

Effect of Gama Irradiation of Bioethanol Producing Microorganisms on Bioethanol Formation from Sugarcane Bagasse and Potato Peels

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THE PRESENT work was designed to investigate the production of bioethanol from agriculture feedstock (sugarcane bagasse and potato peels) using *Saccharomyces cerevisiae* ATCC 7754 and *Zymomonas mobilis* ATCC 29191, exposed to different doses of gamma irradiation (0, 100, 300, 500, 1000 and 1500 Gy). The effect of different hydrolysis pretreatments of feedstock on resulting sugars (initial sugars), which were later fermented to bioethanol, was also tested and compared to non-hydrolyzed feedstock. Hydrolysis of sugarcane bagasse and potato peels was conducted with dilute sulphuric acid (2 and 6 % v/v), running at 100 and 120°C for 30 and 60 min of retention time. The highest bioethanol concentration obtained from sugarcane bagasse was 10.3 gL⁻¹, which was produced by *Sacch. cerevisiae* ATCC 7754 irradiated at 300 Gy from hydrolysate of 2 % (v/v) H₂SO₄ at 120°C for 60 min treatment. From the same treatment, the highest bioethanol concentration obtained by *Z. mobilis* ATCC 29191 was 4.4 gL⁻¹, when irradiated at 100 Gy. This acid treatment produced 23.7 gL⁻¹ of sugars from the feedstock. The highest bioethanol concentration obtained from potato peels was 7.5 gL⁻¹, produced by *Sacch. cerevisiae* ATCC 7754 irradiated at 300 Gy from hydrolysate of 6 % (v/v) H₂SO₄ at 100°C for 60 min treatment, followed by 5.7 gL⁻¹ produced by *Z. mobilis* ATCC 29191 irradiated at 100 Gy. This treatment produced 24 gL⁻¹ of sugars from the feedstock.

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

With the growing crisis in fossil fuel and environmental pollution problems worldwide, bioethanol as a clean-burning renewable resource has become one of the most promising biofuels and many studies have been focused on improving the efficacy of the bioethanol production process. The production of bioethanol

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from biomass materials received great attention in the worldwide. In the U.S., bioethanol is primarily produced from corn starch raw materials while in Brazil it is mainly produced from sugarcane juice and molasses. Together, these two countries account for 89 % of the current global bioethanol production (Limayem & Steven, 2012). Using less valuable materials, like lignocellulosic agricultural waste, could significantly reduce the production expense (Abo-State *et al.*, 2013). 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 by acid or base to produce sugars for bioethanol fermentation (Ferdian *et al.*, 2012). Chemically, about 40–50 % of the dry sugarcane bagasse residue is cellulose, much of which is in a crystalline structure. Another 25–35 % is hemicelluloses. The remainder is mostly lignin plus lesser amounts minerals, waxes and other compounds (Jacobsen & Wyman, 2002). Potato peel waste (PPW) 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 bioethanol by fermentation (Arapoglou *et al.*, 2010). Hydrolysis of sugarcane bagasse is crucial for the conversion of bagasse polysaccharides, mainly cellulose, into valuable products. However, the strong crystalline arrangement of cellulose and the protective effects by lignin and hemicelluloses makes it difficult for enzymes and acid catalysts to cleave the β -1,4 glycosidic bonds, which constitute a serious obstacle to hydrolysis (George *et al.*, 2011).

Acid pretreatments normally aim for high yields of sugars from lignocellulosic materials; includes the use of sulfuric, nitric, or hydrochloric acids to remove hemicellulose components and expose cellulose for enzymatic digestion. The acid pretreatment can operate either under a high temperature and low acid concentration (dilute acid pretreatment) or under a low temperature and high acid concentration (concentrated acid pretreatment) (Karimi *et al.*, 2006).

Gamma irradiation is electromagnetic radiation high-energy with short wavelength, emitted by radioactive isotopes (cobalt-60 or cesium-137) as the unstable nucleus breaks up and decays to reach a stable form. It is widely used for sterilization of medical devices, food preservation and processing of tissue and blood components, obviating the need for high temperatures that can be damaging to such products (Osterholm & Norgan, 2004). The biological effects of ionizing radiation on cells is due to both direct interactions with critical cell components and indirect actions on these targets by molecular entities formed because of the radiolysis of other molecules in the cell, particularly by radicals formed from water. Ionizing radiation is capable of causing a variety of chemical changes in microorganisms, of which DNA is the most critical target of ionizing radiation (Al-Sudany *et al.*, 2010 and Grecz *et al.*, 1983). The low doses of gamma irradiation may enhance the activity of microorganisms in biological processes. *Sacch. cerevisiae* strains, exposed to low doses (>100 Gy) of gamma irradiation, showed increased activity of alcohol-dehydrogenase enzyme (Atia, 2005; Chakravarty & Sen, 2001 and Akacha *et al.*, 2008).

The aim of this work was to study the effect of different doses of gamma irradiation on some bioethanol producing microbes (*Saccharomyces cerevisiae* ATCC 7754 or *Zymomonas mobilis* ATCC 29191) with or without dilute acid hydrolysis of sugarcane bagasse and potato peels and the effect of these treatments on bioethanol production.

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 Univeristy, 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, 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 feedstock were homogenized and oven-dried at 45°C 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 content: Five grams of each feedstock were dried in oven at 45°C overnight and left to cool in a desicator then 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 before and 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 sugarcane bagasse and potato peels were determined according to Stuart (1936).

Irradiation of microorganisms

Effect of gamma irradiation on bioethanol production was investigated by exposing the producing microorganisms 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). For the irradiation of microorganisms, plates containing colonies of *Sacch. cerevisiae* ATCC 7754 grown on YM agar and colonies of *Z. mobilis* on ATCC 948 agar were exposed to doses of γ -radiation as follow: 0, 100, 300, 500, 1000, 1500, 2000, 2500 and 3000 Gy (Gy: Gray is a measurement unit of absorbed dose of gamma radiation, exposure for 1 min = 43.8 Gy) (Thornley, 1963). To determine The D10-value (the dose required to inactivate 90 % of a population), the exposed cells were serially diluted in sterile isotonic saline solution and 0.1 ml suspension of appropriate dilutions was spread on solid YM or ATCC 948 media, incubated at 30°C for 48 h, and the growing colonies were counted. A dose response curve was drawn by plotting the dose (Gy) against log of surviving cells. Surviving colonies resulted after each gamma irradiation dose was plotted on a logarithmic scale as a function of gamma irradiation dose, resulting in survivor curves. The Surviving colonies were tested for bioethanol production. The D10-value was calculated using the following equation (Thornley, 1963).

$$D10 = \frac{\text{Dose (D)}}{\text{Log No} - \text{log N}}$$

where “D” irradiation dose, “No” initial count and “N” the count at specific dose.

Feedstock processing

Bioethanol production from feedstock consisted of two main stages, first: feedstock pretreatment and second: bioethanol production. Feedstock pretreatment was performed by dilute acid hydrolysis. 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.

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 120°C and the reaction time was 30 or 60 min (Pattana *et al.*, 2010). The pretreated sugarcane bagasse and potato peels were 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 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, $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$ 1 and $(\text{NH}_4)_2\text{SO}_4$ 1 (Davis *et al.*, 2009) for *Z. mobilis* ATCC 29191 and yeast extract 3, peptone 3.5, KH_2PO_4 2, $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$ 1 and $(\text{NH}_4)_2\text{SO}_4$ 1 for *Sacch. cerevisiae* ATCC 7754 (Arapoglou *et al.*, 2010). After that, hydrolyzate was autoclaved at 121°C for 20 min and used for bioethanol production. Flasks containing 95 ml of neutralized sterilized acid-hydrolyzates feedstock or sterilized non-hydrolyzed (control), were inoculated with 5 ml of 48 h old liquid seed cultures of *Sacch. cerevisiae* ATCC 7754 or *Z. mobilis* ATCC 29191. Flasks were incubated in anaerobic incubator (Labconco Manufacturing Corp., USA) at $30 \pm 2^\circ\text{C}$ 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 78.5°C . 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 (for *Z. mobilis* ATCC 29191 cultivation) with a full loop of tested culture and incubated at 30°C for 48 h. All tests were performed in triplicates.

Bioethanol determination

Distillate obtained from rotary evaporator was used to determine bioethanol concentration colorimetrically 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).

Bioethanol production parameters

According to Gamal *et al.* (1991):

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

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

Sugar utilizing efficiency (% w/w)

According to Ramadan *et al.* (1985):

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

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

The analysis of sugarcane bagasse and potato peels are shown in Table 1. 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.

TABLE 1. Analysis of raw sugarcane bagasse and potato peels.

Feedstock	Moisture content (w/w %)	Total carbon (w/w %)	Total nitrogen (w/w %)	C/N ratio
Sugarcane bagasse	16.7 ± 3.04	41 ± 1.04	0.52 ± 0.03	79
Potato peels	22.2 ± 5.02	38 ± 2.02	0.69 ± 0.01	55

Effect of gamma irradiation on bioethanol production

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

Effect of gamma irradiation of bioethanol producing organisms to ferment non-hydrolyzed feedstock

In this study, gamma irradiated *Sacch. cerevisiae* ATCC 7754 and *Zymomonas mobilis* ATCC 29191 were used for bioethanol production from non-hydrolyzed sugarcane bagasse and potato peels. Firstly, the effect of exposing these two organisms to different gamma irradiation doses (0, 100, 300, 500, 1000, 1500, 2000, 2500 and 3000 Gy) was tested on the growth of these organisms. *Z. mobilis* ATCC 29191 and *Sacch. cerevisiae* ATCC 7754 were exposed to its specific sublethal dose, which are known to be 3000 Gy for both organisms. The radiation resistance of *Z. mobilis* ATCC 29191 and *Sacch. cerevisiae* ATCC 7754 was expressed as D₁₀ value obtained from the dose response curves which were drawn. Both organisms were lethally affected by increasing irradiation dose up to 3000 Gy. Thus, the range of doses was

narrowed to end at 1500 Gy for next experiment. Within the range of 0, 100, 300, 500, 1000 and 1500 Gy, microbial growth, sugars consumption and bioethanol production, were determined to get correlation between irradiation dose and bioethanol production to select the suitable irradiation treatment. Data presented in Table 2 show that cells growth of both organisms decreased with increasing irradiation dose, regardless of the feedstock type. Therefore, the highest cells count was recorded in the non-irradiated *Sacch. cerevisiae* ATCC 7754 (33.8×10^4 CFU/ml) while it was 29.2×10^4 CFU/ml for the non-irradiated cells *Z. mobilis*. When comparing between the two feedstocks, concentration of the initial total sugars in productive media (Table 2, footnote) obtained by sugarcane bagasse was significantly higher (14.2 gL^{-1}) than that obtained from potato peels (6.7 gL^{-1}), which should explain the difference between the two feedstock in bioethanol production by either organisms.

Regarding the effect of gamma irradiation on the organism productivity of bioethanol, irradiation of *Z. mobilis* ATCC 29191 significantly reduced final bioethanol concentration from 3 gL^{-1} (non-irradiated) down to 1.8 gL^{-1} (at 150 Gy) in case of sugarcane bagasse, and from 2 gL^{-1} (non-irradiated) to 1 gL^{-1} (at 1500 Gy) in case of potato peels. *Sacch. cerevisiae* ATCC 7754 had different response to irradiation, that its productivity from sugarcane bagasse increased from 4.2 g L^{-1} , when non-irradiated to reach 4.9 gL^{-1} , when exposed to 300 Gy, then decreased with more irradiation to reach 3 gL^{-1} , when exposed to 1500 Gy. Moreover, its productivity of bioethanol from potato peels increased only from 2.2 g L^{-1} , at 0 Gy, to 2.4 gL^{-1} , at 100 Gy, then decreased thereafter down to 1.2 gL^{-1} , at 1500 Gy. The highest bioethanol concentration (4.9 gL^{-1}) was obtained from sugar cane bagasse when fermented with *Sacch. cerevisiae* ATCC 7754 irradiated at 300 Gy, where the bioethanol yield, conversion coefficient and sugar utilization efficiency were 34.5 % (w/w), 45.7 % (w/w) and 75.4 % (w/w), respectively.

