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RESEARCH ARTICLE

Economic Exploitation of Rejected Watermelon Fruits as a Potential Source of Renewable Energy

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Abstract

Watermelon is one of the most important crops in Egypt relating to both fruits and vegetables. There is a variety of foliar diseases that affect watermelon plants which include fungal, bacterial and viral diseases. Viral diseases are a major problem in watermelon planting especially watermelon mosaic virus-2 (WMV-2) which causes economic damage reflected mostly as reduced plant growth, yield losses of more than 38%, decrease in fruit quality and consumers refuse it as misshapen and deformated fruit. The present investigation was planned to examine a suitable fermentation process for recycling the refused watermelon fruits for production of bioethanol. Four strains of *Zymomonas mobilis*; *Zymomonas mobilis* ATCC 31821, ATCC 31822, ATCC 31823 and ATCC 10988 were examined for ethanol production using different meida. Such media were prepared from different parts of infected watermelon fruits as red flesh, white tissues, lycopene and lycopene – free juice. Also, Bawa and yoshiyuki medium and molasses were used for the comparison. The watermelon juice was a superior medium for ethanol production applying all *Z. mobilis* strains. *Z. mobilis* ATCC 10988 was the most active ethanol producer giving the largest amount of ethanol with highest efficiency production of 4.26g/100 ml and 97.85%, respectively. The watermelon juice supplemented with salts of Bawa and yoshiyuki medium was tested for bioethanol production. The results showed that addition of minerals decreased ethanol production from 4.26 to 3.86 g/100 ml and reduced efficiency from 97.85% to 93.26% by using *Z. mobilis* ATCC 10988. Comparison between watermelon juice and molasses for ethanol production using *Z. mobilis* ATCC 10988 proved that watermelon juice is more effective than molasses giving ethanol content of up to 4.26 g/100 ml and 2.65 g/100 ml and production efficiency of 97.85% and 60%, respectively.

INTRODUCTION

Watermelon (*Citrullus lanatus* Thunb.) is a vine-like flowering plant originally from southern Africa. It is a worldwide economically important member in family Cucurbitaceae. It has been cultivated for a long time in Africa, the Middle East and Egypt (Huh *et al*., 2008 and Gichimu *et al*., 2009).

The watermelon fruit has a smooth thick rind (exocarp) and fleshy center (mesocarp and endocarp) including red pulp and watery juice. Watermelon fruit contains 60% flesh, of which 90% is a juice that contains 7 to 10% w/v sugars. Thus, over 50% of the watermelon fruit is readily fermentable liquid (Wayne *et al*., 2009). Watermelon is rich in useful antioxidants (mainly lycopene) which has been used to inhibit the growth of cancer cells and to reduce the risk of heart attack (Figueroa *et al*., 2010). Also, watermelon is a rich source of L-citrulline

which is a naturally occurring amino acid involved in the detoxification of catabolic ammonia and also serves as a precursor for L-arginine, the amino acid centrally involved in the production of the circulatory vasodilator, nitric oxide (Collins *et al*., 2007). In addition, watermelon is considered as a source of many vitamins as vitamin A, thiamine, riboflavin, niacin and vitamin C.

Watermelon is susceptible to many bacterial, fungal and viral diseases that cause external blemishes and deformities of the fruit making it unacceptable for consumption by the consumer. Such rejected melons could be exploited economically to extract lycopene and L-citrulline or to produce ethanol.

Bioethanol is considered as a fuel and its production has become a great issue in the world. In concern of energy-related environmental pollution, ethanol has been proposed as a clean and efficient energy carrier. It is already used as a cleaner-burning alternative to gasoline and it is the only fuel whose oxidation products do not contain much carbon dioxide and do not contribute to ozone depletion or acid rain. Normally, this biofuel is produced from sugar or starch crops like corn, sorghum or sugarcane. The watermelon juice could be used as a diluent for concentrated sources of fermentable sugars such as molasses that require an approximate dilution to ~25% (w/v) sugars before fermentation, supplemental feedstock, and nitrogen supplement with other biofuel crops or it can be fermented directly (Wayne *et al*., 2009). Also, ethanol can be fermented from glucose, fructose and sucrose in waste-stream juice which is left after lycopene and citrulline extraction (Wayne *et al*., 2009). On average, 20-pounds of watermelon yield about 1.4 pounds of sugar from the flesh and rind, from which about seven-tenths of a pound of ethanol can be derived.

Consequently, the intentional objective of the present work was to study the possibility of recycling the rejected watermelon fruits for bioethanol production using different strains of *Zymomonas mobilis*.

MATERIALS AND METHODS

Raw materials

The raw materials used in the present work comprise the following:

a. Infected watermelon fruits, collected throughout the work from Kafr El-Dawar, El-Bahera Governorate, were used for ethanol production and lycopene extraction.
b. Egyptian sugar cane molasses which is considered as a conventional industrial waste used to produce ethanol. The samples of molasses, with 50% fermentable sugars and 80.5% total solids, were obtained from El-Hawamdia factory for integrated sugar industry.

