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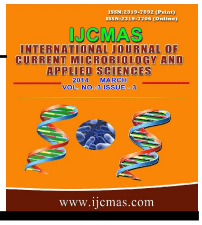

Original Research Article

Production of Ethanol from Molasses and Whey Permeate Using Yeasts and Bacterial Strains

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A B S T R A C T

The aim of this work is to study the application of molasses and whey permeate as potential sources of carbon for ethanol production. Also, to study the elimination of agro-industrial wastes and consequently, decrease the cost of ethanol production. Sugar cane molasses and whey permeate were used as carbon sources for ethanol production by yeasts and bacterial strains. Different concentrations of sugar (10, 15, 20 and 25%) were used to study fermentation by two yeast strains (*Kluyveromyces marxianus NRRL85.54 and Saccharomyces cerevisiae* O-14) and one bacterial strain *(Zymomonas mobilis* ATCC 10988). Also, Ethanol production was examined by mixture of molasses and whey permeate using these strains and their mixed culture.bResults clearly indicated that the optimal sugar concentration was 10% sugar for high efficiency of ethanol fermentation by *Kluyveromyces marxianus* NRRL85.54, *Saccharomyces cerevisiae* O-14 and *Zymomonas mobilis* ATCC 10988. Results also showed that best agro-industrial waste for ethanol production is whey permeate with *K.marixuanus* followed by the mixture of molasses and whey permeate (10% sugar concentration) with mixed culture of three strains then molasses with *K.marixuanus.*

K e y w o r d s

Molasses;
Whey Permeate;
Ethanol Fermentation;
Kluyveromyces marxianus;
Saccharomyces cerevisiae;
Zymomonas mobilis.

Introduction

Ethanol production industry is considered one of the important commercial activities for many countries. There is a world-wide search for alternative methods of energy production from renewable sources .The natural energy resources such as fossil fuel, petroleum and coal are being utilized at a rapid rate and these resources have been estimated to over a few years. Therefore, alternative energy sources such as ethanol, methane and hydrogen are being considered. Ethanol has been trusted as an alternate fuel for the future (Smith, 2007).

Ethanol is made from a variety of agricultural products &wastes such as grain, molasses, fruit, whey and sulfite waste liquor. Generally, most of the agricultural products mentioned above command higher prices as foods, and others, eg, potatoes, are uneconomical

because of their low ethanol yield and high transportation cost. The energy crisis of the early seventies may have generated renewed interest in ethanol fermentation, but its use still depends on the availability and cost of the carbohydrate relative to the availability and cost of ethylene. Sugar and grain prices, like oil prices, have risen dramatically since 1973 (Klein *et al* , 2004).

The many and varied raw materials used in the manufacture of ethanol via fermentation are conveniently classified under three types of agricultural raw materials: sugar, starches, and cellulose materials. Sugars (Sugar cane, sugar beets, molasses, and fruits) can be converted to ethanol directly. Starches (grains, potatoes, root crops) must first be hydrolyzed to fermentable sugars by the action of enzymes from malt or molds. Cellulose (Wood, agricultural residues, waste sulfite liquor from pulp and paper mills) must likewise be converted to sugars generally by the action of mineral acids. Once simple sugars are formed, enzymes from yeast can readily ferment them to ethanol (Dickinson., 1999) .

Molasses is waste product of sugar industry and represents a promising raw material for ethanol production. Brazil is pioneer in large scale motor fuel ethanol production through the fermentation of sugar cane molasses by yeasts. Also in India molasses economically are widely used in alcohol industries. (Schweinitzer and Josenhans., 2010).

Whey permeate from dairy industry contributes a significant liquid waste for ethanol production while minimizing the environmental problems associated with its treatment and disposal (Staniszewski *et al* ., 2007; Fonseca, 2008).

Several microorganisms have been considered as ethanologenic microbes. The yeast *Saccharomyces cerevisiae* and *Kluyveromyces marxianus* and the facultative bacterium *Zymomonas mobilis* are better candidates for industrial alcohol production (Mohammed *et al*., 2001; Alfenore et *al*., 2004)**.** The main aim of the present study to recycling of the agro industrial wastes to reduce the financial cost of the process and the potential of molasses and whey permeate as substrates for ethanol production by yeast and bacterial strains.