From the foregoing results, it could be concluded that the production of bioethanol by either *Sacch. cerevisiae* ATCC 7754 or *Z. mobilis* ATCC 29191 on both non-hydrolyzed sugarcane bagasse and potato peels was not satisfying, which could be attributed to the lower sugar content in non-hydrolyzed sugarcane bagasse or potato peels. Furthermore, the following experiments were conducted to increase the role of irradiation on bioethanol production process. On the contrary, Gunasekaran & Chandra (2007) noticed that the maximum bioethanol yields produced by *Z. mobilis* from cassava peels and sweet potato peels were 23 % (w/w) and 12 % (w/w), respectively, while it was 22 % (w/w) and 12 % (w/w), respectively, when produced by *Sacch. cerevisiae*, which reveals a higher bioethanol production by *Z. mobilis* than *Sacch. cerevisiae*. On the other hand, Carvalho (2009) reported that using sugarcane bagasse directly without pretreatment gave a slow and low biogas yield. Therefore, the pretreatment of residues was needed.

TABLE 2. Effect of exposing *Z. mobilis* ATCC 29191 and *Sacch. cerevisiae* ATCC 7754 cells to different doses of gamma irradiation (Gy) on bioethanol production using non-hydrolyzed sugarcane bagasse and potato peels.

Feedstock	Microorganism	Irradiation dose of microorganisms (Gy ^a)	Bioethanol concentration (g L ⁻¹)	Consumed sugars (mg g ⁻¹)	Residual sugars (mg g ⁻¹)	Bioethanol yield (% w/w)	Conversion coefficient (% w/w)	Sugar utilization efficiency (% w/w)	Cells count (CFUx10 ⁶ ml ⁻¹)				
										Bioethanol concentration (g L ⁻¹)	Consumed sugars (mg g ⁻¹)	Residual sugars (mg g ⁻¹)	
Sugarcane bagasse	<i>Z. mobilis</i> ATCC 29191	0 ^{***}	3 ^L	7.2 ^D	144	7 ^B	140	21.1 ^{GHU}	41.4 ^{AB}	50.7 ^{FOH}	29.2 ^{BC}		
		100	3.2 ^{EF}	64	7.4 ^D	148	6 ^B	136	22.5 ^{FHIL}	43.2 ^{AB}	28.3 ^{CDE}		
		300	2.8 ^{FG}	56	6.8 ^D	136	7.4 ^D	148	19.7 ^{HUK}	41.2 ^{AB}	47.9 ^{FOH}	25 ^{FH}	
		500	2.3 ^{GH}	46	5.5 ^E	110	8.7 ^A	174	16.2 ^{IKL}	41.8 ^{AB}	38.7 ^{KJ}	22.4 ^{HI}	
		1000	2.1 ^{GHI}	42	5.1 ^{EF}	102	9.1 ^A	182	14.8 ^{KL}	41.1 ^{AB}	35.9 ^{KJ}	20.2 ^{IK}	
	<i>Sacch. cerevisiae</i> ATCC 7754	1500	1.8 ^{IKL}	36	4.5 ^{FG}	90	9.7 ^A	194	12.7 ^L	40 ^{AB}	31.7 ^K	18.7 ^{JKL}	
		0	4.2 ^{BC}	84	9.6 ^{BC}	192	4.6 ^{CDE}	92	29.6 ^{BOD}	43.8 ^{AB}	67.6 ^{AB}	33.8 ^A	
		100	4.6 ^{AB}	92	10.4 ^{AB}	208	3.8 ^{DEFG}	76	32.4 ^{ABC}	44.2 ^{AB}	73.2 ^{AB}	31.4 ^{AB}	
		300	4.9 ^A	98	10.7 ^A	214	3.5 ^{FGH}	70	34.5 ^{AB}	45.7 ^A	75.4 ^{AB}	30.6 ^{BC}	
		500	3.9 ^{CD}	78	9.1 ^C	182	5.1 ^{CD}	102	27.5 ^{BOD}	42.9 ^{AB}	64.1 ^{BC}	28.8 ^{BOD}	
	Potato peels	<i>Z. mobilis</i> ATCC 29191	1000	3.5 ^D	70	8.7 ^C	174	5.5 ^C	110	24.6 ^{DEF}	43.7 ^{AB}	61.3 ^{CD}	27 ^{EF}
			1500	3 ^E	60	7.2 ^D	144	7 ^B	140	24.2 ^{DEFG}	41.6 ^{AB}	50.7 ^{FOH}	25.6 ^{DEFG}
			0	2 ^{FH}	40	4.5 ^{FG}	90	2.2 ^{IK}	44	29.9 ^{ABOD}	44.4 ^{AB}	67.2 ^{ABC}	21.4 ^J
			100	1.9 ^{HUK}	38	4.3 ^{FG}	86	2.4 ^{IK}	48	28.3 ^{BOD}	44.2 ^{AB}	64.2 ^{BC}	19.3 ^{JKL}
			300	1.6 ^{IKLM}	32	3.8 ^{GHI}	76	2.9 ^{GHI}	58	23.9 ^{EFH}	42.1 ^{AB}	56.7 ^{DEF}	18.1 ^{JKLM}
<i>Sacch. cerevisiae</i> ATCC 7754	500	1.4 ^{LMN}	28	3.3 ^{HU}	66	3.4 ^{FH}	68	20.9 ^{HUK}	42.4 ^{AB}	49.3 ^{FOH}	16.8 ^{LM}		
	1000	1.3 ^{LMN}	26	3.1 ^{HU}	62	3.6 ^{FGH}	72	19.4 ^{HUK}	41.9 ^{AB}	46.3 ^{GHI}	15 ^M		
	1500	1 ^N	20	2.6 ^I	52	4.1 ^{DEF}	82	14.9 ^{KL}	38.5 ^B	38.8 ^{KJ}	12.7 ^N		
	0	2.2 ^{GHI}	44	5.1 ^{EF}	102	1.6 ^K	32	32.8 ^{ABC}	43.1 ^{AB}	76.1 ^{AB}	28 ^{CDE}		
	100	2.4 ^{FH}	48	5.3 ^E	106	1.4 ^K	28	35.8 ^A	45.3 ^{AB}	79.1 ^A	26.2 ^{DEF}		

* Gy (Gray): is a measurement unit of absorbed dose of gamma radiation, dose rate = 43.8 Gy min⁻¹.

** 0: microorganisms 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.

- Initial sugars concentration in bioethanol production media containing sugarcane bagasse or potato peels were 14.2 g L⁻¹ and 6.7 g L⁻¹, respectively.

- 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.

Effect of gamma irradiation of bioethanol producing organisms on bioethanol production from acid-hydrolyzed feedstock

Acid-hydrolyzed sugarcane bagasse

Acid hydrolysis of sugarcane bagasse was performed using 2 or 6 % (v/v) H₂SO₄ at 100 °C or 120 °C for 30 or 60 min of retention time. The neutralized nutrients-amended acid hydrolyzates of sugarcane bagasse was used as basal media to study the effect of gamma irradiation (as conducted in previous experiment) on bioethanol production by either *Z. mobilis* ATCC 29191 or *Sacch. cerevisiae* ATCC 7754 during 4 days of incubation at 30 °C. Table 3 illustrates results of acid hydrolysis treatment of sugarcane bagasse with 2 % (v/v) H₂SO₄ at 100 °C for 30 and 60 min. Compared with non-hydrolyzed treatment, this treatment increased the initial sugars concentration to 15.7 gL⁻¹ when hydrolysis was run for 30 min and 18.5 gL⁻¹, when hydrolysis was run for 60 min (see footnote of Table 3). However, bioethanol yield was higher in 30 min treatment than 60min, by both organisms, and regardless of which organism was used and irradiation dose.

Overall performance of *Sacch. cerevisiae* ATCC 7754 in producing bioethanol from bagasse was significantly higher in both treatments of retention times than *Z. mobilis* ATCC 29191, regardless of irradiation treatment. Exposing *Z. mobilis* ATCC 29191 to irradiation caused insignificant increase in bioethanol concentration whereas irradiating *Sacch. cerevisiae* ATCC 7754 caused significant increase in bioethanol production up to 300 Gy, where 5.5 gL⁻¹ were obtained from 30min hydrolysis treatment, giving bioethanol yield of 35 % w/w, conversion coefficient of 46.2 % w/w and sugar utilization efficiency of 74 % (w/w). Increasing the irradiation dose over this limit greatly decreased the final bioethanol concentration. The same trend for *Sacch. cerevisiae* ATCC 7754 was observed in 60 min hydrolysis treatment, expect for lower values, where final bioethanol concentration was 4.8 gL⁻¹ when using *Sacch. cerevisiae* ATCC 7754 irradiated at 300 Gy, giving 26 % (w/w) of bioethanol yield, 43.6 % (w/w) for conversion coefficient and 59.5 % (w/w) for sugar utilization efficiency.

It is also important to mention that at this level of irradiation, cell count of *Sacch. cerevisiae* was not at its best, where it was significantly lower than non-irradiated culture, which means that the organism's performance was positively affected by the irradiation, despite the decrease in cell number. The best result of bioethanol production was obtained from 30 min hydrolysis treatment where it showed 5.5 gL⁻¹ of bioethanol concentration. Cell counts of both organisms were negatively affected by irradiation, where the best count was recorded in the non-irradiated culture of *Sacch. cerevisiae* ATCC 7754 (31.4 x 10⁴ CFU ml⁻¹), while it was 27 x 10⁴ CFU ml⁻¹ for the non-irradiated culture of *Z. mobilis* ATCC 29191. In all treatments, *Sacch. cerevisiae* ATCC 7754 had higher cell counts than *Z. mobilis* ATCC 29191. These results are in line with those obtained by Abdel-Fattah *et al.* (2000) who reported that exposing *Sacch. cerevisiae* ATCC 7754 cells to gamma irradiation increased its ability to grow and produce higher ethanol yield in stress conditions.

TABLE 3. Effect of exposing *Z. mobilis* ATCC 29191 and *Sacch. cerevisiae* ATCC 7754 cells to different doses of gamma irradiation (Gy) on bioethanol production using sugarcane bagasse hydrolyzed by 2 % (v/v) H₂SO₄ at 100°C for 30 and 60 min.