Bacterial strains

Different strains of *Zymomonos mobilis* were evaluated for ethanol production from watermelon. These strains, obtained from Cairo MIRCEN, Faculty of Agriculture, Ain Shams University, included *Zymomonos mobilis* ATCC 31821(*Zm* 31821), ATCC 31822 (*Zm* 31822), ATCC 31823 (*Zm* 31823) and ATCC 10988 (*Zm* 10988).

Ethanol production from watermelon

a. Inoculum preparation

The inoculum of each *Zymomonas* strain was prepared using ATCC medium 948 broth containing 2% glucose and 0.5% yeast extract (Atlas, 2004). The inoculated medium (50 ml in 250 ml Erlenmeyer flasks) was incubated at 30°C for 24 hr and then used as seed culture for inoculating the examined fermentation batches for ethanol production.

b. Watermelon pretreatment

Evaluation of ethanol production capability from watermelon fruits was carried out using the previous *Zymomonas* strains. The experimental design included three sets of experiments as the following:

First experiment comprised set of batch fermentations using watermelon or glucose as a carbon source (for comparison). This experiment included the following:

1. Fermentation medium (M1) containing 1% yeast extract, 0.2% potassium dihydrogen orthophosphate, 0.2% ammonium sulphate, 0.2% magnesium sulphate and 10% glucose as a carbon and energy source (Bawa and yoshiyuki, 1992).
2. Watermelon juice (M2) prepared by blending 100 g of watermelon red flesh with 10 ml of water.
3. Filtrated watermelon juice (M3).
4. Residues of juice filtration (M4).
5. White tissue of the watermelon fruit (M5) prepared by blending 100 g of it with 10 ml of water.
6. Lycopene (M6) extracted applying the method of Willstätter and Escher, 1910.
7. Lycopene - free watermelon juice (M7) which is a waste stream from processing watermelon flesh to extract lycopene.

Based on the results of the first experiment, the second experiment included the following:

1. Best medium for ethanol production which was watermelon juice medium (M2).
2. Watermelon juice supplemented with 1% yeast extract, 0.2% potassium dihydrogen orthophosphate, 0.2% ammonium sulphate and 0.2% magnesium sulphate (M8).

Third experiment was conducted to evaluate the efficiency of the most effective strain of *Zymomonos mobilis* (*Zm* 10988) to produce ethanol from M1, M2 and molasses as a commercial source for ethanol production. This experiment included:

1. Fermentation medium (M1)
2. Watermelon juice (M2).
3. Fermentation medium supplemented with clarified molasses 10% as a carbon source instead of glucose (M9). Molasses clarification includes diluting molasses with water in a ratio of 50%, adding concentrated H_2SO_4 (2 ml / 1 kg of the previous mixture), heating the mixture at 100°c / 30 min with stirring, refrigerating the mixture / 12 h and finally sterilizing the clarified molasses (sugar concentration is 25%) at 121°C for 15 min (Amin, 1978).

c. Fermentation conditions

All fermentation batches were run in 250 ml Erlenmeyer flasks containing 100 ml of the examined medium, the pH value of the medium was adjusted to 6 ± 0.3 before autoclaving at 121 °C for 20 min. The sterilized medium was inoculated with the examined *Zymomonas* seed culture (10%) and incubated at 30°C for 2 days. The samples were withdrawn at the beginning of experiment and periodically, 24 intervals, to determine pH value and sugar concentration. Ethanol production kinetics was determined after 24 and 48 hours.

Chemical determinations

Chemical determinations included the following parameters:

a. Sugar determinations comprising:
   1. Total sugars determined using Brix refractometer.
   2. Glucose determined using glucose kits (GOD-POD. Liquid) and spectrophotometer (JENWAY 6300) and measured at 546 nm (Kaplan *et al*., 2001).

b. pH of cultures measured using (pH 211Microprocessor) pH meter.

c. Bio-ethanol production kinetics: Ethanol concentration, ethanol production yield and ethanol production efficiency were calculated using the equations described by Plevako and Bakoshinskaya, 1964 and Laopaiboon *et al*., 2007 and 2009.
   - Ethanol concentration (g/100 ml).
   - Ethanol production yield = (Produced ethanol / Total consumed sugar) x 100.
   - Ethanol production efficiency (%) from the theoretical yield =

$$\frac{\text{Ethanol (g/100 ml)}}{\text{Consumed sugar (g/100 ml) x 0.511}} \times 100$$

Statistical analysis

The experimental design was a completely randomized block design (CRBD) with three replica of each treatment. All percentages were transformed to arcsine to be analyzed. Data were subjected to convenient statistical analysis methods for calculation of means using MSTATC software. Mean separation was estimated by calculating LSD values at alpha 5% according to Snedecor and Cochran, 1980.

RESULTS AND DISCUSSION

The rejected watermelon fruits are considered as feedstock for ethanol production. This is due to the fact that watermelon juice contains about 7-10% (w/v) directly fermentable sugars *i.e.* glucose, fructose and sucrose (El-Adawy *et al*., 2001; Perkins *et al*., 2006; Wayne *et al*., 2009). Also, there is an abundant waste stream of watermelon juice (about 500 l / t of watermelon) that is produced from processing of watermelon for extraction of lycopene and L-citrulline (Wayne *et al*., 2009).