Materials and Methods

Agro-industrial wastes

Egyptian sugar-cane molasses with 50% fermentable sugars and 80% total solids; obtained from El Hawamdia factory for integrated sugar industry was used for ethanol production, after being clarified. Whey permeate(4.2% lactose and pH 4.5) was obtained from Dairy Processing Unit, Animal Production Research Institute, ARC, Ministry of Agriculture, Dokki, Cairo, Egypt.

Microorganisms

Throughout the current investigation, two yeast strains and one of bacteria were tested for their potential to produce ethanol. *Kluyveromyces marxianus* NRRL8554, *Saccharomyces cerevisiae* O-14 and *Zymomonas mobilis* ATCC 10988 were obtained from the culture collection of the Department of Agricultural Microbiology, Faculty of Agriculture, Cairo University.

Culture media

Yeast extract Malt agar medium (YM

media) was used for cultivating and maintaining a yeast strain *Saccharomyces cerevisiae* (Bawa and Yoshiyuki, 1992).
YM broth was also used for preparing yeast cells culture *Kluyveromyces marxianus*. *Zymomonas mobilis* medium was used for cultivating and maintaining *Zymomonas mobilis strain at 4ºC.*
Two different media were used for ethanol production using different carbon sources i.e. glucose, molasses, whey permeate. (Bawa and Yoshiyuk, 1992) The first medium (Medium number 1) composed of 0.5% $(NH_4)_2SO_4$; 0.3% Yeast extract; 0.5% KH_2PO_4 ; 0.1% $MgSO_4$; 0.01%$CaCl_2$; 15.2% Glucose. It was used in the different batch fermentation experiments. Glucose in the fermentation medium was replaced by the examined carbon sources e.g. molasses & whey permeate. The second medium (Medium number 2) is composed of 0.5% peptone; 0.3%Beef extract. Different sugar concentrations were used as carbon sources e.g. Glucose, molasses, whey permeate or mixture of molasses & whey permeate.

Determination of chemical composition of molasses & whey permeate

NPK test and organic matters test were performed on clarified molasses and autoclaved whey permeates samples (Table.1) according to (APHA, 1992). Organic matters were determined by Walkely and Black method (Walkley and Black. 1934).

Molasses Clarification

The clarification of molasses was done chemically by adding 3 ml of concentrated H_2SO_4 to 1kg molasses mixed with 1000 ml distilled water, to reach pH 3.5. Then the mixture was heated in a water bath to boiling for 30 minutes, and after being cooled ,it was completed to 2000 ml, then, it was stand in refrigerator overnight, centrifuged and sterilized at 121ºC for 15 minutes. Sugar concentration was 25% (Amin, 1978).

Table.1 Chemical composition of molasses & whey permeate

Sample	%N	%P	%K	%O.M
Whey permeates	0.06	0.38	0.12	4.5
Molasses	0.20	0.27	0.11	15.2

Whey permeate clarification

Whey permeate clarification was done by heating by adding 3 ml of concentrated H_2SO_4 to 1kg whey permeate Then the mixture was heated in a water bath to boiling for 30 minutes, and after being cooled , it was stand in refrigerator overnight, centrifuged and sterilized at 121ºC for 15 minute (Kitamura *et al*., 1996). Whey permeate clarification also was done by autoclave at 121ºC for 15 min. to precipitate the residual proteins and calcium phosphate. The clarified whey permeate by autoclaving contains 4.5 % sugar was used for ethanol production. Different amounts of clarified molasses were added to whey permeate up to 10,15, 20 or 25% sugar to increase the total sugar content in the fermentation media then used for ethanol production.

Microbiological methods

One slant of yeast culture either *S.cerevisiae or K.marixuanus* was used to inoculate conical flasks (250ml capacity) containing 50ml.of YM broth medium. Then, it was incubated on a rotary shaker (120 rpm) at 30ºC for 24hrs. The bacterial strain *Zymomonas mobilis* inoculums was prepared as the same but without shaking. These active cultures were used as inoculums for ethanol production. Ethanol production was evaluated with the

fermentation media have glucose, molasses, whey permeates and mixture of molasses and whey permeate as carbon sources respectively in both batch flasks and batch bioreactor fermentation. Microbial Growth was determined by dry weight according to the method described by Norris and Ribbons (1970).

