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Microbial Production of Bioethanol from Gamma Irradiated Sugarcane Bagasse and Potato Peels

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RECENTLY, with growing crisis in fossil fuel and the consequent of environmental pollution problems worldwide, bioethanol has become one of the most promising biofuels and many researchers have worked on improving the efficacy of the bioethanol production process. This work was concerned with producing bioethanol from low-cost raw agro-industrial feedstock (sugarcane bagasse and potato peels) and utilizing radiation technology to increase conversion rate of these materials to bioethanol. Both of sugarcane bagasse and potato peels were acid-hydrolyzed and resulted hydrolysates were fermented by either *Zymomonas mobilis* ATCC 29191, *Saccharomyces cerevisiae* ATCC 7754, or both organisms, co-cultured (1:1). The effect of gamma irradiation on bioethanol production was studied by exposing the feedstock to different doses of gamma rays (0, 25, 50 75 kGy). Effect on combining gamma irradiation with acid treatment of feedstock on bioethanol production was also investigated. From sugarcane bagasse, the highest achieved final bioethanol concentration (15.4 gL^{-1}) was obtained from the combined pretreatment by irradiation with 75 kGy followed by hydrolysis with 2 % (v/v) H_2SO_4 at 120°C for 60 min and fermented with co-culture (1:1) of *Z. mobilis* ATCC 29191 and *Sacch. cerevisiae* ATCC 29191. On the other hand, from potato peels the highest bioethanol concentration (12.1 g L^{-1}) was obtained from combined pretreatment by irradiation with 75 kGy and hydrolyzed by 6 % (v/v) H_2SO_4 at 100°C for 60 min then fermented with co-culture (1:1).

Keywords: *Saccharomyces cerevisiae* ATCC 7754, *Zymomonas mobilis* ATCC 29191, Bioethanol, Feedstock, Gamma irradiation, Dilute acid hydrolysis.

The rising concern over depleting fossil fuel and greenhouse gas limits has resulted in a high level of interest in non-conventional fuel originating from bio-renewable sources including sugars, starches and lignocellulosic materials. The importance of the bioethanol production has increased in the last few years, but cost of production is still interfering with the deployment of this new technology, where the cost of used raw materials (sugar and starch-containing materials) represents about 40-70% of the total production cost. Using less valuable materials, like lignocellulosic agricultural waste, could significantly reduce the production

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expense (Abo-State *et al.*, 2013). The lignocelluloses are mainly composed of cellulose, hemicellulose, and lignin. Cellulose chains interact with hemicellulose and lignin forming a lignin-carbohydrate complex, so that they must be pretreated and hydrolyzed to produce sugars for bioethanol fermentation (Ferdian *et al.*, 2012). Because of its lower ash content (1.9 %), sugarcane bagasse offers numerous advantages compared with other agro-based residues such as paddy straw (16 %), rice straw (14.5 %) and wheat straw (9.2 %) (Cardona *et al.*, 2010). Potato peel waste (PPW), also, contains sufficient quantities of starch, cellulose, hemicellulose, lignin and fermentable sugars to warrant use as an ethanol feedstock. Starch is a high yield feedstock for ethanol production, but its hydrolysis is required to produce ethanol by fermentation (Arapoglou *et al*., 2010). Pretreatment is an essential step for practical cellulose conversion processes that is required to modify the structure of cellulosic biomass to make cellulose more accessible to convert the carbohydrate polymers into fermentable sugars (Ribeiro *et al.*, 2013). Recently, use of irradiation for degradation of various lignocellulosic materials, such as sugarcane bagasse, chaff, sawdust, corn stalk and rice straw bunch, to increase sugar yield, has gained great attention. It was demonstrated that irradiation pretreatment can cause significant breakdown of the structure of lignocellulose and increase the rate of enzymatic hydrolysis (Wang *et al.*, 2012). Ribeiro *et al.* (2013) reported positive effect of absorbed doses of gamma irradiation, lower than 150 kGy, on the cleavage of polysaccharides from sugarcane bagasse. High-energy radiation causes a decrease in the degree of polymerization and an increase in the carbonyl content of cellulose due to the chain scission reaction within the cellulose molecules.

The current work aimed to study the effect of different doses of gamma irradiation on the cleavage of polysaccharides from sugarcane bagasse and potato peels with or without combination of dilute acid hydrolysis and the effect of these treatments on bioethanol production compared with dilute acid hydrolysis. Production of bioethanol by fermentation was carried out using *Zymomonas mobilis* ATCC 29191 and/or *Saccharomyces cerevisiae* ATCC 7754.

Materials and Methods

Materials

Microorganisms for bioethanol production

Saccharomyces cerevisiae ATCC 7754 and *Zymomonas mobilis* ATCC 29191 were obtained from The Microbiological Resources Center (Cairo MIRCEN), Faculty of Agriculture, Ain Shams University, Cairo, Egypt.

Agro-industrial feedstock

Sugarcane bagasse was obtained from sugar cane juice shop and potato peels was obtained from local food restaurants, both located in Shibin Al-Qanatir, Al-Qalyubiya Governorate, Egypt. Both sugarcane bagasse and potato peels were sun-dried then milled using a laboratory hammer mill (Retsch GmbH & Co. KG, Germany) to pass through 1 mm screen. These feedstocks were homogenized and oven-dried at 45ºC prior to chemical analysis and pretreatment assays. The dried materials were stored in airtight containers at room temperature before use.

Media used

YM medium (Wickerham, 1946) was used for cultivation, maintenance and seed culture of *Sacch. cerevisiae* ATCC 7754 with the following ingredients (gL^{-1}): Yeast extract 3; malt extract 3; glucose 10; peptone 5; agar 15; pH 6.0 ± 0.2. ATCC medium 948 (Swings & Deley, 1977) was used for cultivation, maintenance and seed culture of *Z. mobilis* ATCC 29191 with the following ingredients (gL^{-1}): Glucose 20; yeast extract 5; agar 15; pH 6.5 ± 0.2.

Methods

Analysis of agro-industrial feedstock

Determination of moisture percentage: Five grams of each feedstock were dried in oven at 45ºC overnight and left to cool in a desiccator and weighed until reach a constant weight. Moisture content of each sample was calculated (George *et al*., 2011).

Determination of total sugars: Total sugars were determined after hydrolysis treatments of sugarcane bagasse and potato peels. Total sugars were extracted according to the method reported by Pak & Simon (2004) and the supernatants were used for sugar analysis. Total sugars analysis was determined by the Phenol-sulfuric acid method (Dubois *et al*., 1956 and Pak & Simon, 2004).