Retention time of hydrolysis (min)	Microorganism	Irradiation dose of microorganisms (Gy*)	Bioethanol concentration (g L ⁻¹)		Consumed sugars (mg g ⁻¹)		Residual sugars (mg g ⁻¹)		Bioethanol yield (% w/w)	Conversion coefficient (% w/w)	Sugar utilization efficiency (% w/w)	Cells count (CFUx10 ⁶ ml ⁻¹)				
			(g L ⁻¹)	(mg g ⁻¹)	(g L ⁻¹)	(mg g ⁻¹)	(% w/w)	(% w/w)								
30	<i>Z. mobilis</i> ATCC 29191	0**	2.8 ^{HJ}	56	6.7 ^{DK}	134	9 ^{FGH}	180	17.8 ^J	41.8 ^A	42.7 ^{GHI}	27 ^{DE}				
		100	3 ^{GHI}	60	7.3 ^{HJ}	146	8.4 ^{GHI}	168	19.1 ^{GHI}	41.1 ^A	46.5 ^{FGH}	24.5 ^{FG}				
		300	2.7 ^{HJK}	54	6.5 ^{IKL}	130	9.2 ^{EFHG}	184	17.2 ^{IKL}	41.5 ^A	41.4 ^{HJK}	22 ^{JK}				
		500	2.3 ^{JKLMN}	46	5.7 ^{KLM}	114	10 ^{FF}	200	14.6 ^{IKL}	40.4 ^A	36.3 ^{IKL}	20 ^{KL}				
		1000	2.1 ^{JKLMN}	42	5.3 ^{KLM}	106	10.4 ^{DEF}	208	13.4 ^{JKLM}	39.6 ^A	33.8 ^{IKL}	17.9 ^M				
		1500	2 ^{LMN}	40	5 ^{LM}	100	10.7 ^{DE}	214	12.7 ^{JKLM}	40 ^A	31.8 ^{LM}	16 ^N				
		30	<i>Sacch. cerevisiae</i> ATCC 7754	0	4.5 ^{BC}	90	10.7 ^{ABCD}	214	5 ^{MN}	100	28.7 ^{ABC}	42.1 ^A	68.2 ^{AB}	31.4 ^A		
				100	4.9 ^B	98	11.3 ^{AB}	226	4.4 ^{MN}	88	31.2 ^{AB}	43.4 ^A	71.9 ^A	29.2 ^{BC}		
				300	5.5 ^A	110	11.9 ^A	238	3.8 ^N	76	35 ^A	46.2 ^A	74 ^A	27.1 ^{DE}		
				500	4.1 ^{CDE}	82	10 ^{BDE}	200	5.7 ^{LM}	114	26.1 ^{CDE}	41 ^A	63.7 ^{BC}	25.6 ^{EF}		
				1000	3.8 ^{DEF}	76	9.2 ^{DEFG}	184	6.5 ^{KL}	130	24.2 ^{CDE}	41.3 ^A	58.6 ^{CDE}	23 ^{GHI}		
				1500	3.6 ^{DEFG}	72	8.8 ^{DEFG}	176	6.9 ^{IKL}	138	22.9 ^{DEFG}	40.9 ^A	56.1 ^{CDE}	20.5 ^{JK}		
				60	<i>Z. mobilis</i> ATCC 29191	0	2.6 ^{HJKL}	52	6.7 ^{DK}	134	11.8 ^{CD}	236	14.1 ^{JKLM}	38.8 ^A	36.2 ^{IKL}	25.7 ^{EF}
						100	3.2 ^{FGH}	64	7.8 ^{GHI}	156	10.7 ^{DE}	214	17.3 ^{IK}	41 ^A	42.2 ^{GHIJ}	22 ^{HU}
						300	2.5 ^{JKLM}	50	5.9 ^{KLM}	118	12.6 ^{ABC}	252	13.5 ^{JKLM}	42.4 ^A	31.9 ^{LM}	20.1 ^{KL}
500	2.3 ^{JKLMN}					46	6.1 ^{IKL}	122	12.4 ^C	248	12.4 ^{LM}	37.7 ^A	32.9 ^{KL}	18.6 ^{LM}		
1000	1.9 ^{MN}					38	5.2 ^{KLM}	104	13.3 ^{AB}	266	10.3 ^M	36.5 ^A	28.1 ^{LM}	15.9 ^N		
1500	1.7 ^N					34	4.5 ^M	90	14 ^A	280	9.2 ^M	37.8 ^A	27.3 ^M	14.1 ^N		
60	<i>Sacch. cerevisiae</i> ATCC 7754					0	4.1 ^{CDE}	82	9.6 ^{CDEF}	192	8.9 ^{EFHG}	178	22.2 ^{EFHG}	42.7 ^A	51.9 ^{DEF}	30.7 ^{AB}
						100	4.3 ^{BCD}	86	10.2 ^{BODE}	204	8.3 ^{HU}	166	23.2 ^{CDEF}	42.2 ^A	55.1 ^{CDEF}	28.2 ^{CD}
						300	4.8 ^B	96	11 ^{ABC}	214	7.5 ^{IK}	150	26 ^{BC}	43.6 ^A	59.5 ^{CD}	26 ^{EF}
		500	4 ^{CDE}			80	9.4 ^{DEFG}	188	9.1 ^{EFHG}	182	21.6 ^{EFHG}	42.6 ^A	50.8 ^{EFG}	23.3 ^{GH}		
		1000	3.8 ^{DEF}			76	9.3 ^{DEFG}	186	9.2 ^{EFHG}	184	20.5 ^{FGH}	40.9 ^A	50.3 ^{EFHG}	21.4 ^{JK}		
		1500	3.5 ^{DEFG}			70	8.6 ^{EFHG}	174	9.9 ^{FGH}	198	18.9 ^{HI}	40.7 ^A	46.5 ^{FGH}	18.8 ^{LM}		

* Gy (Gray): is a measurement unit of absorbed dose of gamma radiation, dose rate = 43.8 Gy min⁻¹.

** 0: microorganisms 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.

- Initial sugars concentration of sugarcane bagasse hydrolyzed by 2 % H₂SO₄ (v/v) at 100°C for 30 and 60 min were 15.7 g L⁻¹, 314 mg g⁻¹ and 18.5 g L⁻¹, 370 mg g⁻¹, respectively.

- 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.

Increasing hydrolysis temperature to 120°C with 2 % (v/v) H₂SO₄ for 30min and 60min increased the initial sugars concentration obtained from sugarcane bagasse to 20.2 gL⁻¹ and 23.7 gL⁻¹, respectively (Table 4, footnote). In the 30 min hydrolysis run, the highest bioethanol concentrations obtained from sugarcane bagasse by *Z. mobilis* ATCC 29191 was 3.9 gL⁻¹ when irradiated at 100 Gy, while that obtained by *Sacch. cerevisiae* ATCC 7754 was 7.3 gL⁻¹, when irradiated at 300 Gy. When hydrolysis was run at the same temperature for 60 min, bioethanol concentration obtained by *Sacch. cerevisiae* ATCC 7754 (irradiated at 300 Gy) significantly increased to 10.3 gL⁻¹, and the bioethanol yield and conversion coefficient and sugar utilization efficiency were 44.7 % (w/w), 46.8 % (w/w) and 92.8 % (w/w), respectively (Table 4). Increasing the irradiation dose over 300 Gy greatly decreased the final bioethanol concentration from *Sacch. cerevisiae* ATCC 7754. In the same hydrolysis run, *Z. mobilis* ATCC 29191 was only able to produce 4.4 gL⁻¹ of bioethanol, when irradiated at 100 Gy. The highest cells count was recorded in the non-irradiated culture of *Sacch. cerevisiae* ATCC 7754 (31 x 10⁴ CFU ml⁻¹), while it was 24.4 x 10⁴ CFU ml⁻¹ for the same treatment of *Z. mobilis* ATCC 29191. In all treatments, *Sacch. cerevisiae* ATCC 7754 gave higher cell counts than *Z. mobilis* ATCC 29191.

These results are in partial agreement with those reported by Aguilar *et al.* (2002) who found that the best acid hydrolysis treatment of sugarcane bagasse was 2 % (v/v) H₂SO₄ at 122°C for 24 min which hydrolyzed around 90 % of hemicellulose to xylose and glucose (21.6 gL⁻¹ and 3 gL⁻¹, respectively). They also detected low concentration of by-products (furfural and acetic acid) and low degradation of the cellulose fraction.

Hydrolysis of sugarcane bagasse with 6 % (v/v) H₂SO₄ at 100°C for 30 and 60 min increased the initial released sugars concentration to 27.2 gL⁻¹ and 28.6 gL⁻¹, respectively (Table 5, footnote). However, final bioethanol concentration decreased in almost all treatments inoculated by *Z. mobilis* ATCC 29191 and *Sacch. cerevisiae* ATCC 7754, which could be attributed to the formation of furfural and hydroxymethylfurfural (HMF), which are known as the most important inhibitors during fermentation of dilute-acid hydrolyzates.

The highest final bioethanol concentration from sugarcane bagasse (6.9 gL⁻¹) was obtained by *Sacch. cerevisiae* ATCC 7754 irradiated at 300 Gy from 60 min hydrolysis treatment (compared to 10.3 gL⁻¹, obtained by the same irradiation treatment of *Sacch. cerevisiae* ATCC 7754 but using bagasse hydrolyzed by 2 % (v/v) H₂SO₄ at 100°C for 60 min). In this treatment, the bioethanol yield, conversion coefficient and sugar utilization efficiency were 24.1 % (w/w), 44.8 % (w/w) and 53.8 % (w/w), respectively. On the other hand, the highest final bioethanol concentration obtained by *Z. mobilis* ATCC 29191 was 3.4 gL⁻¹, from 100 Gy treatment and utilizing sugarcane bagasse hydrolyzed for 60 min. In this treatment, the bioethanol yield, conversion coefficient and sugar utilization efficiency were 12.5 % (w/w), 45.3 % (w/w) and 26.8 % (w/w), respectively (Table 5).

TABLE 4. Effect of exposing *Z. mobilis* ATCC 29191 and *Sacch. cerevisiae* ATCC 7754 cells to different doses of gamma irradiation (Gy) on bioethanol production using sugarcane bagasse hydrolyzed by 2% (v/v) H₂SO₄ at 120°C for 30 and 60 min.

Retention time of hydrolysis (min)	Microorganism	Irradiation dose of microorganism (Cyt*)	Bioethanol concentration (g/L)		Consumed sugars (mg g ⁻¹)		Residual sugars (mg g ⁻¹)		Bioethanol yield (% w/w)	Conversion coefficient (% w/w)	Sugar utilization efficiency (% w/w)	Cells count (CFUx10 ⁶ ml ⁻¹)
			(g/L)	(mg g ⁻¹)	(g/L)	(mg g ⁻¹)	(g/L)	(mg g ⁻¹)				
30	<i>Z. mobilis</i> ATCC 29191	0**	2.8 ^H	56	6.6 ^I	132	13.6 ^F	272	13.9 ^J	42.4 ^{ABC}	32.7 ^I	24.4 ^{GH}
		100	3.9 ^G	78	8.9 ^J	178	11.3 ^{FG}	226	19.3 ^{GH}	43.8 ^{ABC}	44.1 ^{GH}	23.8 ^{HI}
		300	2.7 ^{HI}	54	6.4 ^{JK}	128	13.8 ^E	276	13.4 ^{IJ}	42.9 ^{ABC}	31.7 ^J	22.1 ^{IK}
		500	2.4 ^{HIJ}	48	5.9 ^{JKL}	118	14.3 ^{DE}	286	11.9 ^{JK}	40.7 ^{ABC}	29.2 ^{JK}	15.4 ^N
		1000	2.1 ^{HIK}	42	5.2 ^{KL}	104	15 ^{CD}	300	10.4 ^{KLM}	40.4 ^{ABC}	25.7 ^{KL}	13.9 ^O
		1500	1.8 ^{JK}	36	4.7 ^L	92	15.6 ^C	312	8.9 ^{LM}	39.1 ^{BC}	22.8 ^{KL}	12 ^P
		0	4.7 ^{EF}	94	11.1 ^{FG}	222	9.1 ^I	182	23.3 ^F	42.3 ^{ABC}	54.9 ^F	31 ^A
		100	6 ^D	120	14.2 ^E	284	6 ^I	120	29.7 ^D	42.3 ^{ABC}	70.3 ^D	29.7 ^{AB}
		300	7.3 ^C	146	16 ^C	320	4.2 ^L	84	36.1 ^B	45.6 ^{ABC}	79.2 ^B	28 ^{CD}
		500	6.5 ^D	130	14.8 ^D	296	5.4 ^{KL}	108	32.2 ^C	43.9 ^{ABC}	73.3 ^C	26.2 ^{EF}
		1000	4.5 ^{FG}	90	10.1 ^{GH}	202	10.1 ^{HI}	202	22.2 ^F	44.6 ^{ABC}	50 ^{EF}	24.6 ^{GH}
		1500	4.2 ^{FG}	84	9.7 ^{HI}	194	10.5 ^{HI}	210	20.8 ^{GH}	43.3 ^{ABC}	48 ^{FG}	21.8 ^K
60	<i>Z. mobilis</i> ATCC 29191	0	2.5 ^{HJ}	50	6.1 ^{JKL}	122	17.6 ^B	352	10.5 ^{KLM}	40.9 ^{ABC}	25.7 ^{KL}	22.5 ^{HIK}
		100	4.4 ^{FG}	88	9.8 ^{HI}	196	13.9 ^{DE}	278	18.6 ^H	44.9 ^{ABC}	41.4 ^H	20.2 ^L
		300	2.8 ^{HI}	56	6.3 ^{JK}	126	17.4 ^B	348	11.8 ^{KL}	44.4 ^{ABC}	26.6 ^{JK}	18.6 ^M
		500	2.4 ^{HIJ}	48	5.8 ^{JKL}	120	17.7 ^{AB}	354	10.1 ^{KLM}	40 ^{ABC}	25.3 ^{KL}	16.4 ^N
		1000	2.3 ^{HIJK}	46	5.6 ^{JKL}	116	17.9 ^{AB}	358	9.7 ^{KLM}	39.7 ^{BC}	24.5 ^{KL}	13.8 ^O
		1500	1.9 ^{JK}	38	4.9 ^L	98	18.8 ^A	376	8 ^M	38.8 ^C	20.7 ^L	12 ^P
		0	8.1 ^B	162	18.2 ^B	364	5.5 ^K	110	34.2 ^B	44.3 ^{ABC}	75.8 ^{BC}	30.6 ^A
		100	9.8 ^A	196	21.4 ^A	428	2.3 ^M	46	41.4 ^A	45.8 ^{ABC}	90.3 ^A	29 ^{FC}
		300	10.3 ^A	206	22 ^A	440	1.7 ^M	34	44.7 ^A	46.8 ^A	92.8 ^A	27.3 ^{DE}
		500	8.6 ^B	172	18.8 ^B	376	4.9 ^{KL}	98	36.3 ^B	45.7 ^{ABC}	79.3 ^B	29 ^{FG}
		1000	6.4 ^D	128	14.5 ^D	290	9.2 ^I	184	27 ^E	44.1 ^{ABC}	61.2 ^D	23.4 ^{HI}
		1500	5.1 ^E	102	11.8 ^F	236	11.9 ^J	238	21.5 ^{FG}	43.2 ^{ABC}	49.8 ^{EF}	21.2 ^{KL}

Gy (Gray): is a measurement unit of absorbed dose of gamma radiation, dose rate = 43.8 Gy min⁻¹.