Analytical methods

Total soluble sugars (TSS) were determined using phenol sulfuric acid method described by (Smith *et al*., 1956). Ethanol was estimated according to the methods of Martin after being modified by Plevako and Bakoshinskaya (1964).

Statistical analysis

The data in triplicate for the parameters in various experiments were subjected to ANOVA (Analysis of variance).

Results and Discussion

Ethanol production from pure glucose

Comparison between ethanol production using medium No.(1) and medium No.(2)

In both cases ethanol production increased when the incubation time increased. **(**Table.2). Although with increasing glucose concentration, the consumed sugar also increased and that affected negatively on yield and efficiency. That is in disagreement with Sengupta and Sadhukan(1992) who found that increasing sugar concentration resulted in an increase in the efficiency of ethanol production. Also, results showed that medium No(2) is better than medium No.(1) as ethanol kinetics production was highly recorded with sugar conc.10%,15%,20% and 25%. In medium No.(2) ethanol production was 2.15, 3.34, 2.34 and 2.92 g/100ml, respectively with efficiency 88.19, 50.24, 31.56 and 29.92%, respectively. So this medium was used in all experiment carried out as it gave high ethanol kinetics production.

Ethanol production by the tested strains using different glucose concentrations

In this experiment, different glucose concentrations (10,15,20 and 25%)were added to media No.(2), the two yeast strains (*K.marixuanus & S.cerevisiae*) and one bacterial strain(*Z.mobilis*) were examined for ethanol production. Inoculum size was 5% (v/v) and the temperature was held at 30ºc for 48 hr . Results in Table (3) show ethanol production by different microorganisms grown in medium No.(2). In all cases, ethanol production increased during 48hr. It could be noticed that the maximum level of ethanol was recorded with *Z.mobilis* followed by *S.cerevisiae* then *K.marixuanus.*

The efficiency was at maximum level with *S.cerevisiae* (88.19%) *then Z.mobilis (*81.59%) *then K.marixuanus* (70.34%).That is because most of the consumed sugar was achieved by *Z.mobilis* and that affected negatively on efficiency. Also, results showed that 10% glucose concentration was the best concentration on the basis of economic and ethanol kinetics production aspects. That is in agreement with Srivastava *et al.*, (1997) who noticed that the higher sugar concentrations of 15,20 and 25% inhibit ethanol kinetics production.

Ethanol production from sugar cane molasses

Sugar cane molasses is widely used as the raw material for alcohol production with

Table.2 Ethanol production using media No.(1) and media No.(2)

Conc of sugar (%)	*Incubation Time (hr)*	Media									
		Medium No.1					*Medium No.2*				
		D.W.(g/100ml)	*Consumed sugar (g/100ml)*	*Ethanol Kinetics Production*			*D.W.(g/100ml)*	*Consumed sugar (g/100ml)*	*Ethanol Kinetics Production*		
				g/100ml	*Yield (%)*	*Efficiency (%)*			*g/100ml*	*Yield (%)*	*Efficiency (%)*
10%	**0**	**0.13**	**0**	**0**	**0**	**0**	**0.4**	**0**	**0**	**0**	**0**
	24	**0.79**	**1.63**	**0.6**	**36.84**	**72.09**	**0.46**	**4.03**	**1.506**	**37.31**	**73.01**
	48	**1.1**	**2.37**	**1.03**	**43.71**	**85.55**	**0.53**	**5.43**	**2.15**	**45.06**	**88.19**
15%	**0**	**0.13**	**0**	**0**	**0**	**0**	**0.4**	**0**	**0**	**0**	**0**
	24	**0.74**	**2.86**	**0.63**	**22.14**	**43.32**	**0.6**	**8.16**	**1.92**	**23.58**	**46.14**
	48	**1.17**	**3.9**	**0.92**	**23.59**	**46.16**	**0.93**	**9.81**	**2.344**	**25.67**	**50.24**
20%	**0**	**0.13**	**0**	**0**	**0**	**0**	**0.4**	**0**	**0**	**0**	**0**
	24	**0.73**	**5.07**	**0.7**	**13.77**	**26.96**	**1**	**13.69**	**1.998**	**14.59**	**28.56**
	48	**1.3**	**7.65**	**1.2**	**15.69**	**30.7**	**1.27**	**14.75**	**2.344**	**16.13**	**31.56**
25%	**0**	**0.13**	**0**	**0**	**0**	**0**	**0.4**	**0**	**0**	**0**	**0**
	24	**0.71**	**7.49**	**0.93**	**12.46**	**24.38**	**1.27**	**18.22**	**2.53**	**13.9**	**27.2**
	48	**1.5**	**10.08**	**1.4**	**13.89**	**27.17**	**1.53**	**19.33**	**2.92**	**15.29**	**29.92**