Carbon and nitrogen content of feedstock: Carbon content of sugarcane bagasse and potato peels were determined according to Tiessen & Moir (1993). Nitrogen content of feedstock was determined according to Stuart, (1936).

Feedstock processing

Bioethanol production from feedstock consisted of two main stages, first: Feedstock pretreatment and second: Bioethanol production. Feedstock pretreatment was performed by either dilute acid hydrolysis or gamma irradiation or the combination of both pretreatments. Bioethanol production was performed using neutralized (to pH 5.8) pretreated feedstock, on which *Sacch. cerevisiae* ATCC 7754 and *Z. mobilis* ATCC 29191 were inoculated to ferment released sugars into alcohol.

Irradiation of feedstock

Effect of gamma irradiation on bioethanol production was investigated by exposing feedstock to gamma "γ" radiation (using Indian cobalt-60 gamma cell at the National Center for Radiation Research and Technology, Egyptian Atomic Energy Authority "EAEA", Cairo, Egypt). Irradiation of feedstock was examined to facilitate sugar release from feedstock, thus improving bioethanol production. Irradiation of feedstock was performed in a batch process and the delivered irradiation absorbed doses were 25, 50 and 75 kGy (kiloGray); where Gray is a measurement unit of absorbed dose of gamma radiation, and exposure for 1 min = 43.8 Gray) (Thornley, 1963). Single and combined effect of irradiation and dilute acid treatments was studied by treating irradiated feedstock with 2 % and 6 % (v/v) sulphuric acid (98 %) at 120°C for 30 or 60 min. Sterilized flasks containing treated feedstock were inoculated with 5 ml of 48 h old seed culture of tested microorganisms. Bioethanol production and extraction

were done as described below. Flasks containing treated uninoculated or inoculated untreated feedstock were used as controls. Untreated feedstock was without acid hydrolysis or irradiation, contained 95 ml distilled water.

Dilute acid hydrolysis

To determine the effect of acid concentration, retention time and hydrolysis temperature, 5 grams of feedstock were added to 250 ml Erlenmeyer flask containing 95 ml of 2 % or 6 % (v/v) of sulphuric acid (98 %) or 95 ml of tap water (the control treatment), 6.7 ± 0.2 (using pH meter EPH211-Hanna Instruments Inc),. Hydrolysis was run at either 100 or 120ºC and the reaction time was 30 or 60 min (Pattana *et al*., 2010). The pretreated feedstock was left to cool then filtered to remove the solid fraction and the sugar-rich liquid filtrate was neutralized, as follows: the pH of the separated hydrolyzate was adjusted from around 0.001 to 5.8 in two steps, first by NaOH pellets to pH=3 and second by Ammonia solution (33 %) to pH=5.8.

Bioethanol fermentation

Before sterilization, neutralized hydrolyzate was supplemented with the following nutrients (g L^{-1}): KH_2PO_4 2, $MgSO_4.7H_2O$ 1 and $(NH_4)_2SO_4$ 1 (Davis *et al*., 2006) for *Z. mobilis* ATCC 29191 and yeast extract 3, peptone 3.5, KH_2PO_4 2, $MgSO_4.7H_2O$ 1 and $(NH_2)_2SO_4$ 1 for *Sacch. cerevisiae* ATCC 7754 (Arapoglou *et al*., 2010). After that, hydrolyzate was autoclaved at 121 ºC for 20 min and used for bioethanol production. Flasks containing 95 ml of neutralized sterilized feedstock (non-hydrolyzed, dilute acid-hydrolyzed, gamma-irradiated or combined treated with gamma irradiation and dilute acid) were inoculated with 5 ml of 48 h old liquid seed cultures of *Sacch. cerevisiae* ATCC 7754, *Z. mobilis* ATCC 29191 or co-cultures of both organisms (at 1:1 ratio). Flasks were incubated in anaerobic incubator (Labconco Manufacturing Corp., USA) at 30 ± 2ºC for 4 days. After incubation, bioethanol was extracted by transferring 100 ml of the grown culture to a rotary evaporator (R206D 2L–SENCO) and the apparatus was run for 10–20 min at 78.5ºC. The distillate was used to determine bioethanol concentration as described later. Standard inoculum (seed culture) of each organism was prepared by inoculating test tubes containing 5 ml broth media of YM (for *Sacch. cerevisiae* ATCC 7754 cultivation) or ATCC 948 medium (for *Z. mobilis* ATCC 29191 cultivation) with a full loop of tested culture and incubated at 30ºC for 48 h. All tests were performed in triplicates.

Bioethanol determination

Distillate obtained from rotary evaporator was used to determine bioethanol concentration colormetrically using potassium dichromate method (Crowell & Ough, 1979).

Determination of viable cells count

Viable cells count of both organisms was carried out by plate count method (Talyour, 1962).

(Gamal *et al*. 1991).

Bioethanol production parameters:

$$\text{Conversion coefficient (\%)} = \frac{\text{Bioethanol concentration produced (g L}^{-1})}{\text{Consumed sugars (g L}^{-1})} \times 100$$

$$\text{Bioethanol yield (\% w/w)} = \frac{\text{Bioethanol concentration produced (g L}^{-1})}{\text{Initial sugars (g L}^{-1})} \times 100$$

Sugar utilizing efficiency (% w/w):
(Ramadan *et al*., 1985).

$$\text{Sugar utilizing efficiency (\% w/w)} = \frac{\text{Consumed sugars (g L}^{-1})}{\text{Initial sugars (g L}^{-1})} \times 100$$

Statistical analysis

Data was analyzed by the method of SAS, (1996). Differences between means were compared using Duncan's Multiple Range Test according to Duncan, (1955).

Results and Discussion

Analysis of agro-industrial feedstock

For sugarcane bagasse and potato peels the moisture content was 16.7 % (w/w) and 22.2 % (w/w), total carbon was 41 % (w/w) and 38 % (w/w), total nitrogen was 0.52 % (w/w) and 0.69 % (w/w) and C/N ratio was 79 and 55, respectively.

Effect of gamma irradiation on bioethanol production

Throughout this work, the effect of gamma irradiation was conducted on cellulosic feedstock to enhance the bioethanol production process. Two locally available low-price agricultural wastes, sugarcane bagasse and potato peels, were used for bioethanol production by *Saccharomyces cerevisiae* ATCC 7754 and *Zymomonas mobilis* ATCC 29191 in batch culture process.