Zymomona mobilis is known to be efficient ethanol producer, especially from glucose, fructose and sucrose (Tano and Buzato, 2003; Zafar and Owais, 2006). Therefore, this study was conducted to evaluate the efficiency of four different *Zymomonas mobilis* strains *i.e. Zm* 31821, *Zm* 31822, *Zm* 31823, *Zm* 10988 and seven media prepared from different parts of watermelon for ethanol production. Additionally, glucose and molasses were used as carbon sources for comparison.

Ethanol production by all examined *Zymomonas mobilis* strains using either glucose or the tested media prepared from watermelon was dependent on the *Z. mobilis* genotype (Table 1 and figures 1&2). In this context, *Zm* 10988 was the best strain for ethanol production (4.43g ethanol / 100 ml of glucose batch fermentation) with high

yield and efficiency of 48.15% and 94.23%, respectively. On the other hand, *Zm* 31822 produced the lowest amount of ethanol, which is 3.9 g ethanol / 100 ml after 48h of glucose batch fermentation.

The same trend was observed for *Z. mobilis* strains cultivated in different media prepared from watermelon with the exception of media M3 and M5. In both cases, the lowest amount of ethanol (2.95 and 2.42g / 100 ml) was produced using *Zm* 31821 after 48h of fermentation using media M3 and M5, respectively.

Table 1. Ethanol production by four *Z. mobilis* genotypes using batches containing different media prepared from watermelon.

Media	Initial sugar concentration %	Incubation time (hr)	Zymomonas mobilis											$\bar{x}$ media for Ethanol determination
			ATCC 31821			ATCC 31822			ATCC 31823			ATCC 10988		
			D.W (g/100ml)	Consumed sugar (g/100ml)	Ethanol (g/100ml)	D.W (g/100ml)	Consumed sugar (g/100ml)	Ethanol (g/100ml)	D.W (g/100ml)	Consumed sugar (g/100ml)	Ethanol (g/100ml)	D.W (g/100ml)	Consumed sugar (g/100ml)	Ethanol (g/100ml)	
M1	**10**	24	0.35	8.46	3.88	0.32	8.24	3.58	0.34	8.23	3.79	0.35	8.91	4.15	**4.39**
		48	0.67	8.88	4.13	0.62	8.64	3.90	0.65	8.84	4.17	0.63	9.20	4.43	
M2	**9.43**	24	0.59	8.30	3.67	0.43	7.80	3.50	0.65	8.00	3.76	0.70	8.00	3.80	**4.26**
		48	0.85	8.50	4.00	0.69	8.00	3.67	0.88	8.50	4.00	0.92	8.52	4.26	
M3	**9.22**	24	0.74	5.90	2.79	0.77	6.34	2.68	0.72	6.91	2.96	0.78	6.99	3.16	**3.52**
		48	0.88	6.11	2.95	0.87	6.91	3.00	0.86	7.30	3.19	0.88	7.54	3.26	
M4	**9.37**	24	0.76	7.29	3.40	0.80	7.14	3.16	0.82	7.64	3.70	0.77	7.93	3.76	**4.07**
		48	0.92	8.13	3.93	0.91	7.40	3.33	0.90	8.10	3.93	0.89	8.16	3.97	
M5	**7.23**	24	0.71	5.88	2.27	0.65	6.34	2.27	0.66	6.88	2.52	0.67	6.41	2.71	**3.24**
		48	0.78	5.99	2.42	0.74	6.71	2.55	0.74	6.99	2.63	0.78	6.80	2.79	
M6	**4.96**	24	0.31	3.21	0.90	0.33	2.99	0.90	0.32	2.60	0.90	0.31	2.88	0.90	**1.42**
		48	0.37	3.41	0.91	0.35	3.32	0.90	0.35	2.88	0.96	0.33	2.92	0.98	
M7	**4.43**	24	0.29	3.21	0.93	0.28	2.75	0.88	0.30	2.67	0.93	0.27	2.81	0.93	**1.42**
		48	0.31	3.21	1.20	0.30	2.90	0.95	0.32	2.71	1.33	0.30	2.90	1.50	
$\bar{x}$ Strains for ethanol determination			**2.67**			**2.51**			**2.77**			**2.90**			

Dry weight at the beginning of each batch was 0.21g / 100 ml, Values are means of 3 replica, standard error 0.031

$\bar{x}$ of t(24) = 2.60 $\bar{x}$ of t(48) = 2.81

$LSD_{(0.05)}$ Media = 0.10 $LSD_{(0.05)}$ Strain = 0.078 $LSD_{(0.05)}$Time = 0.05 $LSD_{(0.05)}$ MST = 0.28