Values are means of 3 replicates, LSD value = 0.1874 at P = (05)

Table.3 Ethanol production by the tested strains using different glucose concentrations

Conc of sugar (%)	Incubation Time (hr)	Strains														
		S.cerevisiae O-14					*Z.mobilis ATCC 10988*					*K.marxianus NRRL 85.54*				
		D.W.(g/100ml)	Consumed sugar (g/100ml)	Ethanol Kinetics Production			D.W.(g/100ml)	Consumed sugar (g/100ml)	Ethanol Kinetics Production			D.W.(g/100ml)	Consumed sugar (g/100ml)	Ethanol Kinetics Production		
				g/100ml	Yield (%)	Efficiency (%)			g/100ml	Yield (%)	Efficiency (%)			g/100ml	Yield (%)	Efficiency (%)
10%	0	0.4	0	0	0	0	0.4	0	0	0	0	0.09	0	0	0	0
	24	0.46	4.03	1.506	37.31	73.01	0.67	4.66	1.89	40.72	79.69	0.47	3.42	1.13	33.16	64.89
	48	0.53	5.43	2.15	45.06	88.19	0.83	6.24	2.6	41.7	81.59	0.51	4.59	1.65	35.94	70.34
15%	0	0.4	0	0	0	0	0.4	0	0	0	0	0.09	0	0	0	0
	24	0.6	8.16	1.92	23.58	46.14	0.6	8.6	2.256	26.25	51.27	0.3	8.03	1.57	19.63	38.25
	48	0.93	9.81	2.344	25.67	50.24	0.8	9.24	2.76	29.9	58.52	0.71	8.9	2	22.47	43.98
20%	0	0.4	0	0	0	0	0.4	0	0	0	0	0.09	0	0	0	0
	24	1	13.69	1.998	14.59	28.56	0.7	13.9	3.01	21.68	42.37	0.6	12.43	2.1	16.88	33.03
	48	1.27	14.75	2.344	16.13	31.56	0.93	14.7	3.4	23.15	45.29	0.83	13.3	2.43	17.98	35.83
25%	0	0.4	0	0	0	0	0.4	0	0	0	0	0.09	0	0	0	0
	24	1.27	18.22	2.53	13.9	27.2	1.07	18.66	2.74	14.68	28.74	0.8	18.33	2.33	12.72	24.89
	48	1.53	19.33	2.92	15.29	29.92	1.53	19.3	3.05	15.8	30.94	1.13	19.33	2.73	14.3	28.28

Values are means of 3 replicates, LSD value = 0.2676 at P = (05)

economic reasons. It is the most important substrate used as a carbon source for yeasts and bacteria Kazuhiko and Kozo(1992).In this experiment, fermentation medium No.(2)containing different sugar concentrations(10, 15 ,20 and 25% sugar obtained from sugar cane molasses) were examined in batch fermentation processes for alcohol production by either yeasts or bacteria.

In this experiment, different sugar concentrations of sugar cane molasses (10,15,20 and 25%) were added to media No.(2) and two yeast strains (*K.marixuanus & S.cerevisiae*) and one bacterial strain(*Z.mobilis*) were examined for ethanol production. Inoculum size was 5% (v/v) and the temperature was held at 30ºc for 48 hr.

Table (4) show ethanol production by different microorganisms grown in media No.(2). It was obvious that the yeast strain *K.marixuanus* was the most efficient for alcohol production from molasses followed by *Z.mobilis* and *S.cerevisiae* in descending order after 48hrs of fermentation. These strains produced 2.65, 3.14 and 2.03 g ethanol/100ml with an efficiency of 74.6, 60.12 and 47.04% respectively from the theoretical yield with sugar consumption rate 8.25, 8.63 and 8.47 g/100ml respectively.