Bioethanol production

Bioethanol production was examined on neutralized acid hydrolyzed feedstock using a co-culture (1:1) of *Sacch. cerevisiae* ATCC 7754 and *Z. mobilis* ATCC 29191 (Table 1). The highest final bioethanol concentration, bioethanol yield and conversion coefficient were obtained by the cultivation on neutralized sugarcane bagasse hydrolyzed by 2 % (v/v) H_2SO_4 at 120°C for 60 min being 11.3 g L^{-1}, 47.7 % w/w and 48.3 % w/w, respectively. This treatment also achieved the highest sugar utilization efficiency (98.7 % w/w) and highest cells count (10.8 x 10^5 CFU ml^{-1}). On the other hand, the highest final bioethanol concentration, bioethanol yield and conversion coefficient obtained from potato peels were from hydrolysis treatment by 6 % (v/v) H_2SO_4 at 100°C for 60 min being 10.7 g L^{-1}, 44.6 % w/w and 46.9 % w/w, respectively.

TABLE 1. Effect of acid hydrolysis of sugarcane bagasse or potato peels on bioethanol production by co-culture (1:1) of *Z. mobilis* ATCC 29191 and *Sacch. cerevisiae* ATCC 7754 .

Feedstock	Acid hydrolysis			Bioethanol concentration		Initial sugars		Consumed sugars		Residual sugars		Bioethanol yield (% w/w)	Conversion coefficient (% w/w)	Sugar utilization efficiency (% w/w)	Cells count (CFUx10^5 ml^{-1})
	Acid conc. (v/v)	Hydrolysis temp. (°C)	Retention time (min)	(g L^{-1})	(mg g^{-1})	(g L^{-1})	(mg g^{-1})	(g L^{-1})	(mg g^{-1})	(g L^{-1})	(mg g^{-1})				
Sugarcane bagasse	Without hydrolysis			5.6^{J}	112	14.2^{K}	284	12.8^{H}	256	1.4^{FGH}	28	39.4^{EF}	43.8^{AB}	90.1^{FG}	4.1^{MN}
	2% H_2SO_4	100	30	7.1^{G}	142	15.7^{J}	314	15.2^{G}	304	0.5^{IJK}	10	44.9^{AB}	46.7^{AB}	96.8^{ABC}	6^{HI}
			60	8.2^{E}	164	18.5^{I}	370	17.9^{D}	358	0.6^{IJK}	12	44.3^{BC}	45.8^{AB}	96.8^{ABC}	7.5^{DE}
		120	30	9.5^{C}	190	20.2^{H}	404	19.8^{C}	396	0.4^{JK}	8	46.5^{A}	48^{A}	98^{AB}	8.4^{C}
			60	11.3^{A}	226	23.7^{F}	474	23.4^{B}	468	0.3^{K}	6	47.7^{A}	48.3^{A}	98.7^{A}	10.8^{A}
	6% H_2SO_4	100	30	10.8^{B}	216	27.2^{D}	544	25.5^{A}	510	1.7^{FG}	34	39.7^{EF}	42.4^{B}	93.8^{CDE}	9.4^{B}
			60	7.8^{EF}	156	28.6^{C}	572	17.2^{DE}	344	11.4^{D}	228	27.3^{H}	45.3^{AB}	60.1^{J}	6.7^{FG}
		120	30	6.1^{HI}	122	30.8^{B}	616	13.5^{H}	270	17.3^{B}	346	19.8^{I}	45.2^{AB}	43.8^{L}	5.3^{JK}
			60	5.8^{IJ}	116	32.1^{A}	642	13.6^{H}	272	18.5^{A}	370	18^{I}	42.6^{AB}	42.4^{L}	4.8^{KL}
Potato peels	Without hydrolysis			2.6^{M}	52	6.7^{N}	134	5.8^{K}	116	0.9^{HIJK}	18	38.8^{F}	44.8^{AB}	86.6^{H}	3.1^{O}
	2% H_2SO_4	100	30	4.3^{L}	86	10.7^{M}	214	9.5^{J}	190	1.2^{FGHI}	24	40.2^{DEF}	45.3^{AB}	88.8^{FGH}	3.8^{N}
			60	5.1^{K}	102	12^{L}	240	10.9^{I}	218	1.1^{GHIJ}	22	42.5^{BCD}	46.8^{AB}	90.8^{EFG}	4.5^{LM}
		120	30	6.5^{H}	130	14.6^{K}	292	13.8^{H}	276	0.8^{HIJK}	16	44.5^{BC}	47.1^{AB}	94.5^{BCDE}	5.7^{IJ}
			60	7.6^{F}	152	18.1^{I}	362	16.3^{EF}	326	1.8^{F}	36	42^{CDE}	46.6^{AB}	90^{FG}	6.5^{GH}
	6% H_2SO_4	100	30	9.2^{CD}	184	21.3^{G}	514	19.8^{C}	396	1.5^{FGH}	30	43.2^{BC}	46.5^{AB}	93^{DEF}	8.1^{CD}
			60	10.7^{B}	214	24^{F}	480	22.8^{B}	456	1.2^{FGHI}	24	44.6^{BC}	46.9^{AB}	95.4^{BCD}	9.2^{B}
		120	30	8.9^{D}	178	25.7^{E}	514	20.1^{C}	402	5.6^{E}	112	34.6^{G}	44.3^{AB}	78.2^{I}	7.2^{EF}
			60	7.1^{G}	142	28.6^{C}	572	15.6^{FG}	312	13^{C}	260	24.8^{H}	45.5^{AB}	54.5^{K}	6^{HI}

- (mg g^{-1}): weight in mg of bioethanol or sugars per 1 g of dry feedstock.
- Conversion coefficient (w/w %) = [Bioethanol concentration (g L^{-1}) ÷ consumed sugars (g L^{-1})]x100, Bioethanol yield (w/w %) = [Bioethanol concentration (g L^{-1}) ÷ initial sugars (g L^{-1})]x100 (Gamal *et al.*, 1991) and sugar utilization efficiency (w/w %) = consumed sugars (g L^{-1}) ÷ initial sugars (g L^{-1}) (Ramadan *et al.*, 1985).
- Cells count was determined after 4 days of fermentation period.
- The values are mean of three replicates. Standard deviation was within 10 %.
- Means with the same letter are not significantly different according to Duncan's at 5% level (Duncan, 1955).