** 0: microorganisms 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)]x100 ÷ consumed sugars (g/L) ÷ initial sugars (g/L) ÷ initial sugars (g/L) ÷ initial sugars (g/L) (Ramadan *et al.*, 1985).

- Cells count was determined after 4 days of fermentation period.

- Initial sugars concentration of sugarcane bagasse hydrolyzed by 2 % H₂SO₄ (v/v) at 120°C for 30 and 60 min were 20.2 g L⁻¹, 40.4 mg g⁻¹ and 23.7 g L⁻¹, 47.4 mg g⁻¹, respectively.

- 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.

TABLE 5. Effect of exposing *Z. mobilis* ATCC 29191 and *Sacch. cerevisiae* ATCC 7754 cells to different doses of gamma irradiation (Gy) on bioethanol production using sugarcane bagasse hydrolyzed by 6 % (v/v) H₂SO₄ at 100°C for 30 and 60 min.

Retention time of hydrolysis (min)	Microorganism	Irradiation dose of microorganisms (Gy ^a)	Bioethanol concentration (g L ⁻¹)		Consumed sugars (mg g ⁻¹)		Residual sugars (mg g ⁻¹)		Bioethanol yield (% w/w)	Conversion coefficient (% w/w)	Sugar utilization efficiency (% w/w)	Cells count (CFUx10 ⁴ ml ⁻¹)
			(g L ⁻¹)	(mg g ⁻¹)	(mg g ⁻¹)	(g L ⁻¹)	(mg g ⁻¹)					
30	<i>Z. mobilis</i> ATCC 29191	0**	2.6 ^{HIK}	52	6.7 ^L	134	20.5 ^{FG}	410	9.6 ^{JK}	38.8 ^{GH}	24.6 ^{HI}	18 ^{HI}
		100	3.4 ^{FG}	68	7.3 ^K	146	19.9 ^{GH}	398	12.5 ^{OH}	45.3 ^A	26.8 ^G	17 ^{HI}
		300	2.8 ^{GHI}	56	6.4 ^{LM}	128	20.8 ^{FG}	416	10.3 ^{HU}	43.8 ^{BCD}	23.5 ^{HU}	16.4 ^J
		500	2.7 ^{HU}	54	6.4 ^{LM}	128	20.8 ^{FG}	416	9.9 ^J	42.2 ^F	23.5 ^{HU}	14.5 ^{JKL}
		1000	2.3 ^{HIK}	46	5.7 ^{LM}	114	21.5 ^{DE}	430	8.4 ^{JKL}	40.4 ^{GH}	20.9 ^K	12.8 ^{LMN}
		1500	1.9 ^{KLM}	38	4.9 ^{NR}	98	22.3 ^{CD}	446	6.9 ^{KL}	38.8 ^{GH}	18 ^{LM}	11.2 ^{NO}
	<i>Sacch. cerevisiae</i> ATCC 7754	0	5.4 ^{CD}	108	12.5 ^{FG}	250	14.7 ^K	294	20 ^{CD}	43.2 ^{ODE}	46 ^{BCDE}	28.6 ^A
		100	5.6 ^{CD}	112	12.7 ^{DE}	254	14.5 ^K	290	20.6 ^C	44.1 ^{BC}	46.7 ^{BCD}	27.4 ^B
		300	6.3 ^{AB}	126	13.9 ^{BC}	278	13.3 ^{LM}	266	23.2 ^{AB}	45.3 ^A	51.1 ^{AB}	25.6 ^{BC}
		500	5.7 ^{BC}	114	13 ^{CD}	260	14.2 ^{KL}	284	20.9 ^{BC}	43.8 ^{BCD}	47.8 ^{BC}	23.8 ^{CD}
		1000	5.1 ^{DE}	102	11.5 ^{FG}	230	15.7 ^J	314	18.8 ^{DE}	44.3 ^B	42.3 ^{ODE}	21.5 ^{EF}
		1500	4.9 ^{DE}	98	11.1 ^{GH}	222	16.1 ^J	322	18 ^{EF}	44.1 ^{BC}	40.8 ^{DE}	20 ^{FG}
60	<i>Z. mobilis</i> ATCC 29191	0	2.5 ^{HIK}	50	6.5 ^{LM}	130	22.1 ^{CD}	442	8.7 ^{JKL}	38.5 ^{HI}	22.7 ^{JK}	17.6 ^{HI}
		100	3.1 ^{GH}	62	7.6 ^{IK}	152	21 ^{EF}	420	10.8 ^{HI}	40.8 ^{GH}	26.6 ^{HI}	16.4 ^J
		300	2.3 ^{JKL}	46	6.2 ^{LM}	124	22.4 ^{CD}	448	8 ^{JKL}	37.1 ^{HIK}	21.7 ^{JK}	15 ^{IK}
		500	2 ^{JKL}	40	5.8 ^{LM}	116	22.8 ^{BC}	456	6.9 ^{KL}	34.5 ^{JK}	20.3 ^K	13.5 ^{KLM}
		1000	1.7 ^{LM}	34	5.2 ^{LM}	104	23.4 ^B	468	5.9 ^{LM}	32.7 ^{JK}	18.2 ^{KL}	11.9 ^{MN}
		1500	1.3 ^{NR}	26	4.3 ^N	86	26.3 ^A	466	4.5 ^M	30.7 ^{JK}	15 ^M	9.7 ^O
	<i>Sacch. cerevisiae</i> ATCC 7754	0	4.9 ^{DE}	98	11.4 ^{GH}	228	17.2 ^I	344	17.1 ^{EF}	43 ^{DE}	40 ^{DEF}	26 ^B
		100	6.7 ^A	134	14.6 ^{AB}	292	14 ^{KLM}	280	23.4 ^A	45.9 ^A	51 ^{BC}	25.8 ^B
		300	6.9 ^A	138	15.4 ^A	308	13.2 ^M	264	24.1 ^A	44.6 ^B	53.8 ^A	24.6 ^{BCD}
		500	5.4 ^{CD}	108	12.6 ^{EF}	252	16 ^I	320	18.9 ^{DE}	42.9 ^F	44.1 ^{BODE}	22.7 ^{DE}
		1000	4.6 ^{DE}	92	11 ^{HU}	220	17.6 ^I	352	16.1 ^F	41.8 ^{FG}	38.5 ^{EF}	20.3 ^{FG}
		1500	3.9 ^E	78	9.5 ^{JK}	190	19.1 ^H	382	13.6 ^G	41.1 ^{GH}	33.2 ^{FG}	18.7 ^{GH}

Gy (Gray): is a measurement unit of absorbed dose of gamma radiation, dose rate = 43.8 Gy min⁻¹.

** 0: microorganisms 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)x100] ÷ 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.

- Initial sugars concentration of sugarcane bagasse hydrolyzed by 6 % H₂SO₄ (v/v) at 100°C for 30 and 60 min were 27.2 g L⁻¹, 544 mg g⁻¹ and 28.6 g L⁻¹, 272 mg g⁻¹, respectively.

- 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.

Results of increasing hydrolysis temperature to 120°C are illustrated in Table 6. Hydrolysis with 6 % (v/v) H₂SO₄ at 120°C for 30 and 60 min increased the initial released sugars concentration obtained from sugarcane bagasse to 30.8 gL⁻¹ and 32.1 gL⁻¹, respectively (Table 6, footnote). Final bioethanol concentration decreased in all treatments inoculated by irradiated *Z. mobilis* ATCC 29191 and *Sacch. cerevisiae* ATCC 7754 subjected to all irradiation doses. The highest final bioethanol concentration obtained by *Z. mobilis* ATCC 29191 irradiated at 100 Gy on sugarcane bagasse hydrolyzed for 30 min was 2.9 gL⁻¹. In this treatment, the bioethanol yield, conversion coefficient and sugar utilization efficiency were 9.4 % (w/w), 38.2 % (w/w) and 24.7 % (w/w), respectively.

The highest final bioethanol concentration was 6.8 gL⁻¹, which was obtained by *Sacch. cerevisiae* ATCC 7754 irradiated at 300 Gy on sugarcane bagasse hydrolyzed for 60 min and the bioethanol yield and conversion coefficient and sugar utilization efficiency were 21.2 % (w/w), 45 % (w/w) and 47 % (w/w), respectively. Increasing the irradiation dose over 300 Gy greatly decreased the final bioethanol concentration. The highest cells count was recorded in the non-irradiated culture of *Sacch. cerevisiae* (25.6 x 10⁴ CFU ml⁻¹), while it was 16.7 x 10⁴ CFU ml⁻¹ for the same treatment of *Z. mobilis* ATCC 29191. Similar to these findings, many investigators found that exposing strains of *Saccharomyces cerevisiae* to lower doses of gamma irradiation (100 - 1000 Gy) increased its growth and its ability of producing ethanol in stress conditions (Abo-Sereh *et al.*, 2006; Edgardo *et al.*, 2008 and Abdel-Fattah *et al.*, 2000).

Acid-hydrolyzed potato peels

Similar to what have been conducted on sugarcane bagasse, potato peels were used as substrate for bioethanol production after been hydrolyzed using the same set of treatments. Acid hydrolysis of potato peels was performed using 2 and 6 % (v/v) H₂SO₄ acid at 100°C and 120°C and for 30 and 60 min retention time. The neutralized acid-hydrolyzates of potato peels, amended with nutrients, was used as basal media to study the effect of gamma irradiation (doses of 0, 100, 300, 500, 1000 and 1500 Gy) on bioethanol production by either *Z. mobilis* ATCC 29191 or *Sacch. cerevisiae* ATCC 7754 incubated for 4 days at 30°C.

As can be seen in Table 7, acid hydrolysis treatment of potato peels with 2 % (v/v) H₂SO₄ at 100°C for 30 and 60 min increased initial sugars concentration from 6.7 gL⁻¹ (Table 2) to 10.7 gL⁻¹ and 12 gL⁻¹, respectively. Accordingly, the final bioethanol concentration significantly increased in all hydrolysis treatments of potato peels.

Irradiation of *Z. mobilis* ATCC 29191 slightly increased its productivity of bioethanol concentration only at 100 Gy, after which increasing the irradiation greatly decreased the final bioethanol concentration. *Sacch. cerevisiae* ATCC 7754 (irradiated at 300 Gy) achieved the highest bioethanol concentration (5 gL⁻¹) when used on potato peels hydrolyzed for 60 min of retention time. This treatment, recorded the highest bioethanol yield, 41.7 % (w/w), conversion coefficient, 46.3 % (w/w), and sugar utilization efficiency was 90 % (w/w). The highest cells count was recorded in the non-irradiated culture of *Sacch. cerevisiae* ATCC 7754 (29.3 x 10⁴ CFU ml⁻¹), while it was 20.7 x 10⁴ CFU ml⁻¹ for the same treatment of *Z. mobilis* ATCC 29191.

TABLE 6. Effect of exposing *Z. mobilis* ATCC 29191 and *Sacch. cerevisiae* ATCC 7754 cells to different doses of gamma irradiation (Gy) on bioethanol production using sugarcane bagasse hydrolyzed by 6 % (v/v) H₂SO₄ at 120°C for 30 and 60 min.