This ethanol yield using *Z.mobilis* is in disagreement with those obtained by Diez and Yokoya (1996) who noticed that ethanol yield was 94.5% of theoretical when *Z.mobilis* CP4 applied. Also, results show that 10% glucose concentration was the best concentration in all aspects (Economic , Ethanol kinetics production aspect). That is in agreement with Srivastava et al.(1997) who noticed that the higher sugar concentrations of 15, 20 and 25% inhibit ethanol kinetics production.

Ethanol production from whey permeates

Whey is produced in huge quantities by processing dairy industries and often considered as an environmental threat. Several processes have been proposed for whey utilization largely based on fermentation by microorganisms (*Kluyveromyces sp.,Candida sp.,Lactobacillus sp.,etc*) that utilize lactose naturally (O'Leary et al (1977) and Moulin & Galzy (1984)). Whey permeate was examined as a raw material for ethanol production

Comparison between ethanol production by using clarified and autoclaved whey permeates.

Clarification of Whey permeate was applied with acid and heat then added to medium No.(2) and inoculated with the same strain *S.cerevisiae* to examine which method of clarification is better for ethanol production, Inoculums' size was 5%(v/v) and the temperature was held at 30ºc for 48 hr. Results in table (5) show ethanol production by two different clarification methods. It could be indicated that clarified whey permeate using heat is better than clarified one using acid as ethanol kinetics production is higher in autoclaved whey. Clarified whey using heat produced 1.61 g/100ml with efficiency 87.75% with consumed sugar 3.61g/100ml while clarified whey using acid produced 0.885 g/100ml with efficiency 69.21% with consumed sugar 2.5g/100ml.The big difference between them may be due to exposure to high temperature in clarified whey using heat or to acidity in clarified whey permeate using acid.

Table.4 Ethanol production by the tested strains using different sugar concentrations of sugar cane molasses

Conc of sugar (%)	Incubation Time (hr)	Strains														
		S.cerevisiae O-14					Z.mobilis ATCC 10988					K.marxianus NRRL 85.54				
		D.W.(g/100ml)	Consumed sugar (g/100ml)	Ethanol Kinetics Production			D.W.(g/100ml)	Consumed sugar (g/100ml)	Ethanol Kinetics Production			D.W.(g/100ml)	Consumed sugar (g/100ml)	Ethanol Kinetics Production		
				g/100ml	Yield (%)	Efficiency (%)			g/100ml	Yield (%)	Efficiency (%)			g/100ml	Yield (%)	Efficiency (%)
10%	0	0.2	0	0	0	0	0.5	0	0	0	0	0.09	0	0	0	0
	24	0.43	7.61	1.25	16.37	32.04	0.87	7.57	1.53	20.24	39.61	0.43	7.74	2.63	33.98	66.5
	48	0.6	8.47	2.03	24.03	47.04	1.1	8.63	2.651	30.75	60.12	0.46	8.25	3.14	38.12	74.6
15%	0	0.2	0	0	0	0	0.5	0	0	0	0	0.09	0	0	0	0
	24	0.97	10.76	1.597	14.95	29.25	0.97	10.43	1.87	17.97	35.17	0.48	11.93	3.29	27.62	54.04
	48	1.27	12.13	2.048	16.98	33.23	1.33	11.13	2.67	24.03	47.03	0.55	13.43	4.3	22.89	63.06
20%	0	0.2	0	0	0	0	0.5	0	0	0	0	0.09	0	0	0	0
	24	1	1.07	1.293	13.38	26.19	0.85	16.5	2.3	13.96	27.32	0.39	10.34	2.33	22.57	44.17
	48	1.47	11.86	1.714	14.44	28.25	1.47	17.73	3.24	18.28	35.78	0.51	11.93	4.06	32.89	66.55
25%	0	0.2	0	0	0	0	0.5	0	0	0	0	0.09	0	0	0	0
	24	1.57	13.7	1.472	10.74	21.02	1.13	20.9	2.53	12.11	23.7	0.44	5.57	0.77	13.81	27.02
	48	1.83	14.77	1.712	11.59	22.69	1.67	21.6	3.83	17.72	34.67	0.61	7.78	1.82	23.41	45.81

Values are means of 3 replicates, LSD value = 0.2725 at P = (05)