Our results were comparative to those of Oyeleke *et al*. (2012) who reported that using mixed culture of *Sacch. cerevisiae* and *Z. mobilis* produced maximum bioethanol yield of 26 % from cassava peels and 12 % from sweet potato peels and these results were attributed due to the combined activity of the two organisms to produce bioethanol. Their results also revealed that cassava peels produced higher bioethanol yield than sweet potato peels, which was due to the presence of more carbohydrate in cassava peels than in sweet potato peels. Another related study (Hashem & Darwish, 2010) observed that maximum bioethanol yield (5.5 g L^{-1}) was achieved by *Sacch. cerevisiae* y-1646 after 36 h in batch fermentation using dilute acid hydrolysis of potato residue by 1 % (v/v) H_2SO_4, which was efficient enough to hydrolyze all starch content of potato residue.

Effect of gamma irradiation of non-hydrolyzed feedstock on bioethanol production

Bioethanol production was examined on non-hydrolyzed irradiated sugarcane bagasse and potato peels (at 0, 25, 50 and 75 kGy) using single or co-culture of *Z. mobilis* ATCC 29191 and *Sacch. cerevisiae* ATCC 7754. As shown in Table 2, a significant increase in final bioethanol concentration was recorded by the co-culture cultivation on irradiated sugarcane bagasse compared to that obtained from non-irradiated sugarcane bagasse (Table 1). The highest final bioethanol concentration, bioethanol yield and conversion coefficient (8.2 g L^{-1}, 43.2 % w/w and 46.3 % w/w, respectively) were obtained from sugarcane bagasse irradiated at the dose of 75 kGy by co-culture cultivation. In this treatment, the highest cells count was recorded in the co-culture (7.6 x 10^5 CFU ml^{-1}). The same treatments were applied to potato peels, of which data Table 3 demonstrated that bioethanol concentration slightly increased by the co-culture cultivation on irradiated potato peels compared with that obtained from non-irradiated potato peels (Table 1). The highest final bioethanol concentration, bioethanol yield and conversion coefficient (3.5 g L^{-1}, 36.5 % w/w and 43.8 % w/w, respectively) were obtained from potato peels irradiated at the dose of 75 kGy inoculated with co-culture. In this treatment, the highest cell count was recorded in the co-culture (4.7 x 10^5 CFU ml^{-1}). These results are in agreement with those of Qian *et al.* (2006), who demonstrated that using co-culture of *Sacch. cerevisiae* and recombinant *Escherichia coli* (carrying both *pdc* and *adhB* genes derived from *Z. mobilis*) to ferment acid hydrolyzate of softwood bioethanol production achieved a high ethanol yield of 0.49 g ethanol/g sugars, corresponding to 96.1 % of the maximum theoretical bioethanol yield after 24 h. However, our results disagreed with those of Duarte *et al*. (2008), who found that irradiation of sugarcane bagasse with low doses (lower than 20 kGy) can cleave the external structure of sugarcane bagasse without destroying the cellulose or losing sugars.

Effect of combining dilute acid hydrolysis with gamma irradiation of feedstock on bioethanol production

As illustrated in Table 4, bioethanol production was conducted on sugarcane bagasse irradiated at doses of 25, 50 and 75 kGy, followed by hydrolysis with 2 % (v/v) H_2SO_4 at 120°C for 30 or 60 min and fermented using single or co-culture of *Z. mobilis* ATCC 29191 and *Sacch. cerevisiae* ATCC 7754. A significant increase in final bioethanol concentration was recorded by the co-culture treatment compared with that obtained by the co-culture cultivated on

sugarcane bagasse treated only with dilute acid (Table 1). The highest final bioethanol concentration, bioethanol yield and sugar utilization efficiency were obtained from sugarcane bagasse irradiated at the dose of 75 kGy followed by acid hydrolysis with 2 % (v/v) H_2SO_4 at 120°C for 60 min (15.6 g L^{-1}, 44.8 % w/w and 93.7 % w/w, respectively). In this treatment, the highest cells count was recorded in the co-culture (13.6 x 10^5 CFU ml^{-1}).

Similarly, bioethanol production was also examined on potato peels irradiated at doses of 25, 50 and 75 kGy, followed by hydrolysis with 6 % (v/v) H_2SO_4 at 100°C for 30 and 60 min and using single or co-culture of *Z. mobilis* ATCC 29191 and *Sacch. cerevisiae* ATCC 7754 (Table 5). A significant increase in final bioethanol concentration was recorded comparing with that obtained by the co-culture cultivation on the acid hydrolyzed potato peels (Table 1). The highest final bioethanol concentration, bioethanol yield and sugar utilization efficiency were obtained from potato peels irradiated at the dose of 75 kGy followed by acid hydrolysis with 6 % (v/v) H_2SO_4 at 120°C for 60 min (12.1 g L^{-1}, 41.7 % w/w and 87.6 % w/w, respectively). In this treatment, the highest cells count was observed by the co-culture (11.8 x 10^5 CFU ml^{-1}).

Generally, all combined treatments led to increasing the total sugars (initial sugars) of both sugarcane bagasse and potato peels compared with dilute acid-hydrolyzed feedstock. In the case of sugarcane bagasse, the highest total sugars (34.8 g L^{-1}, 696 mg/g sugarcane bagasse) was obtained by the combined treatment of feedstock composed of irradiation at 75 kGy with hydrolysis by 2 % (v/v) H_2SO_4 at 120˚C for 60 min. Similarly, the highest total sugars (31 g L^{-1}, 620 mg/ g potato peels) was obtained by the combined treatment of feedstock composed of irradiation at 75 kGy and hydrolysis by 6 % (v/v) H_2SO_4 at 100˚C for 60 min.

Finally, it can be recommended that the best method for bioethanol production from sugarcane bagasse is composed of co-culture cultivation of *Z. mobilis* ATCC 29191 and *Sacch. cerevisiae* ATCC 7754 (1:1) on feedstock irradiated at 75 kGy followed by the dilute acid hydrolysis using 2 % (v/v) H_2SO_4 at 120°C for 60 min. Similarly, the recommended method for bioethanol production from potato peels is composed of the same co-culture treatment on feedstock irradiated at 75 kGy followed by the dilute acid hydrolysis using 6 % (v/v) H_2SO_4 at 120°C for 60 min. These results agreed with those obtained by Duarte *et al.* (2012) and Duarte *et al.* (2013), who found that the combination of dilute acid hydrolysis and irradiation pretreatment of sugarcane bagasse resulted in improving the bioethanol production. Ribeiro *et al*. (2013) also stated that the free radicals produced by interaction of high-energy radiation with polysaccharides resulted in decreasing the degree of polymerization and increasing the carbonyl content due to the chain cleavage in the cellulose and hemicelluloses molecules, in addition to the decrease in the formation of by-products such as furfural, hydroxy-methyl-furfural and acetic acid, which affect the growth of fermentative microorganisms.