Retention time of hydrolysis (min)	Microorganism	Irradiation dose of microorganisms (Gy*)	Bioethanol concentration (g/L ⁻¹)		Consumed sugars (g/L ⁻¹)		Residual sugars (g/L ⁻¹)		Bioethanol yield (% w/w)	Conversion coefficient (% w/w)	Sugar utilization efficiency (% w/w)	Cells count (CFUx10 ⁶ /ml ⁻¹)
			(g/L ⁻¹)	(mg g ⁻¹)	(g/L ⁻¹)	(mg g ⁻¹)	(g/L ⁻¹)	(mg g ⁻¹)				
30	<i>Z. mobilis</i> ATCC 29191	0**	2.2 ^{HI}	44	7.3 ^{EFH}	146	23.5 ^{GHU}	470	7.1 ^{HEL}	30.1 ^{CD}	23.7 ^{FH}	16.7 ^{HI}
		100	2.9 ^{GE}	58	7.6 ^{FGH}	152	23.2 ^{FHI}	464	9.4 ^{FHI}	38.2 ^{BCD}	24.7 ^{FHI}	15.5 ^J
		300	2.6 ^{GH}	52	7 ^{GH}	140	23.8 ^{GHI}	476	8.4 ^{GHI}	37.1 ^{ABCD}	22.7 ^{GH}	13.9 ^K
		500	2.4 ^{GHI}	48	6.4 ^{HI}	128	24.4 ^F	488	7.8 ^{HIK}	37.5 ^{ABCD}	20.8 ^{HI}	12.3 ^L
		1000	2 ^{HIJ}	40	5.5 ^{IK}	110	25.3 ^F	506	6.5 ^{KLM}	36.4 ^{BCD}	17.9 ^{JK}	10.8 ^M
		1500	1.9 ^{JKL}	36	5.1 ^{IK}	102	25.7 ^F	514	5.6 ^{LMN}	35.3 ^{BCD}	16.6 ^{JK}	9.2 ^N
	<i>Sacch. cerevisiae</i> ATCC 7754	0	3.5 ^F	70	8.3 ^{BE}	166	22.5 ^J	450	11.4 ^E	42.2 ^{AB}	27 ^{EF}	25.6 ^A
		100	4.2 ^D	84	9.5 ^C	190	21.3 ^{IK}	426	13.6 ^D	44.2 ^{AB}	31.2 ^D	23.5 ^B
		300	5.3 ^C	106	12 ^B	240	18.8 ^L	376	17.2 ^C	44.2 ^{AB}	38.9 ^B	21.1 ^D
		500	3.6 ^E	72	8.2 ^{EF}	164	22.6 ^J	452	11.7 ^E	43.9 ^{AB}	26.6 ^{FG}	20 ^F
		1000	3.2 ^{EF}	66	7.7 ^{FG}	154	23.1 ^{HU}	462	10.7 ^{EF}	42.9 ^{AB}	26.6 ^{FG}	18.7 ^G
		1500	2.9 ^{FG}	58	7.2 ^{FHI}	144	23.6 ^{GHI}	472	9.4 ^{FHI}	40.3 ^{ABCD}	23.4 ^{GH}	17.4 ^H
60	<i>Z. mobilis</i> ATCC 29191	0	2.5 ^{GHI}	50	6 ^{IJ}	120	26.1 ^{EF}	522	7.6 ^{HIK}	41.7 ^{ABC}	18.7 ^{IJ}	13.6 ^K
		100	2.5 ^{GHI}	50	6 ^{IJ}	120	26.1 ^{EF}	522	7.6 ^{HIK}	41.7 ^{ABC}	18.7 ^{IJ}	13.6 ^K
		300	2.2 ^{HIJ}	44	4.1 ^{LM}	82	28.8 ^{BC}	536	6.9 ^{JKL}	41.5 ^{ABCD}	16.5 ^{JK}	12.1 ^L
		500	1.7 ^{JKL}	34	4.1 ^{LM}	82	28.8 ^{BC}	536	6.9 ^{JKL}	41.5 ^{ABCD}	16.5 ^{JK}	12.1 ^L
		1000	1.6 ^{JKL}	32	3.9 ^{LMN}	78	28.2 ^{AB}	564	5 ^{NO}	41.5 ^{ABCD}	12.1 ^{LMN}	10.7 ^M
		1500	1.3 ^L	26	3.2 ^H	64	28.9 ^A	578	4 ^O	40.6 ^{ABCD}	10 ^N	6.7 ^P
	<i>Sacch. cerevisiae</i> ATCC 7754	0	3.2 ^{EF}	64	9 ^{CD}	180	23.1 ^{HU}	462	10 ^{FG}	35.6 ^{BCD}	28 ^E	23.6 ^B
		100	6.2 ^B	124	14.3 ^A	286	17.8 ^{LM}	356	19.3 ^B	43.4 ^{AB}	44.5 ^A	22.2 ^C
		300	6.8 ^A	136	15.1 ^A	302	17 ^M	340	21.2 ^A	45 ^A	47 ^A	20.6 ^{DE}
		500	4.7 ^D	94	11.2 ^B	224	20.9 ^K	418	14.6 ^D	41.9 ^{ABC}	34.9 ^C	18.9 ^{FG}
		1000	2.9 ^{FG}	58	9.2 ^C	184	22.9 ^{JU}	458	9 ^{SH}	31.5 ^{CD}	28.7 ^{DE}	17 ^H
		1500	2.4 ^{GHI}	62	8 ^{FG}	160	24.1 ^{GH}	482	7.5 ^{JKL}	30 ^P	24.9 ^{FG}	15.7 ^{IJ}

* Gy: is a measurement unit of absorbed dose of gamma radiation, dose rate = 43.8 Gy min⁻¹.

** 0: microorganisms 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)x100] ÷ 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.

- Initial sugars concentration of sugarcane bagasse hydrolyzed by 6 % H₂SO₄ (v/v) at 120°C for 30 and 60 min were 30.8 g/L⁻¹, 616 mg g⁻¹ and 32.1 g/L⁻¹, 642 mg g⁻¹, respectively.

- 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.

TABLE 7. Effect of exposing *Z. mobilis* ATCC 29191 and *Sacch. cerevisiae* ATCC 7754 cells to different doses of gamma irradiation (Gy) on bioethanol production using potato peels hydrolyzed by 2% (v/v) H₂SO₄ at 100°C for 30 and 60 min.

Retention time of hydrolysis (min)	Microorganism	Irradiation dose of microorganisms (Gy*)	Bioethanol concentration (g L ⁻¹)		Consumed sugars (mg g ⁻¹)		Residual sugars (mg g ⁻¹)		Bioethanol yield (w/w%)	Conversion coefficient (w/w%)	Sugar utilization efficiency (w/w%)	Cells count (CFUx10 ⁶ ml ⁻¹)
			(g L ⁻¹)	(mg g ⁻¹)	(mg g ⁻¹)	(g L ⁻¹)	(mg g ⁻¹)					
30	<i>Z. mobilis</i> ATCC 29191	0**	2.6 ^{HIJK}	52	6.5 ^{JK}	130	4.2 ^{DEF}	84	24.3 ^{HIJ}	40 ^A	60.7 ^{FG}	20.7 ^E
		100	3.2 ^{CDEF}	64	7.5 ^{FG}	150	3.2 ^{GH}	64	22.9 ^{DEFG}	43.8 ^A	70.1 ^D	19.2 ^{EF}
		300	2.8 ^{HIJ}	56	6.6 ^{JK}	132	4.1 ^{DEF}	82	26.2 ^{FGHI}	42.4 ^A	61.7 ^{EF}	17.6 ^{GH}
		500	2.5 ^{HIJK}	50	6.1 ^{JK}	120	4.7 ^D	94	23.4 ^{HIJK}	41.7 ^A	56.1 ^{GH}	15.4 ^J
		1000	2.1 ^{JKLM}	42	5.1 ^{KL}	102	5.6 ^{BC}	112	19.6 ^{JKLM}	41.2 ^A	47.7 ^J	13.5 ^{KL}
		1500	1.8 ^L	36	4.7 ^{LM}	94	6 ^B	120	16.8 ^{LM}	38.3 ^A	43.9 ^K	12 ^L
	<i>Sacch. cerevisiae</i> ATCC 7754	0	3.1 ^{DEFG}	62	7.1 ^{GH}	142	3.6 ^{FG}	72	29.9 ^{DEFGH}	43.7 ^A	66.4 ^D	29.3 ^A
		100	4 ^{BC}	80	8.8 ^{DE}	176	1.9 ^{JK}	38	37.4 ^{BCD}	45.4 ^A	82.9 ^{BC}	28.5 ^A
		300	4.3 ^{BC}	86	9.6 ^{BC}	192	1.1 ^L	22	40.2 ^{AB}	44.8 ^A	89.7 ^A	26.8 ^B
		500	3.5 ^{BCDE}	70	7.9 ^{EF}	158	2.8 ^{HI}	56	32.7 ^{CD}	44.3 ^A	73.6 ^C	24 ^{CD}
		1000	2.9 ^{FGH}	58	6.8 ^{HI}	136	3.9 ^{FG}	78	27.1 ^{EFHI}	42.6 ^A	63.6 ^{DEF}	22.5 ^E
		1500	2.8 ^{GHI}	56	6.7 ^J	134	4 ^{EF}	80	26.2 ^{FGHI}	41.8 ^A	62.6 ^{EF}	22.6 ^D
60	<i>Z. mobilis</i> ATCC 29191	0	2.9 ^{FGH}	58	6.7 ^J	134	5.3 ^C	106	27.5 ^{DEFGH}	43.3 ^A	55.6 ^{GH}	18.5 ^{FGH}
		100	3.5 ^{CDE}	70	7.9 ^{EF}	158	4.1 ^{EF}	82	29.9 ^{DEFG}	44.3 ^A	65.8 ^{DE}	17.3 ^{HI}
		300	3 ^{DEFG}	60	7.5 ^{FG}	150	4.5 ^{DE}	80	25.9 ^{HIJ}	40 ^A	62.5 ^{EF}	16 ^I
		500	2.6 ^{HIJK}	52	6.5 ^{JK}	124	5.8 ^{BC}	116	21.7 ^{JKLM}	40 ^A	54.2 ^{HI}	14.6 ^{JK}
		1000	2.2 ^{JKLM}	44	5.4 ^L	108	6.6 ^A	132	18.3 ^{JKLM}	40.7 ^A	49 ^{JK}	12.8 ^{KL}
		1500	2 ^{KL}	40	5 ^{LM}	100	7 ^A	140	16.7 ^{LM}	40 ^A	41.7 ^{JK}	11.2 ^M
	<i>Sacch. cerevisiae</i> ATCC 7754	0	4 ^{BC}	80	9.9 ^{CD}	184	2.8 ^{HI}	56	33.3 ^{CD}	43.5 ^A	76.7 ^C	26.8 ^B
		100	4.5 ^{AB}	9	10.1 ^{AB}	202	1.9 ^{JK}	38	37.5 ^{ABC}	44.6 ^A	84.2 ^{AB}	25.1 ^{BC}
		300	5 ^A	100	10.8 ^A	216	1.2 ^{KL}	24	41.7 ^A	46.3 ^A	90 ^A	24 ^{CD}
		500	4.1 ^{BC}	82	10.1 ^{AB}	202	1.9 ^{JK}	38	34.2 ^{BCD}	40.6 ^A	84.2 ^{AB}	22.7 ^D
		1000	3.9 ^{BC}	78	9.7 ^{BC}	194	2.3 ^{JK}	46	32.5 ^{CDE}	40.2 ^A	80.8 ^{BC}	20.6 ^{EF}
		1500	3.8 ^{CD}	72	9.5 ^{CD}	186	2.7 ^{JK}	54	30 ^{DEF}	38.7 ^A	77.5 ^{BC}	18.8 ^{FG}

* Gy (Gray): is a measurement unit of absorbed dose of gamma radiation, dose rate = 43.8 Gy min⁻¹.

** 0: microorganisms 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.

-Initial sugars concentration of potato peels hydrolyzed by 2 % H₂SO₄ (v/v) at 100°C for 30 and 60 min were 10.7 g L⁻¹, 214 mg g⁻¹ and 12 g L⁻¹, 240 mg g⁻¹, respectively.

-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.

These results are in line with those obtained by Tasić *et al.* (2009) who reported that acid hydrolysis of potato tuber mash by 1 M HCl at 1:1 (w/v) ratio, at (100°C) for 60 min, gave the highest dextrose equivalent (94 %) and the best bioethanol yield (31 gL⁻¹) in batch fermentation for 18 h by *Sacch. cerevisiae* with inoculum rate of 3 % (w/v).