Table.5 Ethanol production using clarified whey permeates using acid & heat

Incubation Time (hr)	Clarification methods									
	Using heat					*Using acid*				
	D.W.(g/100ml)	*Consumed sugar (g/100ml)*	*Ethanol Kinetics Production*			*D.W.(g/100ml)*	*Consumed sugar (g/100ml)*	*Ethanol Kinetics Production*		
			g/100ml	*Yield (%)*	*Efficiency (%)*			*g/100ml*	*Yield (%)*	*Efficiency (%)*
0	**0.5**	**0**	**0**	**0**	**0**	**0.5**	**0**	**0**	**0**	**0**
24	**0.72**	**1.74**	**0.718**	**41.28**	**80.78**	**1.35**	**1.66**	**0.334**	**20.17**	**39.48**
48	**0.88**	**3.61**	**1.61**	**44.84**	**87.75**	**1.72**	**2.5**	**0.885**	**35.36**	**69.21**

Values are means of 3 replicates, LSD value = 0.8136 at P = (05)

Table.6 Ethanol production by the tested three strains using whey permeates

Incubation Time (hr)	*Strains*														
	S.cerevisiae O-14					*Z.mobilis ATCC 10988*					*K.marxianus NRRL 85.54*				
	D.W.(g/100ml)	*Consumed sugar (g/100ml)*	*Ethanol Kinetics Production*			*D.W.(g/100ml)*	*Consumed sugar (g/100ml)*	*Ethanol Kinetics Production*			*D.W.(g/100ml)*	*Consumed sugar (g/100ml)*	*Ethanol Kinetics Production*		
			g/100ml	*Yield (%)*	*Efficiency (%)*			*g/100ml*	*Yield (%)*	*Efficiency (%)*			*g/100ml*	*Yield (%)*	*Efficiency (%)*
0	**0.5**	**0**	**0**	**0**	**0**	**0.5**	**0**	**0**	**0**	**0**	**0.09**	**0**	**0**	**0**	**0**
24	**0.72**	**1.77**	**0.73**	**41.02**	**80.28**	**0.88**	**2.41**	**1.07**	**44.27**	**86.63**	**0.53**	**45.32**	**0.98**	**45.32**	**88.68**
48	**0.88**	**3.61**	**1.61**	**44.84**	**87.75**	**1.17**	**3.46**	**1.62**	**46.79**	**91.57**	**0.8**	**48.81**	**1.74**	**48.81**	**95.53**

Values are means of 3 replicates, LSD value = 0.1896 at P = (05)

Ethanol production by the tested strains using whey permeates

Autoclaved whey permeate was added to media No.(2) and two yeast strains (*K.marixuanus & S.cerevisiae*) and one bacterial strain(*Z.mobilis*) were examined for ethanol production. Inoculum size was 5% (v/v) and the temperature was held at 30ºc for 48 hr .

Results in Table (6) show ethanol production by different microorganisms grown in media No.(2).It could be explained that *K.marixuanus* is the efficient strain with whey followed by *Z.mobilis then S.cerevisiae. K.marixuanus* produced 1.74 g/100 ml with efficiency 95.53% but *Z.mobilis* produced 1.62 g/100ml with efficiency 91.57%. *S.cerevisiae* produced 1.61 g/100ml with efficiency 87.75%. In this regard Brady et al.(1994) stated that *K.marixuanus* fermented lactose more rapidly than others sugar.

Effect of inoculum size on ethanol production

Autoclaved whey permeate was added as carbon source to medium No.(2) followed by inoculation with *S.cerevisiae and Z.mobilis.E*thanol production. Was examined. Inoculum size was 5 %(v/v) and the temperature was held at 30ºC for 48 hr.

Results in table (7) show the effect of inoculum size on ethanol production. It could be noticed that 5% inoculum size with two different strains is better than 2.5%. Also as previously mentioned that clarified whey permeate using heat is better than clarified one using acid. *Z.mobilis* is more efficient because it is produced 1.89 g ethanol/100ml with efficiency 94.48% with inoculum size 5%. *S.cerevisiae* produced 1.67 g ethanol/100ml with efficiency 90.42% with inoculum size 5%.Chahal (1991) stated that *Z.mobilis* has the highest specific rate of ethanol production that means *Z.mobilis* is able to produce ethanol appreciably faster than comparable yeast. The obtained results are in agreement with those obtained by Davison and Scott.(1988)and Webb et al.(1995) who found that *Z.mobilis* had the capability to produce 50-200g ethanol/L/hr with yield around 97% of theoretical one. In contrast; Ghasem-Najafpour et al.(2004) found that in batch fermentation of 5% sugar by *S.cerevisiae*, the productivity of ethanol was calculated as 0.29 g/L/hr. Also, from this results showed that 10% sugar concentrations of autoclaved whey permeate was the best concentration.