TABLE 2. Effect of exposing sugarcane bagasse to different gamma irradiation doses on bioethanol production by *Z. mobilis* ATCC 29191 and *Sacch. cerevisiae* ATCC 7754 and co-culture of both microorganisms (1:1).

Irradiation dose of feedstock (kGy*)	Microorganism	Bioethanol concentration		Initial sugars		Consumed sugars		Residual sugars		Bioethanol yield (% w/w)	Conversion coefficient (% w/w)	Sugar utilization efficiency (% w/w)	Cells count (CFUx10^5 ml^{-1})
		(gL^{-1})	($mg\ g^{-1}$)	(gL^{-1})	($mg\ g^{-1}$)	(gL^{-1})	($mg\ g^{-1}$)	(gL^{-1})	($mg\ g^{-1}$)				
0**	***Z. mobilis***	3.4^{J}	68	14.2^{CD}	284	7.9^{I}	158	6.3^{A}	126	24^{G}	43^{A}	55.6^{G}	1.5^{H}
	Sacch. cerevisiae	4.9^{H}	98	14.2^{CD}	284	10.7^{G}	214	3.5^{C}	70	34.5^{D}	45.7^{A}	75.4^{D}	3.1^{G}
	Co-culture (1:1)	5.6^{FG}	112	14.2^{CD}	284	12.8^{E}	256	1.2^{EF}	28	39.4^{CD}	43.8^{A}	90.1^{BC}	4.1^{EF}
25	***Z. mobilis***	4.2^{I}	84	15.3^{C}	306	9.7^{H}	194	5.6^{B}	112	27.5^{FG}	43.3^{A}	63.4^{F}	3^{G}
	Sacch. cerevisiae	6^{EF}	120	15.3^{C}	306	13.3^{DE}	266	2^{D}	40	39.2^{CD}	45.1^{A}	86.9^{C}	4.2^{DEF}
	Co-culture (1:1)	6.7^{CD}	134	15.3^{C}	306	14.2^{C}	290	0.8^{F}	16	45.1^{A}	47.2^{A}	92.8^{AB}	5.3^{C}
50	***Z. mobilis***	5^{GH}	100	17^{B}	340	11.6^{F}	232	5.4^{B}	108	29.4^{F}	43.1^{A}	68.2^{E}	3.9^{F}
	Sacch. cerevisiae	6.8^{CD}	136	17^{B}	340	15.3^{B}	306	1.7^{ED}	34	40^{CD}	44.4^{A}	90^{BC}	4.8^{CDE}
	Co-culture (1:1)	7.5^{BC}	150	17^{B}	340	15.8^{B}	316	1.2^{EF}	24	44.1^{AB}	47.5^{A}	92.9^{AB}	6.3^{B}
75	***Z. mobilis***	6.4^{DE}	128	19^{A}	380	13.9^{CD}	278	5.1^{B}	102	33.5^{E}	46^{A}	73.2^{D}	5^{CD}
	Sacch. cerevisiae	7.8^{B}	156	19^{A}	380	17.3^{A}	346	1.7^{ED}	34	41.1^{BCD}	45.1^{A}	91.1^{AB}	6.6^{B}
	Co-culture (1:1)	8.2^{A}	164	19^{A}	380	17.7^{A}	354	1.3^{EF}	26	43.2^{ABC}	46.3^{A}	93.2^{A}	7.6^{A}

* kGy (Kilogray): is a measurement unit of absorbed dose of gamma radiation, dose rate = 2.6 kGy h^{-1}.

** 0: feedstock without exposing to gamma irradiation.

- (mg g^{-1}): weight in mg of bioethanol or sugars per 1 g of dry feedstock.
- Conversion coefficient (w/w %) = [Bioethanol concentration (g L^{-1}) ÷ consumed sugars (g L^{-1})]x100, Bioethanol yield (w/w %) = [Bioethanol concentration (g L^{-1}) ÷ initial sugars (g L^{-1})]x100 (Gamal *et al*., 1991) and sugar utilization efficiency (w/w %) = consumed sugars (g L^{-1}) ÷ initial sugars (g L^{-1}) (Ramadan *et al*., 1985).
- Cells count was determined after 4 days of fermentation period.
- The values are mean of three replicates. Standard deviation was within 10 %.
- Means with the same letter are not significantly different according to Duncan's at 5% level (Duncan, 1955).

TABLE 3. Effect of exposing potato peels to different gamma irradiation doses on bioethanol production by *Z. mobilis* ATCC 29191 and *Sacch. cerevisiae* ATCC 7754 and co-culture of both microorganisms (1:1).

Irradiation dose of feedstock (kGy*)	Microorganism	Bioethanol concentration		Initial sugars		Consumed sugars		Residual sugars		Bioethanol yield (w/w%)	Conversion coefficient (w/w%)	Sugar utilization efficiency (w/w%)	Cells count (CFUx10^5 ml^{-1})
		(gL^{-1})	($mg\,g^{-1}$)	(gL^{-1})	($mg\,g^{-1}$)	(gL^{-1})	($mg\,g^{-1}$)	(gL^{-1})	($mg\,g^{-1}$)				
0**	***Z. mobilis***	1.9^{E}	38	6.7^{C}	134	4.3^{H}	86	2.4^{C}	48	28.3^{D}	44.2^{A}	64.2^{E}	2.2^{F}
	Sacch. cerevisiae	2.4^{DE}	48	6.7^{C}	134	5.3^{G}	106	1.4^{EF}	28	35.8^{BC}	45.3^{A}	79.1^{ABCD}	2.6^{EF}
	Co-culture (1:1)	2.6^{CD}	52	6.7^{C}	134	5.8^{EFG}	116	0.9^{G}	18	38.8^{AB}	44.8^{A}	86.6^{A}	3.1^{CDE}
25	***Z. mobilis***	2.6^{CD}	52	7.8^{BC}	156	5.8^{EFG}	116	2^{CD}	40	33.3^{C}	44.8^{A}	76.3^{CD}	3.2^{CDE}
	Sacch. cerevisiae	2.8^{BCD}	56	7.8^{BC}	156	6.1^{DE}	122	1.7^{DE}	34	35.9^{BC}	45.9^{A}	78.2^{BCD}	3.5^{BCD}
	Co-culture (1:1)	3.2^{ABC}	64	7.8^{BC}	156	6.8^{BC}	136	1^{FG}	40	41^{A}	47.1^{A}	87.2^{A}	4.1^{AB}
50	***Z. mobilis***	2.4^{DE}	48	8.5^{B}	170	5.4^{FG}	108	3.1^{BC}	62	28.2^{D}	44.4^{A}	63.5^{EF}	2.9^{DE}
	Sacch. cerevisiae	3^{ABCD}	60	8.5^{B}	170	6.5^{BC}	130	2^{CD}	40	35.3^{BC}	46.2^{A}	76.5^{CD}	3.8^{BC}
	Co-culture (1:1)	3.3^{AB}	66	8.5^{B}	170	6.9^{B}	138	1.6^{DE}	32	38.8^{AB}	47.8^{A}	81.2^{ABC}	4.5^{A}
75	***Z. mobilis***	2.5^{DE}	50	9.6^{A}	192	6.2^{CD}	124	3.4^{B}	44	26^{E}	40.3^{B}	64.6^{D}	3.2^{CDE}
	Sacch. cerevisiae	2.7^{BCD}	54	9.6^{A}	192	5.9^{EF}	118	3.7^{A}	74	28.1^{D}	45.8^{A}	58.3^{F}	3.7^{BC}
	Co-culture (1:1)	3.5^{A}	70	9.6^{A}	192	8^{A}	160	1.6^{DE}	32	36.5^{BC}	43.8^{A}	83.3^{AB}	4.7^{A}