Results presented in Table 8 showed the effect of increasing hydrolysis temperature to 120°C using the same acid concentration, *i.e.* 2 % H₂SO₄ (v/v). Results showed hydrolysis of potato peels at 120°C for 30 and 60 min increased the initial sugars concentration from potato peels to 14.6 gL⁻¹ and 18.1 gL⁻¹, respectively, and logically, the final bioethanol concentration significantly increased in all hydrolysis treatments. The highest bioethanol concentration obtained by *Z. mobilis* ATCC 29191 was 3.8 gL⁻¹, which was obtained from 100 Gy treatment on potato peels hydrolyzed for 30 min and 4.3 gL⁻¹, obtained from 300 Gy treatment used on potato peels hydrolyzed for 60 min. On the other hand, *Sacch. cerevisiae* ATCC 7754 had better results, that is when irradiated at 300 Gy, it produced bioethanol concentration of 6 gL⁻¹, from potato peels hydrolyzed for 30 min and 6.5 gL⁻¹, from potato peels hydrolyzed for 60 min. In the last treatment, bioethanol yield, conversion coefficient and sugar utilization efficiency were 35.9 % (w/w), 44.8 % (w/w) and 80.1 % w/w, respectively. Bioethanol concentration decreased in the culture of *Z. mobilis* ATCC 29191 (irradiated more than 100 and 300 Gy) on potato peels hydrolyzed for 30 and 60 min.

As in previous experiment, irradiation had negative effect on cell counts of both organisms that was recorded in the non-irradiated culture of *Sacch. cerevisiae* ATCC 7754 (28.9 x 10⁴ CFU ml⁻¹), while it was 18.3 x 10⁴ CFU ml⁻¹ for the same treatment of *Z. mobilis* ATCC 29191. In this respect, Mehdikhani *et al.* (2011) found that *Sacch. cerevisiae* cells exposed to 100 Gy of γ -irradiation produced a high yield of bioethanol (23.50 % w/v) at 42°C compared with the non-irradiated strain.

Results of hydrolyzing potato peels with 6 % (v/v) H₂SO₄ at 100°C, presented in Table 9, showed that running hydrolysis for 30 and 60 min increased the initial released sugars concentration obtained to 21.3 g L⁻¹ and 24 g L⁻¹, respectively. Final bioethanol concentration increased in all treatments inoculated with irradiated *Z. mobilis* ATCC 29191 and *Sacch. cerevisiae* ATCC 7754. The highest final bioethanol concentration obtained by *Z. mobilis* ATCC 29191 (5.7 gL⁻¹) was in the treatment irradiated at 100 Gy on potato peels hydrolyzed for 60 min. In this treatment, the bioethanol yield and conversion coefficient were 23.8 % (w/w) and 45.2 % (w/w), respectively. Exposing *Z. mobilis* ATCC 29191 to irradiation above 100 Gy significantly decreased its productivity of bioethanol, whether hydrolysis was run for 30 min or 60 min. The highest final bioethanol concentration obtained by *Sacch. cerevisiae* ATCC 7754 (7.5 gL⁻¹) was in the treatment irradiated at 300 Gy on potato peels hydrolyzed for 60 min. In this treatment, the bioethanol yield and conversion coefficient were 31.3 % (w/w) and 45.7 % (w/w), respectively. Increasing the irradiation dose to *Sacch. cerevisiae* ATCC 7754 over 300 Gy decreased greatly the final bioethanol concentration. The highest cells count was recorded in the non-irradiated culture of *Sacch. cerevisiae* ATCC 7754 (26.7 x 10⁴ CFU ml⁻¹), while it was 16.9 x 10⁴ CFU ml⁻¹ for the same treatment of *Z. mobilis*.

TABLE 8. Effect of exposing *Z. mobilis* ATCC 29191 and *Sacch. cerevisiae* ATCC 7754 cells to different doses of gamma irradiation (Gy) on bioethanol production using potato peels hydrolyzed by 2% (v/v) H₂SO₄ at 120°C for 30 and 60 min.

Retention time of hydrolysis (min)	Microorganism	Irradiation dose of microorganisms (Gy ^{**})	Bioethanol concentration (g L ⁻¹)		Consumed sugars (mg g ⁻¹)		Residual sugars (mg g ⁻¹)		Bioethanol yield (% w/w)	Conversion coefficient (% w/w)	Sugar utilization efficiency (% w/w)	Cells count (CFUx10 ⁶ ml ⁻¹)
			30	60	30	60	30	60				
30	<i>Z. mobilis</i> ATCC 29191	0**	3.8 ^{JK}	60	7.1 ^{LM}	142	7.6 ^E	152	20.5 ^{HU}	42.3 ^{AB}	48.8 ^{HI}	18.3 ^{HI}
		100	3.8 ^{HI}	76	8.7 ^{HIK}	174	5.9 ^{HI}	118	2.6 ^{FG}	43.7 ^{AB}	59.2 ^{FG}	17.7 ^J
		300	3.4 ^J	68	8.2 ^{JKL}	164	6.4 ^E	128	23.3 ^{SHI}	41.5 ^{AB}	56.2 ^{FG}	16.1 ^K
		500	2.9 ^{HL}	58	7.1 ^M	140	7.7 ^E	154	19.9 ^J	41.4 ^{AB}	47.9 ^{HI}	14.4 ^M
		1000	2.4 ^{LMN}	48	5.9 ^I	118	8.7 ^D	174	16.4 ^{KL}	40.7 ^{AB}	40.4 ^J	12.5 ^N
		1500	2.8 ^{JK}	40	5.1 ^O	102	9.5 ^F	190	13.7 ^{LM}	39.1 ^B	34.9 ^{JK}	10.9 ^O
	<i>Sacch.</i> <i>cerevisiae</i> ATCC 7754	0	4.6 ^{FG}	92	10.6 ^{DEF}	212	4.1 ^K	82	31.5 ^{DE}	43.4 ^{AB}	72.6 ^D	28.9 ^A
		100	5.8 ^{BC}	112	12.6 ^{BCD}	252	2.1 ^N	42	38.4 ^{AB}	44.4 ^{AB}	86.3 ^{AB}	27.5 ^{AB}
		300	6.8 ^{AB}	120	13.8 ^C	260	1.6 ^O	32	41.1 ^A	46.2 ^A	88 ^A	25.0 ^B
		500	5.3 ^{CD}	106	11.7 ^{BCD}	234	2.9 ^M	58	36.3 ^{BC}	45.3 ^A	80.1 ^B	23.5 ^E
		1000	4.4 ^{FGH}	84	10.9 ^{HI}	200	4.7 ^I	94	30.1 ^{EF}	44.4 ^{AB}	68.4 ^{DE}	20.8 ^{FG}
		1500	3.8 ^{HI}	76	8.6 ^{JK}	172	6 ^{HI}	120	26.7 ^F	43.6 ^{AB}	58.9 ^{EF}	17.3 ^{JK}
60	<i>Z. mobilis</i> ATCC 29191	0	4.8 ^{HI}	80	9.4 ^{GHU}	188	8.7 ^D	174	22.1 ^{HU}	42.6 ^{AB}	51.6 ^{GH}	15.6 ^{KL}
		100	4.3 ^{GH}	86	10.1 ^{GH}	202	8 ^E	160	23.8 ^{GH}	42.9 ^{AB}	55.8 ^{FG}	14.4 ^{LM}
		300	2.7 ^{KL}	54	6.3 ^{MN}	126	11.8 ^B	236	14.9 ^{LM}	42.9 ^{AB}	34.8 ^K	12.7 ^N
		500	2.8 ^{KL}	52	6 ^{MN}	120	12.1 ^A	242	14.4 ^{LM}	43.3 ^{AB}	33.2 ^K	10.6 ^O
		1000	2.9 ^{LMN}	44	5.2 ^{NO}	104	12.9 ^A	258	12.2 ^M	42.3 ^{AB}	28.7 ^K	8.6 ^P
		1500	5.1 ^{DE}	102	12.4 ^{BCD}	248	5.7 ^{HI}	114	28.2 ^F	41.1 ^{AB}	68.4 ^{DE}	25.8 ^{BC}
	<i>Sacch.</i> <i>cerevisiae</i> ATCC 7754	0	5.9 ^B	118	13.3 ^B	266	4.8 ^I	96	32.6 ^{DE}	44.4 ^{AB}	73.3 ^{BC}	24 ^{DE}
		100	6.5 ^A	130	14.5 ^A	290	3.6 ^L	72	35.9 ^{CD}	44.3 ^{AB}	80.1 ^B	22.9 ^E
		300	5.8 ^{BC}	112	12.8 ^{BC}	256	5.3 ^I	106	30.9 ^{DE}	43.8 ^{AB}	70.7 ^D	21.3 ^F
		500	4.7 ^{FG}	94	11.2 ^{CDE}	224	6.9 ^F	138	26.7 ^F	41.9 ^{AB}	61.9 ^{DE}	19.5 ^{GH}
		1000	4.9 ^{GH}	84	10.9 ^{FG}	204	7.9 ^F	158	23.9 ^{GH}	41.2 ^{AB}	56.4 ^{FG}	16.7 ^{JK}
		1500	4.9 ^{GH}	84	10.9 ^{FG}	204	7.9 ^F	158	23.9 ^{GH}	41.2 ^{AB}	56.4 ^{FG}	16.7 ^{JK}

* Gy (Gray): is a measurement unit of absorbed dose of gamma radiation, dose rate = 43.8 Gy min⁻¹.

** 0: microorganisms 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.

-Initial sugars concentration of potato peels hydrolyzed by 2% H₂SO₄ (v/v) at 120°C for 30 and 60 min were 14.6 g L⁻¹, 292 mg g⁻¹ and 18.1 g L⁻¹, 362 mg g⁻¹, respectively.

-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.

TABLE 9. Effect of exposing *Z. mobilis* ATCC 29191 and *Sacch. cerevisiae* ATCC 7754 cells to different doses of gamma irradiation (Gy) on bioethanol production using potato peels hydrolyzed by 6% (v/v) H₂SO₄ at 100°C for 30 and 60 min.

