Method	Hydrolysis	
		Acid	enzymatic
Orange peel	DNS method (t) (glucose)	0.013	0.05 [a]; 0.004 [b]
	HPLC technique (t) (cellobiose+glucose+arabinose)	0.012	0.005 [a]; 0.004 [b]
	Ethanol (e)	0.029	0.018 [a]; 0.020 [b]; 0.031 [c]
Orange bagasse	DNS method (t) (glucose)	0.012	0.008 [a]; 0.004 [b]
	HPLC technique (t) (cellobiose+glucose+arabinose)	0.009	0.003 [a]; 0.003 [b]
	Ethanol (e)	0.028	0.016 [a]; 0.008 [b]; 0.012 [c]

[a] results from Amyloglucosidase; [b] results from α-Amylase; [c] results from Amyloglucosidase and α-Amylase mixed
(t) Theoretical; (e) experimental.

and bagasse given its higher production yield for orange peel, experimental production yields are corrected by industrial scale factor of 11% (maximum standard deviation found in laboratory), being the corrected production yields for orange peel and bagasse 27.59 and 14.24 bioethanol liters per ton, respectively.

Cost for producing 8,081.2 bioethanol liters from 380 tons of orange waste cost is determined by necessary inputs costs for the enzymatic production process; the labor and equipment available in the company will be used, so adsorption tower and distillation column as investment cost are incurred (USD 3,000) with depreciation cost 300$ per year. Additionally, process requirement are: 1,900 m^3 water (USD 1,090.39), 3 tons NaOH (USD 1,160.17), 6.2 tons $CaSO_4$ (USD 636.15), 13.3 m^3 enzymes (USD 1,335.72), 10 kg Saccharomyces cerevisiae yeast (USD 10.04), 20 kg HCl (USD 86.08), and 12,000 kWh electricity (USD 1,721.66); being the production cost USD 0.78 per bioethanol liter.

Although bioethanol price from orange waste is major than ethanol producer price in Bolivia, the bioethanol production from waste generates environmental benefits related to GHG emissions and polluting gases reduction; also it is soluble in water and more degradable than hydrocarbons, as in case of accidental spills elimination, oil can take many years, and ethanol would be a matter of days and with less danger of toxicity for living beings.

Environmental impacts of 2G bioethanol production are presented in Table 3. Considering quantification from three areas of emission reduction: by bioethanol use as a gasoline additive, by the waste volume reduction in a landfill, and by bioethanol production process. The emissions reduction by bioethanol use as fuel is 408.75 tons of CO_2-eq per year, considering that CO_2 emissions quantification by bioethanol use is zero according the guidelines of the Intergovernmental Panel on Climate Change (IPCC). In addition, bioethanol combustion process is considered a neutral cycle respect to CO_2 emissions, since all carbon emitted in the combustion of this alcohol corresponds to carbon that had been previously removed from the atmosphere by its cultivation. Polluting and greenhouse gases are also reduced compared to those produced by gasoline use, with the exception of nitrogen oxides that increase by 5%. In conclusion, environmental impact is positive by GHG reduction, considering that total estimated GHG emissions in Bolivia were 19.46 Gkg of CO_2 in 2016, bioethanol use would reduce 0.0021% of it, quantity that would increase with this type of projects since GHG mitigation approach.

Table 3. CO_2-eq emissions by 2G bioethanol use.

Area	Detail	Emission factor	Emissions[CO_2-eq tons/year]
Bioethanol use as a gasoline additive	Gasoline use	2.38	-19.23
	Ethanol use	0.00	0.00
Waste volume reduction in a landfill	Waste deposition in a landfill without selective collection	1.03	-391.40
Bioethanol production process	Electric power use	0.30	0.15
	NaOH use	0.47	1.41
	Enzyme use	1.00	0.32
	Bioethanol combustion	0.00	0.00

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