* kGy (Kilogray): is a measurement unit of absorbed dose of gamma radiation, dose rate = 2.6 kGy h^{-1}.

** 0: feedstock without exposing to gamma irradiation.

- ($mg\,g^{-1}$): weight in mg of bioethanol or sugars per 1 g of dry feedstock.
- Conversion coefficient (w/w %) = [Bioethanol concentration (g L^{-1}) ÷ consumed sugars (g L^{-1})]x100, Bioethanol yield (w/w %) = [Bioethanol concentration (g L^{-1}) ÷ initial sugars (g L^{-1})]x100 (Gamal *et al*., 1991) and sugar utilization efficiency (w/w %) = consumed sugars (g L^{-1}) ÷ initial sugars (g L^{-1}) (Ramadan *et al*., 1985).
- Cells count was determined after 4 days of fermentation period.
- The values are mean of three replicates. Standard deviation was within 10 %.
- Means with the same letter are not significantly different according to Duncan's at 5% level (Duncan, 1955).

TABLE 4. Effect of combination treatment of sugarcane bagasse by exposing to different gamma irradiation doses and hydrolysis by 2 % (v/v) H_2SO_4 at 120ºC for 30 and 60 min on bioethanol production by *Z. mobilis* ATCC 29191 and *Sacch. cerevisiae* ATCC 7754 and co-culture of both microorganisms (1:1).

Retention time of hydrolysis (min)	Irradiation dose of feedstock (kGy*)	Microorganism	Bioethanol concentratio		Initial sugars		Consumed sugars		Residual sugars		Bio-ethanol yield (w/w%)	Conversion coefficient (w/w%)	Sugar utilization efficiency (w/w%)	Cells count (CFUx10^5ml l^{-1})
			(gL^{-1})	($mg\,g^{-1}$)	(gL^{-1})	($mg\,g^{-1}$)	(gL^{-1})	($mg\,g^{-1}$)	(gL^{-1})	($mg\,g^{-1}$)				
30	0***	*Z. mobilis*	3.2^{Q}	64	20.2^{G}	404	7.7^{N}	154	12.5^{G}	250	15.8^{K}	41.6^{ABC}	38.1^{K}	2.4^{KLM}
		Sacch. cerevisiae	7.3^{K}	146	20.2^{G}	404	16^{I}	320	4.2^{I}	84	36.1^{G}	45.6^{AB}	79.2^{F}	3^{JK}
		Co-culture (1:1)	9.5^{I}	190	20.2^{G}	404	19.8^{G}	396	0.4^{O}	8	46.5^{AB}	48^{A}	$98A^{B}$	8.4^{G}
	25	*Z. mobilis*	5.1^{M}	102	21.6^{F}	432	11.4^{K}	228	10.2^{H}	204	23.6^{I}	44.7^{AB}	52.8^{H}	3.3^{J}
		Sacch. cerevisiae	8^{J}	160	21.6^{F}	432	17.7^{H}	354	3.9^{I}	78	37^{G}	45.2^{AB}	81.9^{EF}	5.5^{I}
		Co-culture (1:1)	9.8^{HI}	196	21.6^{F}	432	20.6^{G}	412	1^{O}	20	45.4^{BC}	47.6^{A}	95.4^{BC}	7.6^{H}
	50	*Z. mobilis*	4.3^{O}	86	24.8^{D}	940	9.7^{LM}	194	15.1^{E}	302	17.3^{KL}	44.3^{A}	39.1^{JK}	3.2^{JK}
		Sacch. cerevisiae	9.4^{I}	188	24.8^{D}	940	20.1^{G}	402	4.7^{I}	94	37.9^{FG}	46.8^{AB}	81^{F}	7.4^{H}
		Co-culture (1:1)	10.6	212	24.8^{D}	940	22.3^{F}	446	2.5^{JK}	50	42.7^{DE}	47.5^{A}	89.9^{D}	9.9^{EF}
	75	*Z. mobilis*	3.7^{PQ}	74	30.4^{B}	608	8.9^{M}	178	21.5^{B}	430	12.2^{M}	41.6^{ABC}	29.3^{L}	2.6^{JKLM}
		Sacch. cerevisiae	6.4^{L}	128	30.4^{B}	608	13.7^{J}	274	16.4^{D}	328	21.1^{J}	46.7^{AB}	45.1^{I}	4.8^{I}
		Co-culture (1:1)	12.9^{B}	258	30.4^{B}	608	27.4^{B}	548	3^{JK}	60	42.4^{DE}	47.1^{A}	90.1^{D}	11.7^{B}
60	0****	*Z. mobilis*	4.4^{NO}	88	23.7^{E}	474	9.8^{L}	196	13.9^{F}	278	18.6^{K}	45^{AB}	41.4^{J}	2^{M}
		Sacch. cerevisiae	10.3^{GH}	206	23.7^{E}	474	22^{F}	440	1.7^{LM}	34	44.7^{CD}	46.8^{AB}	92.8^{CD}	2.9^{JKL}
		Co-culture (1:1)	11.3^{DE}	226	23.7^{E}	474	23.4^{E}	468	0.3^{O}	6	47.7^{A}	48.3^{A}	98.7^{A}	10.8^{CD}
	25	*Z. mobilis*	4.9^{MN}	98	26^{C}	520	10.8^{K}	216	15.2^{E}	304	18.8^{JK}	45.4^{AB}	41.5^{J}	2.5^{JKLM}
		Sacch. cerevisiae	11.1^{DEF}	222	26^{C}	520	24^{DE}	480	2^{LM}	80	42.7^{DE}	46.3^{AB}	92.3^{CD}	10^{DEF}
		Co-culture (1:1)	11.6^{D}	232	26^{C}	520	24.4^{D}	488	1.6^{MN}	32	44.6^{CDE}	47.5^{A}	93.8^{C}	10.5^{CDE}
	50	*Z. mobilis*	3.3^{Q}	66	29.2^{B}	584	9.1^{LM}	182	20.1^{C}	402	11.3^{M}	36.3^{C}	31.2^{L}	2.1^{LM}
		Sacch. cerevisiae	11.6^{D}	232	29.2^{B}	584	24.7^{D}	494	4.5^{I}	90	39.7^{F}	46.9^{AB}	84.6^{E}	10.4^{CDE}
		Co-culture (1:1)	12.3^{C}	246	29.2^{B}	584	26.1^{C}	522	3.1^{J}	62	42.1^{E}	47.1^{AB}	89.4^{D}	11^{BC}
	75	*Z. mobilis*	3.9^{OP}	78	34.8^{A}	696	9.7^{L}	194	25^{A}	500	11.2^{M}	40.2^{BC}	27.9^{L}	2.4^{KLM}
		Sacch. cerevisiae	10.8^{EF}	216	34.8^{A}	696	24.5^{D}	490	10.2^{H}	204	31^{H}	44.1^{AB}	70.4^{G}	9.3^{F}
		Co-culture (1:1)	15.6^{A}	316	34.8^{A}	696	32.6^{A}	646	2.2^{KL}	44	44.8^{BCD}	47.9^{A}	93.7^{C}	13.6^{A}