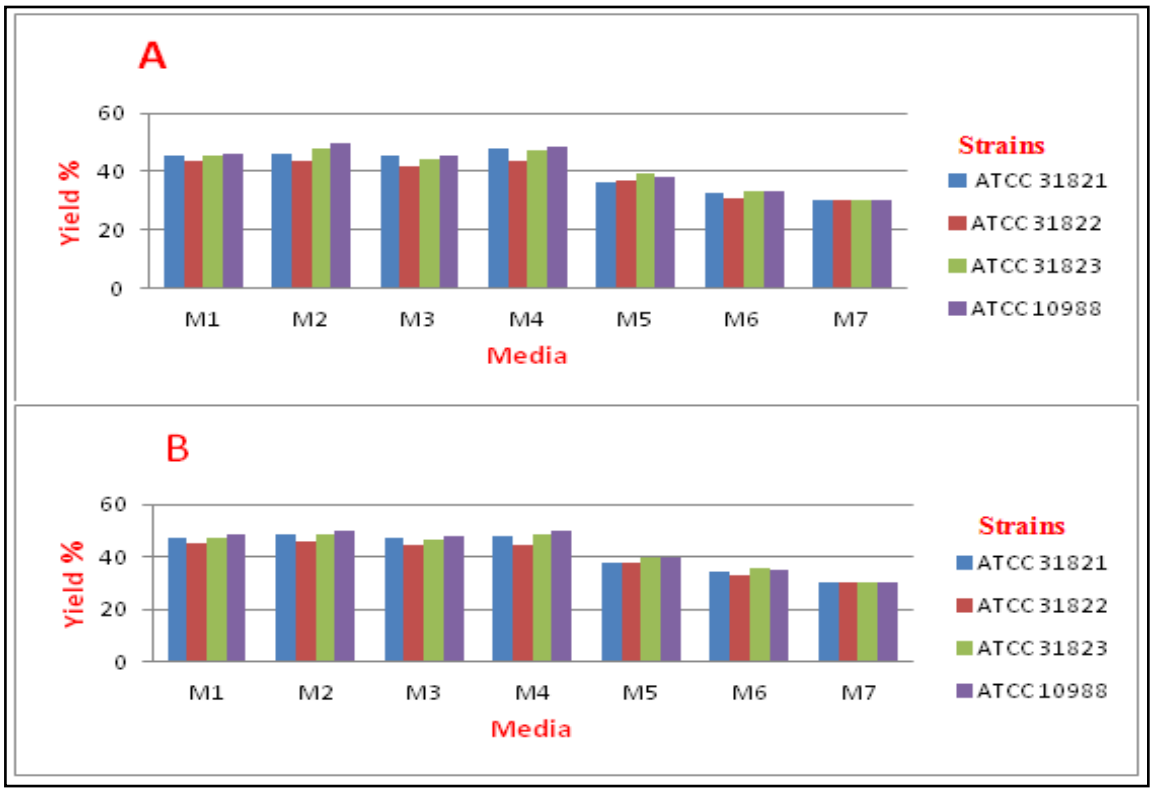

Fig.1. Effect of medium type, incubation period and strain genotype on yield (%) of ethanol production: A, after 24h. B, after 48h.

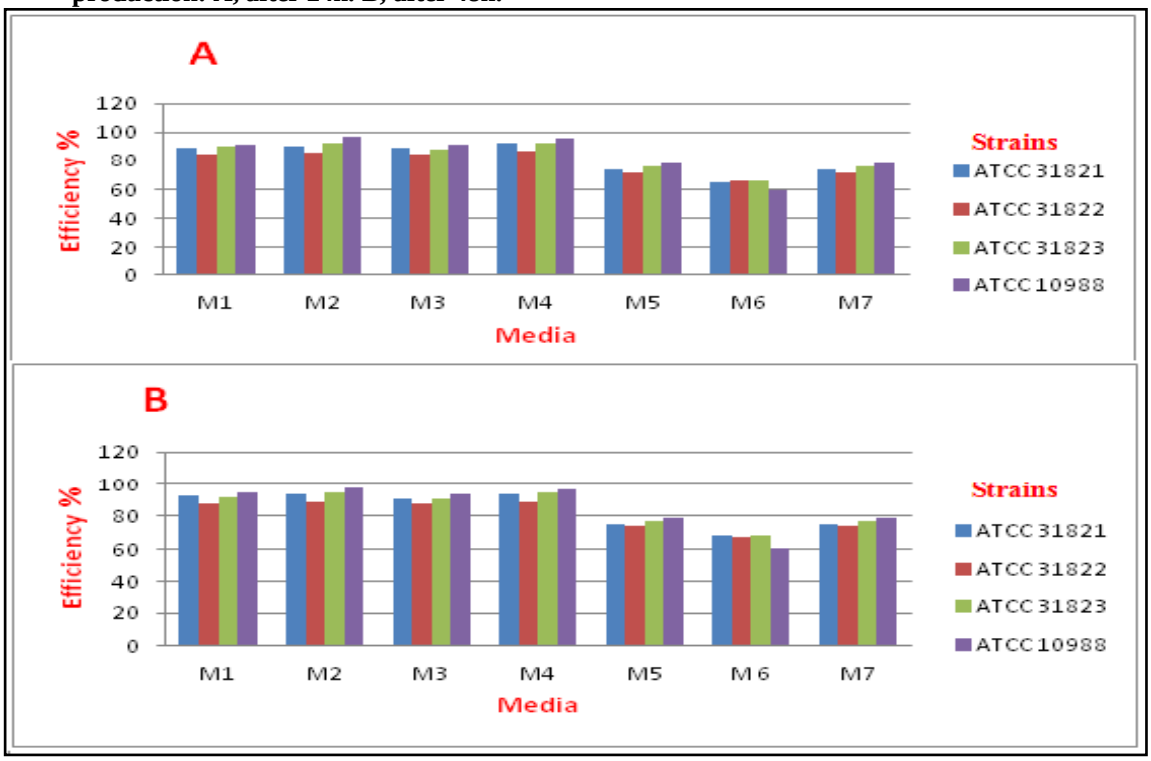

Fig.2. Effect of medium type, incubation period and strain genotype on efficiency (%) of ethanol production: A, after 24h. B, after 48h.