Retention time of hydrolysis (min)	Microorganism	Irradiation dose of microorganisms (Gy*)	Bioethanol concentration (g/L)		Consumed sugars (mg g ⁻¹)		Residual sugars (mg g ⁻¹)		Bioethanol yield (% w/w)	Conversion coefficient (% w/w)	Sugar utilization efficiency (% w/w)	Cells count (CFUx10 ⁴ ml ⁻¹)	
			30 min	60 min	30 min	60 min	30 min	60 min					
30	<i>Z. mobilis</i> ATCC 29191	0**	3.8 ^{HIK}	6.6 ^{HIK}	160	13.3 ^{DEFGH}	266	15.5 ^{FGHI}	41.3 ^A	41.3 ^A	37.6 ^{GHIKL}	16.9 ^B	
		100	4.8 ^{DEFGH}	9.6 ^{DEFGH}	214	10.6 ^{HIK}	212	22.5 ^{BCDE}	44.9 ^A	42.7 ^A	50.2 ^{DEF}	15 ^B	
		300	4.1 ^{FGHIK}	8.2 ^{FGHIK}	192	11.7 ^{GHI}	234	14.4 ^{CHGHI}	280	14.8 ^{FGHI}	42.5 ^A	34.3 ^{HIK}	10.6 ^B
		500	3.1 ^{IKL}	6.2 ^{IKL}	146	14.4 ^{CHGHI}	298	12.7 ^{HIJ}	42.2 ^A	42.2 ^A	30.9 ^{IKL}	8.2 ^B	
		1000	2.7 ^{IKL}	5.4 ^{IKL}	128	14.9 ^{BCDEF}	298	10.8 ^J	41.6 ^A	41.6 ^A	25.8 ^L	7 ^B	
		1500	4.5 ^{DEFGH}	9.0 ^{DEFGH}	220	10.3 ^{IKL}	206	21.1 ^{CDE}	40.9 ^A	40.9 ^A	51.6 ^{DEF}	26.7 ^A	
	<i>Sacch. cerevisiae</i> ATCC 7754	0	6.1 ^{ABCD}	12.2 ^{AB}	282	7.9 ^{JKLMN}	144	28.6 ^{ABC}	43.3 ^A	43.3 ^A	66.2 ^{AB}	25.3 ^B	
		100	6.8 ^{AB}	13.6 ^{AB}	300	6.3 ^{LMN}	126	31.9 ^A	45.3 ^A	45.3 ^A	70.4 ^A	23.8 ^B	
		300	5.1 ^{ABCEDEF}	10.2 ^{BCDEF}	232	9.7 ^{IKL}	194	23.9 ^{BCD}	44 ^A	44 ^A	54.6 ^{BCDE}	21.7 ^B	
		500	4.3 ^{FGHIK}	8.6 ^{FGHIK}	208	10.9 ^{GHIK}	218	20.2 ^{DEF}	41.3 ^A	41.3 ^A	48.8 ^{DEFG}	20.1 ^B	
		1000	3.8 ^{GHIK}	7.6 ^{GHIK}	184	12.1 ^{FGHI}	242	17.8 ^{DEFGH}	41.3 ^A	41.3 ^A	43.2 ^{FGHIK}	19 ^B	
		1500	3.1 ^{IKL}	6.2 ^{IKL}	148	16.6 ^{ABCD}	332	12.9 ^{HIJ}	41.3 ^A	41.3 ^A	30.8 ^{IKL}	15.6 ^B	
60	<i>Z. mobilis</i> ATCC 29191	0	5.7 ^{ABCDE}	11.4 ^{ABCDE}	252	11.4 ^{GHI}	228	23.8 ^{BCDE}	45.2 ^A	45.2 ^A	52.5 ^{CDEF}	13.8 ^B	
		100	5.8 ^{BCDEF}	11.6 ^{BCDEF}	230	12.5 ^{FGHI}	250	20.8 ^{CDE}	43.5 ^A	43.5 ^A	47.9 ^{DEFGH}	12.1 ^B	
		300	3 ^{IKL}	6.0 ^{IKL}	142	16.9 ^{ABC}	338	12.5 ^I	42.3 ^A	42.3 ^A	30.9 ^{IKL}	10.3 ^B	
		500	2.8 ^{IKL}	5.6 ^{IKL}	136	17.2 ^{AB}	344	11.7 ^J	41.2 ^A	41.2 ^A	28.3 ^{IKL}	8.7 ^B	
		1000	2.5 ^{IKL}	5.0 ^{IKL}	124	17.8 ^A	356	10.4 ^J	40.3 ^A	40.3 ^A	25.8 ^L	7 ^B	
		1500	4.8 ^{DEFGH}	9.6 ^{DEFGH}	224	12.8 ^{FGHI}	256	20.8 ^{CDE}	44.6 ^A	44.6 ^A	46.7 ^{DEFGH}	24.7 ^B	
	<i>Sacch. cerevisiae</i> ATCC 7754	0	6.2 ^{ABC}	12.4 ^{ABC}	274	10.3 ^{HIK}	206	25.8 ^{BCD}	45.3 ^A	45.3 ^A	57.1 ^{ABC}	23.8 ^B	
		100	7.5 ^A	15.0 ^A	328	7.8 ^{JKLM}	152	31.3 ^{AB}	45.7 ^A	45.7 ^A	68.3 ^A	22.2 ^B	
		300	5.8 ^{ABCD}	11.6 ^{ABCD}	270	10.5 ^{HIK}	210	24.2 ^{BCD}	43 ^A	43 ^A	56.3 ^{BCD}	21 ^B	
		500	4.6 ^{DEFGH}	9.2 ^{DEFGH}	212	13.2 ^{DEFGH}	264	19.2 ^{DEFG}	43.4 ^A	43.4 ^A	44.2 ^{FGHI}	19.5 ^B	
		1000	4.1 ^{FGHIK}	8.2 ^{FGHIK}	196	14.2 ^{DEFGH}	284	17.1 ^{DEFGH}	41.8 ^A	41.8 ^A	40.8 ^{GHIK}	17.8 ^B	
		1500	4.1 ^{FGHIK}	8.2 ^{FGHIK}	196	14.2 ^{DEFGH}	284	17.1 ^{DEFGH}	41.8 ^A	41.8 ^A	40.8 ^{GHIK}	17.8 ^B	

Gy (Gray): is a measurement unit of absorbed dose of gamma radiation, dose rate = 43.8 Gy min⁻¹.

** 0: microorganisms 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) ÷ consumed sugars (g/L)] x 100. Bioethanol yield (% w/w) = [Bioethanol concentration (g/L) ÷ initial sugars (g/L)] x 100 (Gamal *et al.*, 1991) and sugar utilization efficiency (% w/w) = consumed sugars (g/L) ÷ initial sugars (g/L) (Ramadan *et al.*, 1985).

-Cells count was determined after 4 days of fermentation period.

-Initial sugars concentration of potato peels hydrolyzed by 6% H₂SO₄ (v/v) at 100°C for 30 and 60 min were 21.3 g/L⁻¹, 426 mg g⁻¹ and 24 g/L⁻¹, 480 mg g⁻¹, respectively.

-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.

Data presented in Table 10 show the effect of increasing hydrolysis temperature to 120°C. Hydrolysis using 6 % (v/v) H₂SO₄ at 120°C for 30 and 60 min increased the initial released sugars concentration obtained from sugarcane bagasse to 25.7 gL⁻¹ and 28.6 gL⁻¹, respectively (compared with the hydrolysis with 6 % (v/v) H₂SO₄ at 100°C). However, final bioethanol concentration decreased in all treatments inoculated by *Z. mobilis* ATCC 29191 and *Sacch. cerevisiae* ATCC 7754 subjected to all irradiation doses.

The highest final bioethanol concentration obtained by *Z. mobilis* ATCC 29191 was 3.4 gL⁻¹ from irradiation culture at 100 Gy on potato peels hydrolyzed for 30 min. In this treatment, the bioethanol yield, conversion coefficient and sugar utilization efficiency were 13.2 % (w/w), 41.5 % (w/w) and 31.9 % (w/w), respectively. Comparatively, the highest final bioethanol concentration obtained by *Sacch. cerevisiae* ATCC 7754 was 5.6 gL⁻¹ from irradiated culture at 500 Gy on potato peels hydrolyzed for 60 min. In this treatment, the bioethanol yield, conversion coefficient and sugar utilization efficiency were 19.6 % (w/w), 46.3 % (w/w) and 42.3 % (w/w), respectively. Increasing the irradiation dose over 100 for *Z. mobilis* ATCC 29191 and over 300 Gy for *Sacch. cerevisiae* significantly decreased the final bioethanol concentration. The highest cell counts for *Sacch. cerevisiae* ATCC 7754 was in non-irradiated culture which was 23 x 10⁴ CFU ml⁻¹, while it was 13.6 x 10⁴ CFU ml⁻¹ for the non-irradiated treatment of *Z. mobilis* ATCC 29191.

From the aforementioned results, several points could be noticed. Treatment of gamma irradiation to *Sacch. cerevisiae* ATCC 7754 or *Z. mobilis* ATCC 29191, prior to inoculation of the neutralized acid hydrolyzates of either sugarcane bagasse or potato peels (Tables 3-10), significantly increased the final bioethanol concentration compared with the treatment of non-irradiated organisms and non-hydrolyzed feedstock (Table 2). Apparently, exposing both *Z. mobilis* ATCC 29191 and *Sacch. cerevisiae* ATCC 7754 to gamma irradiation helped these microorganisms to tolerate the toxic residues formed in the feedstock acid hydrolyzates, which was reflected on increasing the final bioethanol concentration.

The most favorable treatment of sugarcane bagasse was using the irradiated *Sacch. cerevisiae* at 300 Gy on the neutralized acid hydrolyzates using 2 % (v/v) H₂SO₄ at 120°C for 60 min (Table 4). This treatment achieved a maximum final bioethanol concentration of 10.3 gL⁻¹ (equivalent to 206 mg g⁻¹) which represents 2.5 fold of final bioethanol concentration obtained by non-irradiated strain from non-hydrolyzed sugarcane bagasse. While the best treatment in case of potato peels was using the irradiated *Sacch. cerevisiae* ATCC 7754 at 300 Gy on the neutralized acid hydrolyzates using 6 % (v/v) H₂SO₄ at 100°C for 60 min (Table 9), which achieved a maximum final bioethanol concentration of 7.5 gL⁻¹ (150 mg g⁻¹), representing 3.4 fold of the final bioethanol concentration obtained by non-irradiated strain from non-hydrolyzed potato peels.

TABLE 10. Effect of exposing *Z. mobilis* ATCC 29191 and *Sacch. cerevisiae* ATCC 7754 cells to different doses of gamma irradiation (Gy) on bioethanol production using potato peels hydrolyzed by 6% (v/v) H₂SO₄ at 120°C for 30 and 60 min.

Retention time of hydrolysis (min)	Microorganism	Irradiation dose of microorganisms (Gy*)	Bioethanol concentration (mg g ⁻¹)		Consumed sugars (mg g ⁻¹)	Residual sugars (mg g ⁻¹)		Bioethanol yield (% w/w)	Conversion coefficient (% w/w)	Sugar utilization efficiency (% w/w)	Cells count (CFUx10 ⁴ ml ⁻¹)	
			(g L ⁻¹)	(mg g ⁻¹)		(g L ⁻¹)	(mg g ⁻¹)					
30	<i>Z. mobilis</i> ATCC 29191	0**	2.7 ^H	54	6.6 ^{FG}	132	19.1 ^I	382	10.5 ^H	41 ^A	25.7 ^E	13.6 ^B
		100	3.4 ^{EF}	68	8.2 ^{DE}	164	17.5 ^J	350	13.2 ^{EF}	41.5 ^A	31.9 ^{CD}	12.3 ^B
		300	2.8 ^{GH}	56	6.7 ^F	134	19.1 ^I	382	10.5 ^H	41.8 ^A	26.1 ^E	11 ^B
		500	2.1 ^{IJ}	42	5.1 ^{IK}	100	20.7 ^H	414	8.1 ^{JK}	42 ^A	19.5 ^{GH}	9.7 ^B
		1000	1.7 ^{JK}	34	4.2 ^{KL}	84	21.5 ^G	430	6.6 ^{KL}	40.5 ^A	16.3 ^J	7.8 ^B
		1500	1.6 ^K	32	4 ^L	80	21.7 ^{FG}	434	6.2 ^{LM}	40 ^A	15.6 ^I	6.1 ^B
	<i>Sacch. cerevisiae</i> ATCC 7754	0	4.1 ^{CD}	82	9.4 ^C	188	16.3 ^K	326	16 ^{AB}	43.6 ^A	36.6 ^B	23 ^A
		100	4.5 ^{BC}	90	10.3 ^B	206	15.4 ^L	308	17.5 ^{BC}	43.7 ^A	40.1 ^A	21.7 ^B
		300	4.7 ^B	94	10.4 ^B	208	15.3 ^L	306	18.3 ^{AB}	45.2 ^A	40.5 ^A	20.4 ^B
		500	3.6 ^{EF}	72	8.3 ^{DE}	166	17.4 ^I	348	14 ^{EF}	43.4 ^A	32.3 ^{CD}	18.6 ^B
		1000	3.2 ^{FG}	64	7.6 ^E	152	18.1 ^J	362	12.5 ^{FG}	42.1 ^A	29.6 ^D	16.8 ^B
		1500	2.8 ^{GH}	56	6.7 ^F	134	19 ^I	380	10.9 ^{GH}	41.8 ^A	26.1 ^E	14.5 ^B
60	<i>Z. mobilis</i> ATCC 29191	0	2.2 ^{IJ}	44	5.6 ^{IJ}	112	23 ^{BCD}	460	7.6 ^{KL}	39.3 ^A	21.8 ^{FHIJ}	13.1 ^B
		100	2.8 ^{GH}	56	6.4 ^{FG}	128	22.2 ^{BCD}	444	9.8 ^{HI}	43.8 ^A	22.4 ^{FG}	11.8 ^B
		300	2.4 ^{HI}	48	5.8 ^{GH}	116	22.8 ^{CDE}	456	8.4 ^{JK}	41.4 ^A	20.3 ^{FHI}	10.1 ^B
		500	2.1 ^{IJ}	42	5.2 ^{IJ}	104	23.4 ^{BC}	468	7.3 ^{KL}	40.3 ^A	18.2 ^{FHIJ}	9.6 ^B
		1000	1.9 ^{JK}	38	4.7 ^{KL}	94	23.9 ^{AB}	478	6.6 ^{KL}	40.4 ^A	16.4 ^{IJ}	8 ^B
		1500	1.6 ^K	32	4.3 ^{KL}	86	24.3 ^A	486	5.6 ^M	37.2 ^A	15 ^J	6.4 ^B
	<i>Sacch. cerevisiae</i> ATCC 7754	0	3.5 ^{EF}	70	8.5 ^D	170	20.2 ^H	402	12.2 ^{FG}	41.2 ^A	29.7 ^D	20.4 ^B
		100	3.8 ^{DE}	76	8.6 ^D	172	20 ^H	400	13.5 ^{EF}	44.2 ^A	30.1 ^D	19.6 ^B
		300	4.2 ^{CD}	84	9.5 ^C	190	19.1 ^I	384	14.7 ^{DE}	44.2 ^A	33.2 ^C	17.8 ^B
		500	5.6 ^A	112	12.1 ^A	242	16.5 ^K	330	19.6 ^A	46.3 ^A	42.3 ^A	16.1 ^B
		1000	2.7 ^H	54	6.7 ^F	134	21.9 ^{FG}	438	9.4 ^{HIJ}	40.3 ^A	23.4 ^{EF}	14.5 ^B
		1500	2.4 ^{HI}	48	6.1 ^{GH}	122	22.5 ^{DEF}	450	8.4 ^{JK}	39.3 ^A	21.3 ^{FHI}	12.8 ^B

* Gy (Gray): is a measurement unit of absorbed dose of gamma radiation, dose rate = 43.8 Gy min⁻¹.