* kGy (Kilogray): is a measurement unit of absorbed dose of gamma radiation, dose rate = 2.6 kGy h^{-1}.

** 0 (control) = sugarcane bagasse was hydrolyzed by 2 % H_2SO_4 (v/v) at 120 °C for 30 min.

*** 0 (control) = sugarcane bagasse was hydrolyzed by 2 % H_2SO_4 (v/v) at 120 °C for 60 min

- ($mg\,g^{-1}$): weight in mg of bioethanol or sugars per 1 g of dry feedstock.
- Conversion coefficient (w/w %) = [Bioethanol concentration ($g\,L^{-1}$) ÷ consumed sugars ($g\,L^{-1}$)]x100, Bioethanol yield (w/w %) = [Bioethanol concentration ($g\,L^{-1}$) ÷ initial sugars ($g\,L^{-1}$)]x100 (Gamal *et al*., 1991) and sugar utilization efficiency (w/w %) = consumed sugars ($g\,L^{-1}$) ÷ initial sugars ($g\,L^{-1}$) (Ramadan *et al*., 1985).
- Cells count was determined after 4 days of fermentation period.
- The values are mean of three replicates. Standard deviation was within 10 %.
- Means with the same letter are not significantly different according to Duncan's at 5% level (Duncan, 1955).

TABLE 5. Effect of combination treatment of potato peels by exposing to different gamma irradiation doses and hydrolysis by 6 % (v/v) H_2SO_4 at 100°C for 30 and 60 min on bioethanol production by each of *Z. mobilis* ATCC 29191 and *Sacch. cerevisiae* ATCC 7754 and co-culture of both microorganisms (1:1).

Retention time of hydrolysis (min)	Irradiation dose of feedstock (kGy*)	Microorganism	Bioethanol concentratio		Initial sugars		Consumed sugars		Residual sugars		Bioethanol yield (w/w%)	Conversion coefficient (w/w%)	Sugar utilization efficiency (w/w%)	Cells count (CFUx10^5 ml^{-1})
			(g L^{-1})	(mg g^{-1})	(g L^{-1})	(mg g^{-1})	(g L^{-1})	(mg g^{-1})	(g L^{-1})	(mg g^{-1})				
30	0***	*Z. mobilis*	$4.8L^{M}$	96	21.3^{H}	514	10.7^{P}	214	10.6^{F}	212	22.5^{H}	45^{A}	50.2^{J}	1.5^{M}
		Sacch. cerevisiae	6.8^{HI}	136	21.3^{H}	514	15L	300	6.3^{K}	126	31.9^{EF}	45.3^{A}	70.4^{G}	2.5^{KLM}
		Co-culture (1:1)	9.2^{CD}	184	21.3^{H}	514	19.8^{G}	396	1.5^{QK}	30	43.2^{AB}	46.5^{A}	93^{B}	8.1^{EF}
	25	*Z. mobilis*	5.6^{J}	112	22.4^{G}	448	12.5^{N}	250	9.9^{G}	198	25^{G}	44.8^{A}	55.8^{H}	4.6^{HIJ}
		Sacch. cerevisiae	7.1^{GH}	142	22.4^{G}	448	15.6^{K}	312	6.8^{IJ}	136	31.7^{EF}	45.5^{A}	69.6^{G}	6.3^{FGH}
		Co-culture (1:1)	9.5^{C}	190	22.4^{G}	448	20.3^{F}	406	2.1^{OP}	42	42.4^{AB}	46.8^{A}	85.3^{C}	8.8^{CDEF}
	50	*Z. mobilis*	4.3^{MN}	86	23.7^{F}	474	9.7^{R}	194	14^{D}	280	18.1^{IJ}	44.3^{A}	40.9^{L}	3.6^{KL}
		Sacch. cerevisiae	8.3^{E}	166	23.7^{F}	474	18.3^{H}	366	5.4^{L}	108	35^{CD}	45.4^{A}	77.2^{E}	7.2^{EFG}
		Co-culture (1:1)	10.5^{B}	200	23.7^{F}	474	22^{E}	440	1.7^{PQ}	34	44.3^{A}	47.7^{A}	44.3^{L}	9^{CDE}
	75	*Z. mobilis*	4^{N}	80	25.1^{D}	502	9.2^{R}	184	15.9^{B}	318	15.9^{K}	43.5^{A}	36.7^{L}	3.1^{KL}
		Sacch. cerevisiae	8.9^{D}	178	25.1^{D}	502	19.7^{G}	394	5.4^{L}	108	35.5^{CD}	45.2^{A}	78.5^{D}	8.1^{EFG}
		Co-culture (1:1)	10.8^{B}	224	25.1^{D}	502	22.8^{C}	456	2.3^{NO}	46	43^{AB}	47.4^{A}	90.8^{B}	9.7^{BCD}
60	0****	*Z. mobilis*	5.7^{J}	114	24^{E}	480	12.6^{N}	252	11.4^{E}	228	23.8^{GH}	45.2^{A}	52.5^{I}	1.4^{M}
		Sacch. cerevisiae	7.5^{FG}	150	24^{E}	480	16.4^{J}	328	7.6^{H}	152	31.3^{F}	45.7^{A}	68.3^{G}	2.2^{ML}
		Co-culture (1:1)	10.7^{B}	212	24^{E}	480	22.5^{C}	450	1.2^{RS}	24	44.2^{A}	47.1^{A}	93.8^{A}	9.6^{CD}
	25	*Z. mobilis*	$6.3G^{H}$	126	25.8^{C}	516	14^{M}	280	11.8^{E}	236	24.4^{GH}	45^{A}	54.3^{HI}	4.9^{GHI}
		Sacch. cerevisiae	8^{EF}	160	25.8^{C}	516	17.8^{I}	356	8^{H}	160	31^{F}	44.9^{A}	69^{G}	6.2^{FGH}
		Co-culture (1:1)	11^{B}	220	25.8^{C}	516	23.2^{C}	464	2.6^{N}	52	42.6^{AB}	47.4^{A}	89.9^{C}	10.2^{BC}
	50	*Z. mobilis*	5.4^{JK}	108	27.6^{B}	552	12.2^{N}	244	15.4^{C}	308	19.6^{I}	44.3^{A}	44.2^{K}	4.5^{IJK}
		Sacch. cerevisiae	9.3^{CD}	186	27.6^{B}	552	20.6^{F}	412	7^{I}	140	33.7^{DE}	45.1^{A}	74.6^{F}	8.4^{DEF}
		Co-culture (1:1)	11.8^{A}	236	27.6^{B}	552	26.7^{A}	540	0.9^{S}	18	42.8^{AB}	44.2^{A}	96.7^{A}	10.7^{B}
	75	*Z. mobilis*	5^{KL}	100	29^{A}	580	11.6^{O}	232	17.4^{A}	348	17.2^{JK}	43.1^{A}	40^{L}	4.1^{JK}
		Sacch. cerevisiae	10.6^{B}	212	29^{A}	580	22.5^{D}	452	6.5^{JK}	130	36.6^{C}	47.1^{A}	77.6^{E}	9.1^{CDE}
		Co-culture (1:1)	12.1^{A}	242	29^{A}	580	25.4^{B}	508	3.6^{M}	72	41.7^{B}	47.6^{A}	87.6^{C}	11.8^{A}