With all strains, M2 was the best medium prepared from watermelon for ethanol production followed by M4, M3, M5, M7 and M6, respectively.

Comparing M1 (pure glucose) and M2 (watermelon juice) for ethanol production using *Zm* 10988 leads to the conclusion of the M2 was more favorable for ethanol production. From M2, *Zm* 10988 produced an ethanol amount of 4.26 g / 100 ml after 48 h which was less than that produced from M1 (pure glucose) with a ratio of 3.8%. This might be attributed to the slight reduction of M2 sugar concentration *i.e.* 0.57%. Also, the ethanol yield and production efficiency increased from 48.15% and 94.23 using M1 to 50% and 97.85%, respectively using M2 after incubation period of 48h (figures 1 & 2). The ethanol yield using *Z. mobilis* is in agreement with those obtained by Diez and Yokoya (1996) who noticed that ethanol yield was 94.5% when *Z. mobilis* was grown in medium containg 10% (w/v) sucrose.

Generally, data in table (1) show that the amount of ethanol produced from all the examined media was significantly increased with increasing the fermentation period up to 48h. According to the statistical analysis, there is significant difference among the various media for ethanol production, while there is no significant difference between *Z.mobilis* strains (*Zm* 31821, *Zm* 31822, *Zm* 31823 and *Zm* 10988).

Figure (3) illustrates the changes of pH values during ethanol production using different media and four types of *Z.mobilis* strains. It could be noticed that, the reduction of pH values was accompanied with ethanol production. Consequently, the lowest values of pH in the range of (3.0-3.8) were recorded in batches cotaining the suitable media for ethanol production *i.e.* M1, M2, M3 and M4 after 48h of fermentation with all examined strains.

Interestingly, all tested strains preferred M2 medium (watermelon juice) than other media prepared from watermelon. This could be explained by the fact that M2 contains sufficiently higher nutritional requirements which support good growth of bacteria. However, the efficiency of the watermelon minerals content for microbial growth and ethanol production needed more confirmation.

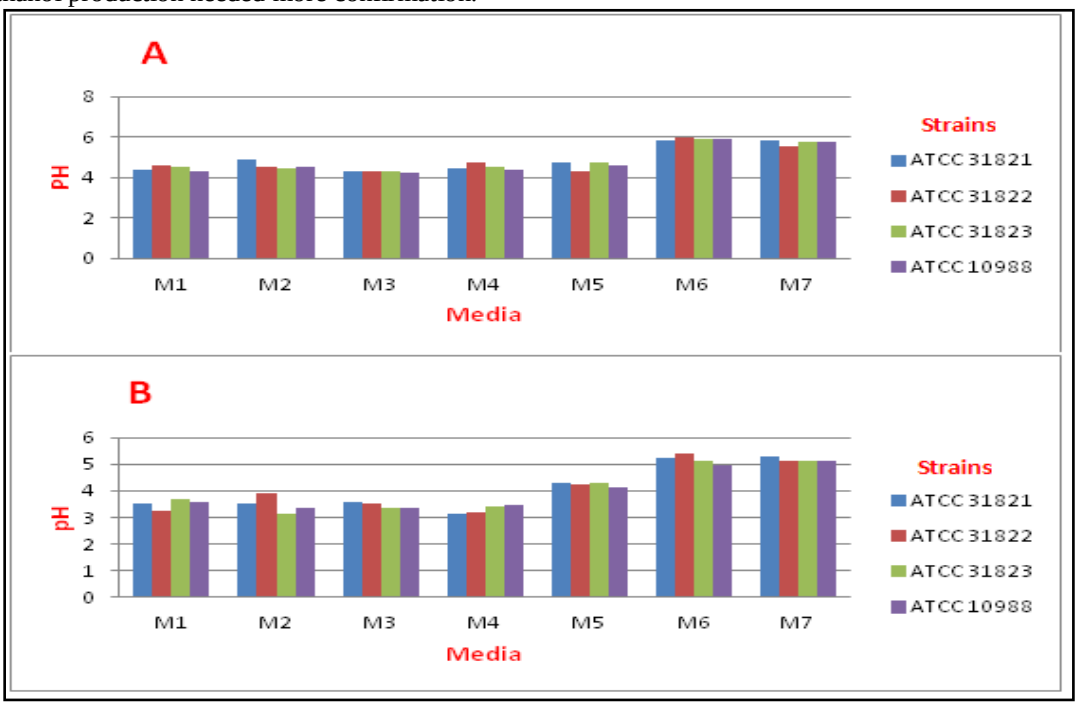

Fig.3. Effect of medium type, incubation period and strain genotype on pH values during ethanol production: A, after 24h. B, after 48h