** 0: microorganisms 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.

-Initial sugars concentration of potato peels hydrolyzed by 6 % H₂SO₄ (v/v) at 120°C for 30 and 60 min were 25.7 g L⁻¹, 51.4 mg g⁻¹ and 28.6 g L⁻¹, 57.2 mg g⁻¹, respectively.

-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.

Conclusions

As mentioned earlier, dilute acid hydrolysis led to increase the total sugars (initial sugars) from both sugarcane bagasse and potato peels compared with non-hydrolyzed feedstock. The highest concentrations of total sugars were 32.1 gL⁻¹ (equivalent to 642 mg g⁻¹) from sugarcane bagasse and 28.6 g L⁻¹ (equivalent to 572 mg g⁻¹) from potato peels, both obtained from hydrolysis by 6 % (v/v) H₂SO₄ at 120°C for 60 min. It is apparent from previous irradiation results to microorganisms, that they were sensitive to high levels of irradiation in general. *Z. mobilis* ATCC 29191 were more sensitive to irradiation and toxic compounds than *Sacch. cerevisiae* ATCC 7754. Therefore, further experiments will be conducted and published in a second manuscript to determine the effect of irradiation, in addition to acid hydrolysis, of feedstock using irradiated microorganisms, which showed the highest bioethanol productivity obtained from current experiments. The production of bioethanol using a co-culture of *Z. mobilis* ATCC 29191 and *Sacch. cerevisiae* ATCC 7754 will also be tested.

References

- Abdel-Fattah, W.R., Fadil, M., Nigam, P. and Banat, I.M. (2000)** Isolation of thermotolerant ethanologenic yeasts and use of selected strains in industrial scale fermentation in an Egyptian distillery. *Biotechnol. Bioeng.* **68**, 531–535.
- Abo-Sereh, N.A., Soliman, E.A.M. and Abd El-Khalek, B.A. (2006)** Mutation Induction for Genetic Improvement of *Saccharomyces boulardii* which used as probiotic. *Yeast Res. J. Agr. Biol. Sci.* **2**, 478–482.
- Abo-State, Mervat A., Ragab, A.M., El-Gendy, N.Sh., Farahat, Laila A. and Madian, Hekmat R. (2013)** Effect of different pretreatments on Egyptian sugarcane bagasse saccharification and bioethanol production. *Egypt J. Petrol.* **22**, 161–167.
- Aguilar, R., Ramírez, J.A., Garrote, G. and Vázquez, M. (2002)** Kinetic study of the acid hydrolysis of sugarcane bagasse. *J. Food Eng.* **55**, 309–318.
- Akacha, N., Zehlila, A., Mejri, S., Taieb, J. and Mohamed, G. (2008)** Effect of gamma ray on activity and stability of alcohol dehydrogenase from *Saccharomyces cerevisiae*. *Biochem. Eng. J.* **40**, 184–188.
- Al-Sudany, A., Wasan, M.Z. and Al-Aubeidi, Hind J.A. (2010)** Detection of gamma radiation effect induced by Cobelt-60 on *Escherichia coli* cells. *J. Al-Nahrain Univ.* **13**, 129–133.
- Arapoglou, D., Varzakas, T., Vlyssides, A. and Israilides, C. (2010)** Ethanol production from potato peel waste (Ppw). *Waste Manage.* **30**, 1898–1902.
- Atia, K.S. (2005)** Co-immobilization of cyclo-hexanone mono-oxygenase and glucose-6-phosphate dehydrogenase onto polyethylenimine-porous agarose polymeric composite using γ -irradiation to use in biotechnological processes. *Radiat. Phys. Chem.* **73**, 91–99.
- Egypt. J. Microbiol.* **49** (2014)

- Carvalho, N.L. (2009)** Dilute acid and enzymatic hydrolysis of sugarcane bagasse for biogas production. *Master Thesis*. Department of Chemical and Biological Engineering, Instituto Superior Técnico, Lisbon, Portugal
- Chakravarty, B. and Sen, S. (2001)** Enhancement of regeneration potential and variability by γ -irradiation in cultured cells of *Scilla indica*. *Biologia Plantarum*, **44**, 189–193.
- Crowell, E.A. and Ough, C.S. (1979)** A Modified procedure for alcohol determination by dichromate oxidation. *Am. J. Enol. Viticult.* **30**, 61–63.
- Davis, Linda, Rogers, P. Pearce, J. and Peiris, P. (2009)** Evaluation of zymomonas-based ethanol production from a hydrolysed waste starch stream. *Biomass Bioenergy*, **30**, 809-814.
- Dubois, M., Gilles, K.A., Hamilton, J.K., Rebers, P.A. and Smith, F. (1956)** Colorimetric method for determination of sugars and related substances. *Anal. Chem.* **28**, 350–356.
- Duncan, D.B. (1955)** Multiple ranges and multiple F test. *Biometrics*, **11**, 1–42.
- Edgardo, A., Parra, C., Manuel, R., Juanita, F. and Jaime, B. (2008)** Selection of thermotolerant yeast strains *Saccharomyces cerevisiae* for bioethanol production. *Enzyme Microbial. Technol.* **2**, 1–7.
- Ferdian, W., Cheng, C., Kao, W., Lee, D.B. and Chang, J. (2012)** Cellulosic ethanol production performance with SSF and SHF processes using immobilized *Zymomonas mobilis*. *Appl. Energy*, **100**, 19–26.
- Gamal, Rawia F., Nassar, Fatma R., Abd El-Hady, Hemmat M. and El-Sawy, M. (1991)** Glycerol production by osmotolerant yeast strain using fermentor as fed batch and continuous culture techniques. *Annal. Agric. Sci., Ain Shams University*, **36**, 319–421.
- George, J., Carlos, M., Isaias, B., Souto, Maria A., Henrique, M. and Cesar, A. (2011)** Dilute mixed-acid pretreatment of sugarcane bagasse for ethanol production. *Biomass Bioenergy*, **35**, 663–670.
- Grecz, N., Rowley, D.B. and Matsuyama, A. (1983)** The action of radiation on bacteria and viruses. In: “*Preservation of Food by Ionizing Radiation*”, Josephson Es; Peterson Ms (Ed). Vol. Ii. pp. 167–218. Boca Raton; Fl; Crc Press.
- Gunasekaran, P. and Chandra, K.R. (2007)** Ethanol fermentation Technology: *Zymomonas mobilis*. pp. 1–22. Madurai Kamary University, Madurai, India,
- Jacobsen, S.E. and Wyman, C.E. (2002)** Xylose monomer and oligomer yields for uncatalyzed hydrolysis of sugarcane bagasse hemicellulose at varying solids concentration. *Ind. Eng. Chem. Res.* **41**, 1454–1461.
- Karimi, K., Emtiazi, G. and Taherzadeh, M.J. (2006)** Ethanol production from dilute-acid pretreated rice straw by simultaneous saccharification and fermentation with *Mucor indicus*; *Rhizopus oryzae* and *Saccharomyces cerevisiae*. *Enzyme Microbial. Technol.* **40**, 138–44.

- Limayem, A. and Steven, C.R. (2012)** Lignocellulosic biomass for bioethanol production: current perspectives; potential issues and future prospects (Review). *Prog. Energy Combust. Sci.* **38**, 449–467.
- Mehdikhani, P., Mahmoud, B.R. and Hrachya, H. (2011)** Screening of *Saccharomyces cerevisiae* for high tolerance of ethanol concentration and temperature. *African J. Microbiol. Res.* **5**, 2654–2660.
- Osterholm, M.T. and Norgan, A.P. (2004)** The Role of irradiation in food safety. *New England J. Med.* **350**, 1898–1901.
- Pak, S.C. and Simon, M.L. (2004)** A Method for routine measurements of total sugar and starch content in woody plant tissues. *Tree Physiol.* **24**, 1129–1136.
- Pattana, L., Thani, A., Leelavatcharamas, V. and Laopaiboon, L. (2010)** Acid hydrolysis of sugarcane bagasse for lactic acid production. *Bioresour. Technol.* **101**, 1036–1043.
- Ramadan, E.M., El-Sawy, M., Gamal, Rawia F. and Abd El-Hady, Hemmat M. (1985)** Growth parameters of yeast grown on agricultural residues using shake flask as a batch culture. *Annals Agric. Sci., Ain Shams University*, **30**, 25–45.
- SAS (1996)** “*Statistical Analysis System, SAS User's Guide*”: Statistics. SAS Institute. Inc. Editors, Cary, NC.
- Stuart, N.W. (1936)** Adaptation of the micro-Kjeldahl method for the determination of nitrogen in plant tissues. *Plant Physiol.* **11**, 173–179.
- Swings, J. and Deley, J. (1977)** The Biology of Zymomonas. *Bact. Reviews*, **41**, 1–46.
- Talyour, J. (1962)** The estimation of numbers of bacteria by tenfold dilution series. *J. Appl. Bacteriol.* **25**, 54–56.
- Tasić, M.B., Konstantinović, B.V., Lazić, M.L. and Veljković, V.B. (2009)** The acid hydrolysis of potato tuber mash in bioethanol production. *Biochem. Engineering J.* **43**, 208–211.
- Thornley, M.J. (1963)** Radiation resistance among bacteria. *J. Appl. Bacteriol.* **26**, 334–345.
- Tiessen, H. and Moir, J.O. (1993)** Total and organic carbon. In: “*Soil Sampling and Methods of Analysis*”, M.E. Carter (Ed.), pp. 187–211. Lewis Publishers, Ann Arbor, MI.
- Wickerham, L.J. (1946)** A critical evaluation of the nitrogen assimilation tests commonly used in the classification of yeasts. *J. Bacteriol.* **52**, 293–301.

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تأثير التشعيع الجامي للميكروبات المنتجة للإيثانول الحيوى على انتاج الايثانول الحيوى من مصاصة قصب السكر وقشور البطاطس

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تم اجراء هذا البحث لدراسة انتاج الايثانول الحيوى من المخلفات الزراعية (مصاصة قصب السكر وقشور البطاطس) باستخدام ATCC 7754 *Saccharomyces cerevisiae* و ATCC 29191 *Zymomonas mobilis* و التى تم تعريضها لجرعات من اشعه جاما (0 , 100 , 300 , 500 , 1000 و 1500 جراى). كما تم اختبار معاملات مختلفة للتحليل المائى للمخلفات و دراسة تأثير ذلك على السكريات الناتجة و تخميرها بعد ذلك الى ايثانول حيوى و مقارنة ذلك عند استخدام المخلفات غير المعاملة فى الانتاج. تم اجراء التحليل المائى باستخدام محلول حامض الكبريتيك المخفف بتركيز 2 و 6 ٪ حجمية , عند 100 م° و 120 م° و لمدة 30 و 60 دقيقة من التعريض. و عند الانتاج على مصاصة القصب المحللة بحامض الكبريتيك 2 ٪ عند 120 م° لمدة 60 دقيقة باستخدام مزرعة ال *Sacch. cerevisiae* ATCC 7754 المشععة عند 300 جراى كان أعلى تركيز من الإيثانول الحيوى هو 10,3 جم/لتر. و كمية السكريات الناتجة من التحليل بالحامض 23,7 جم/لتر. فى حين كانت الكمية الناتجة من الإيثانول الحيوى 4,4 جم/لتر باستخدام *Z. mobilis* ATCC 29191 المشععة عند 100 جراى و تحت نفس الظروف المذكورة أعلاه. أعلى تركيز للإيثانول الحيوى متحصل عليه من قشور البطاطس كان عند التحليل بحامض الكبريتيك بتركيز 6 ٪ عند 100 م° لمدة 60 دقيقة باستخدام *Sacch. cerevisiae* ATCC 7754 المشععة عند 300 جراى (7,5 جم/لتر) , بلى ذلك فى حالة الانتاج بواسطة *Z. mobilis* ATCC 2919 المشععة عند 100 جراى (5,7 جم/لتر). و كانت كمية السكريات الناتجة 24 جم/لتر.