* kGy (Kilogray): is a measurement unit of absorbed dose of gamma radiation, dose rate = 2.6 kGy h^{-1}.

** 0 (control) = potato peels were hydrolyzed by 6 % H_2SO_4 (v/v) at 100 °C for 30 min.

*** 0 (control) = potato peels were hydrolyzed by 6 % H_2SO_4 (v/v) at 100 °C for 60 min.

- (mg g^{-1}): weight in mg of bioethanol or sugars per 1 g of dry feedstock.
- Conversion coefficient (w/w %) = [Bioethanol concentration (g L^{-1}) ÷ consumed sugars (g L^{-1})]x100, Bioethanol yield (w/w %) = [Bioethanol concentration (g L^{-1}) ÷ initial sugars (g L^{-1})]x100 (Gamal *et al*., 1991) and sugar utilization efficiency (w/w %) = consumed sugars (g L^{-1}) ÷ initial sugars (g L^{-1}) (Ramadan *et al*., 1985).
- Cells count was determined after 4 days of fermentation period.
- The values are mean of three replicates. Standard deviation was within 10 %.
- Means with the same letter are not significantly different according to Duncan's at 5% level (Duncan, 1955).

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الإنتاج الميكروبى للإيثانول الحيوى من مصاصة قصب السكر وقشور البطاطس المعاملة الإشعاع

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في الآونة الأخيرة ومع تفاقم أزمة الوقود غير المتجدد في جميع أنحاء العالم وما يترتب على ذلك من مشاكل التلوث البيئي فقد أصبح الإيثانول الحيوي واحداً من أكثر أنواع الوقود الحيوى الواعدة، وقد عمل العديد من الباحثين على تحسين كفاءة عملية إنتاج الإيثانول الحيوى. وقد إهتم هذا العمل بإنتاج الإيثانول الحيوى من المخلفات الزراعية منخفضة التكلفة الناتجة عن الصناعة (مصاصة قصب السكر وقشور البطاطس) والإستفادة من تكنولوجيا الإشعاع لزيادة معدل تحويل هذه المواد إلى إيثانول حيوى. وتم معاملة قصب السكر وقشور البطاطس بالحامض ثم تخمير ناتج التحلل إما عن طريق *Zymomonas mobilis* ATCC 29191 أو *Saccharomyces cerevisiae* ATCC 7754 أو كليهما معاً (مزرعة مختلطة بنسبة 1:1). ودراسة تأثير أشعة جاما على إنتاج الإيثانول الحيوي من خلال تعريض تلك المخلفات لجرعات مختلفة من أشعة جاما (0، 25، 50 ، 75 كيلو جراى) علاوة على دراسة تأثير الجمع بين تشعيع المخلفات بأشعة جاما ثم معاملتها بالحامض على إنتاج الإيثانول الحيوى. وقد كان أعلى تركيز من الإيثانول الحيوى من مصاصة قصب السكر هو 15.4 جم/ لتر ناتجة عن تشعيع مصاصة قصب السكر بجرعة 75 كيلو جراى ثم تحليلها بواسطة محلول حامض الكبريتيك بتركيز 2 ٪ عند 120°م لمدة 60 دقيقة بإستخدام المزرعة المختلطة (1:1) من *Sacch. cerevisiae* ATCC 7754، *Z. mobilis* ATCC 29191. ومن ناحية أخرى كان أعلى تركيز من الإيثانول الحيوى من قشور البطاطس هو 12.1 جم/ لتر ناتجة عن تشعيع قشور البطاطس بجرعة 75 كيلو جراى ثم تحليلها بواسطة محلول حامض الكبريتيك بتركيز 6 ٪ عند 100°م لمدة 60 دقيقة بإستخدام المزرعة المختلطة (1:1).