Therefore, another set of experiments was coducted to study the effect of adding minerals of M1 medium to M2 medium on ethanol production using the examined *Z.mobilis* strains. Table (2) presents a comparison between watermelon juice (M2 medium) and watermelon juice supplemented with salts (M8 medium). Data in table (2) show that, addition of minerals did not enhance ethanol production by examined *Z. mobilis* starins. On the contrary, there was a reduction in the amount of ethanol produced, yield and production efficiency. In this regard, the amount of ethanol produced by *Zm* 10988, the best strain in ethanol production, was 4.26 and 3.86 g / 100 ml after 48h of fermentation using M2 and M8 media, respectively. Also, ethanol production yield was 50% and 47.65% with efficiency of 97.85% and 93.26% using M2 and M8, respectively (figure 4&5). So, it could be said that, watermelon

can be considered as an excellent medium for ethanol production without addition of any minerals. This is in agreement with the results of Aslam (1999), Mabee (2007) and Wayne *et al*. (2009) which reported that watermelon is economically important as a feedstock for ethanol production.

According to the statistical analysis, significant differences could be recorded among the examined media. While no significant differences could be observed between *Zm* 31821, *Zm* 31823 and *Zm* 10988, however there are significant differences between these strains and *Zm* 31822.

As expected, the lowest value of pH was recorded after 48h of cultivation especially with the superior strain (*Zm* 10988) for ethanol production to be 3.34 and 3.66 using M2 and M8 media, respectively.

Data confirmed *Zm* 10988 as a promising ethanol producer from such waste material and hence it was used for studying ethanol production from molasses which is the commercial raw material used for ethanol production.

Table 2. Ethanol production by four *Z. mobilis* genotypes in batches using watermelon juice (M2) and watermelon juice supplemented with salts (M8).

Media	Initial sugar concentration %	Incubation time(hr)	Zymomonas mobilis												$\bar{x}$ media for Ethanol determination
			ATCC 31821			ATCC 31822			ATCC 31823			ATCC 10988			
			D.W(g/100ml)	Consumed sugar(g/100ml)	Ethanol (g/100ml)	D.W(g/100ml)	Consumed sugar(g/100ml)	Ethanol (g/100ml)	D.W(g/100ml)	Consumed sugar(g/100ml)	Ethanol (g/100ml)	D.W(g/100ml)	Consumed sugar(g/100ml)	Ethanol (g/100ml)	
M2	**9.43**	24	0.59	8.30	3.67	0.43	7.80	3.50	0.65	8.00	3.76	0.70	8.00	3.80	**4.26**
		48	0.85	8.50	4.00	0.69	8.00	3.67	0.88	8.50	4.00	0.92	8.94	4.26	
M8	**9.43**	24	0.52	8.20	3.63	0.43	7.66	3.31	0.62	7.56	3.66	0.70	7.88	3.66	**4.10**
		48	0.82	8.50	3.96	0.69	7.94	3.50	0.80	7.77	3.90	0.90	8.10	3.86	
$\bar{x}$ Strains for Ethanol determination			**3.81**			**3.49**			**3.83**			**3.89**			

Dry weight at the beginning of each batch 0.21g / 100ml, Values are means of 3 replica, standard error ± 0.028

$\bar{x}$ of t(24) = 3.63 $\bar{x}$ of t(48) = 3.92 $\bar{x}$ of time = 3.75

$LSD_{(0.05)}$ Media = 0.01 $LSD_{(0.05)}$ Strains = 0.14 $LSD_{(0.05)}$ Time = 0.01 $LSD_{(0.05)}$ MST = 0.28

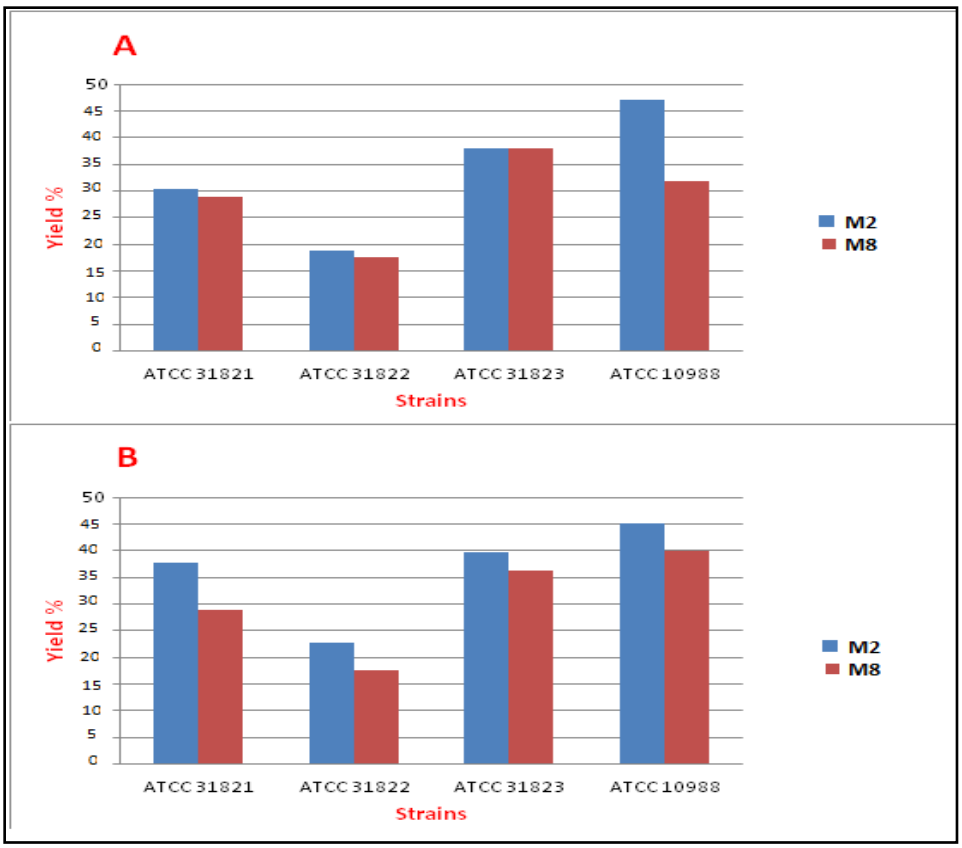

Fig.4. Effect of M2 and M8 media, incubation period and strain genotype on yield (%) of ethanol production: A, after 24h. B, after 48h.

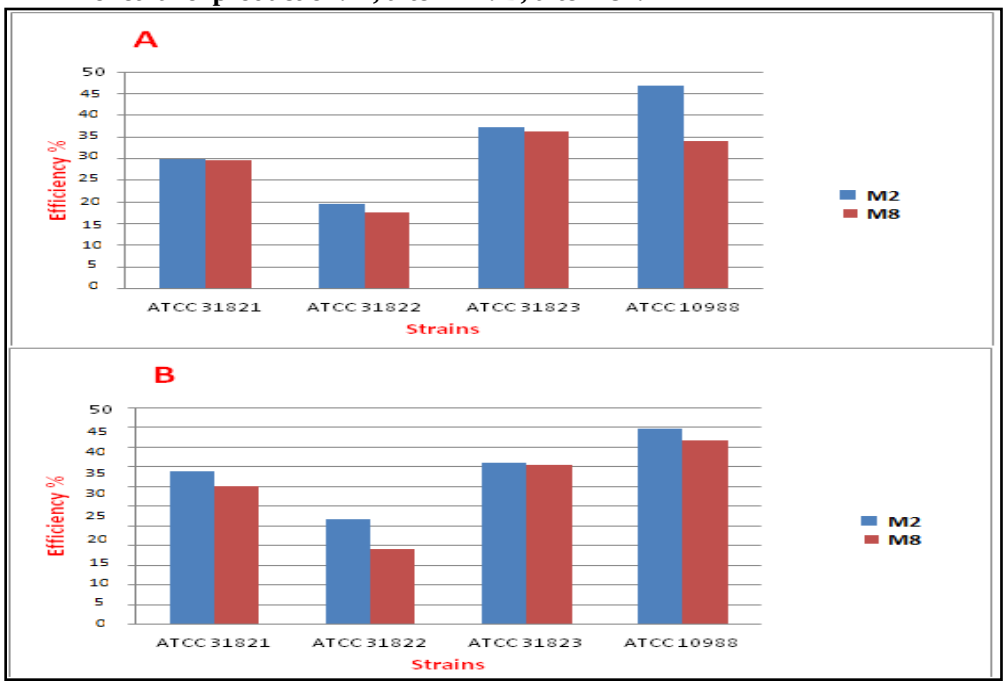

Fig.5. Effect of M2 and M8 media, incubation period and strain genotype on efficiency (%) of ethanol production: A, after 24h. B, after 48h.

Data in table (3) and figure (6) evaluate the potency of using watermelon instead of molasses as a raw material for ethanol production by the most efficient ethanol producing strain *i.e. Zm* 10988. The amount of ethanol produced from molasses (1.53 and 2.65g / 100 ml after 24 and 48 h, respectively) was much lower than that produced from either watermelon or glucose. In this regard, the lowest calculated values of yield and production efficiency were recorded as 30.71% and 60.19% after 48h of batch-fermentation using M9. In contrast, peaks of calculated values of ethanol yield (50%) and production efficiency (97.85%) were reached in fermentation batches containing M2 medium after 48h of incubation; where the lowest pH value (3.2) was recorded (figure 6). The superiority of watermelon in ethanol production over molasses can be explained as watermelon contains suitable amount of directly fermentable sugars, free amino acids, minerals and vitamines (Wayne *et al*., 2009), while molasses contains high sugar concentration and salts except nitrogen (Schweinitzer and Josenhans, 2010). Besides, molasses may contain unknown inhibitory factors such as metals, inorganic salts, ketoses and organic acids which may suppress ethanol production (Kazuhiko and kozo, 1995 and Rapeanu, 2009).

According to the statistical analysis, there are significant differences among different media. However, no significant differences were found between ethanol production from glucose and watermelon after 48h of fermentation

Table 3. Ethanol production by *Zm* 10988 in batches using media prepared from watermelon or molasses

Media	Initial sugar concentration %	Incubation time (hr)	D.W (g/100 ml)	Consumed sugar (g/100 ml)	Ethanol (g/100 ml)	$\bar{x}$ media for ethanol determination
M1	**10**	24	0.35	8.91	4.15	**4.61**
		48	0.63	8.20	4.43	
M2	**9.43**	24	0.70	8.00	3.80	**4.43**
		48	0.92	8.52	4.26	
M9	**10**	24	0.87	7.57	1.53	**3.72**
		48	1.1	8.63	2.65	
$\bar{x}$ time for ethanol determination			**t (24) = 3.16 t(48)=3.78**			

Dry weight at the beginning of each batch is 0.21g / 100 ml,
Values are means of 3 replica, standard error ± 0.02998
$LSD_{(0.05)}$ Media=0.099 $LSD_{(0.05)}$ Time=0.079 $LSD_{(0.05)}$ MT=0.22

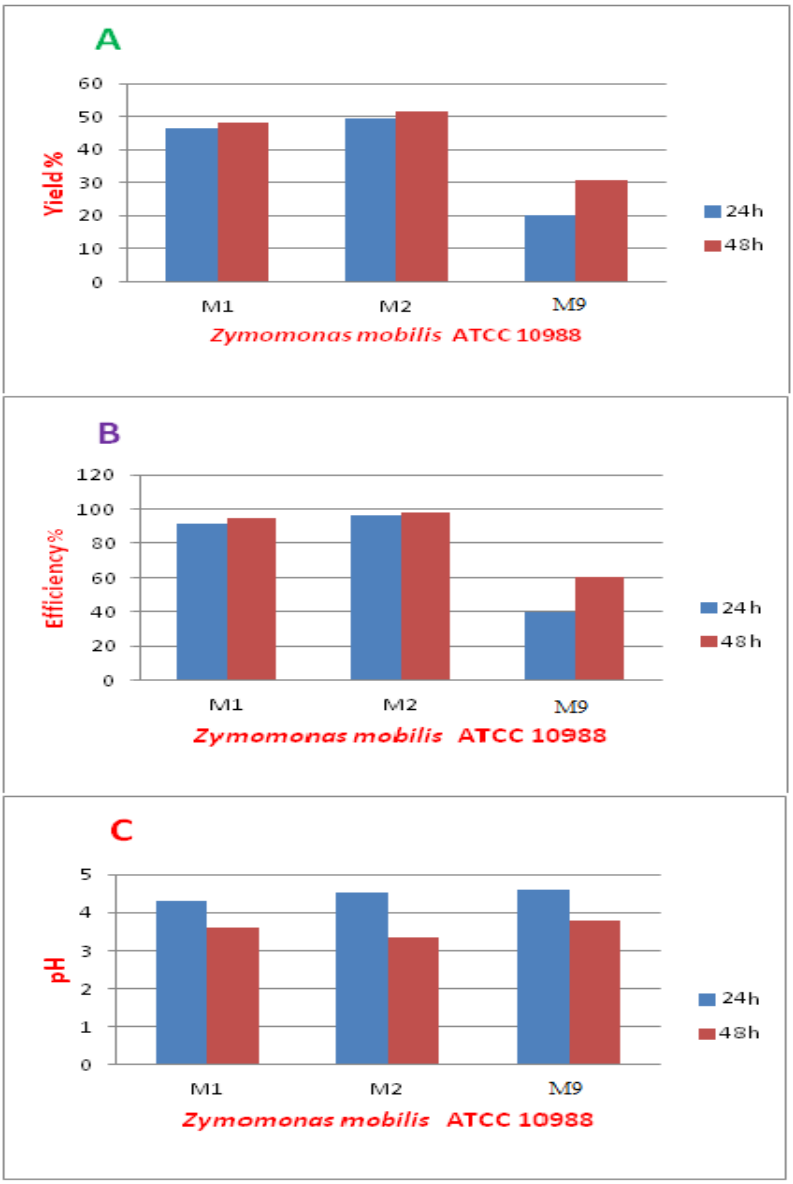

Fig.6. Effect of M1, M2 and M9 media on ethanol yield, ethanol production efficiency and pH values using *Z. mobilis* ATCC 10988.

Finally, the obtained results are in agreement with those obtained by Davison and Scott (1988) and Webb *et al*. (1996) who found that *Z. mobilis* had the capability to produce high amounts of ethanol with high efficiency around 97%. Also, Chandhary (1991) stated that *Z. mobilis* had higher specific rate of ethanol production more than yeasts.

It could be concluded that the best medium prepared from watermelon for ethanol production is the medium prepared from watermelon red flesh (watermelon juice) without any additives. The bio-ethanol production kinetics calculated for all tested *Z. mobilis* strains revealed *Z. mobilis* ATCC 10988 is the most efficient *Z. mobilis* strain for ethanol production.

Beside all of the above, there is an economic advantage for using watermelon juice in bioethanol production where the sugar content of it is fermentable sugar so there is no need for the saccharification process which is highly cost process and is associated with the loss of a part of the carbon source (Fadl, 2006 and Barakat *et al*., 2012).

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