Ethanol production from the mixture of molasses and whey permeate by the tested strains

This experiment was carried out to evaluate ethanol production by two yeast strains *(K.marixuanus & S.cerevisiae)* and one bacterial strain*(Z.mobilis)* grown on medium No.(2) supplemented with sugar cane molasses to reach to 10% sugar concentration. El-Nemr(1999) found that the maximum ethanol productivity from sweet or salted whey was obtained at 10% sugar concentration (Whey lactose 4% and molasses6%). Fermentation process was run for 48hrs at 30 ºc . Results in table (8) show ethanol production from the mixture of molasses and whey permeate by the tested strains. It could be stated that *Z.mobilis* was the best strain maintaining a high efficiency of ethanol production when grown on the mixture of molasses and whey. Its efficiency was 93.74% followed by *S.cerevisiae* was 91.51% then *K.marixuanus* was 86.98%.

Table.7 Effect of inoculum size on ethanol production from whey permeate.

Inoculum size & clarification method	*Incubation Time (hr)*	*Strains*									
		S.cerevisiae O-14					*Z.mobilis ATCC 10988*				
		D.W.(g/100ml)	*Consumed sugar (g/100ml)*	*Ethanol Kinetics Production*			*D.W.(g/100ml)*	*Consumed sugar (g/100ml)*	*Ethanol Kinetics Production*		
				g/100ml	*Yield (%)*	*Efficiency (%)*			*g/100ml*	*Yield (%)*	*Efficiency (%)*
H.W 2.5ml	**0**	**0.29**	**0**	**0**	**0**	**0**	**0.15**	**0**	**0**	**0**	**0**
	24	**0.41**	**2.96**	**1**	**35**	**66.74**	**0.21**	**3.5**	**1.4**	**39.78**	**77.89**
	48	**0.52**	**3.43**	**1.32**	**38.36**	**75.06**	**0.4**	**4.08**	**1.68**	**41.29**	**80.8**
H.W. 5ml	**0**	**0.29**	**0**	**0**	**0**	**0**	**0.15**	**0**	**0**	**0**	**0**
	24	**0.38**	**3.34**	**1.45**	**43.46**	**85.05**	**0.29**	**3.73**	**1.78**	**47.74**	**93.43**
	48	**0.53**	**3.63**	**1.67**	**46.21**	**90.42**	**0.38**	**3.905**	**1.89**	**48.28**	**94.48**
A.W.2.5ml	**0**	**0.29**	**0**	**0**	**0**	**0**	**0.15**	**0**	**0**	**0**	**0**
	24	**0.4**	**4.6**	**0.8**	**17.43**	**34.1**	**0.26**	**3.79**	**1.2**	**31.58**	**61.8**
	48	**0.48**	**5.04**	**1.13**	**22.5**	**44.02**	**0.4**	**5.54**	**1.83**	**33.07**	**65.59**
A.W. 5ml	**0**	**0.29**	**0**	**0**	**0**	**0**	**0.15**	**0**	**0**	**0**	**0**
	24	**0.42**	**2.85**	**1.1**	**38.65**	**75.65**	**0.3**	**3.47**	**1.45**	**41.84**	**81.88**
	48	**0.6**	**3.7**	**1.57**	**42.5**	**83.18**	**0.47**	**4.15**	**1.78**	**42.98**	**84.1**

Values are means of 3 replicates, LSD value = 0.2986at P = (05)
A.W refers to clarified whey using acid., H.W refers to clarified whey using heat.

Table.8 Ethanol production from the mixture of molasses and whey permeate by the tested strains

Incubation Time (hr)	*Strains*														
	S.cerevisiae O-14					*Z.mobilis ATCC 10988*					*K.marxianus NRRL 85.54*				
	D.W.(g/100ml)	*Consumed sugar (g/100ml)*	*Ethanol Kinetics Production*			*D.W.(g/100ml)*	*Consumed sugar (g/100ml)*	*Ethanol Kinetics Production*			*D.W.(g/100ml)*	*Consumed sugar (g/100ml)*	*Ethanol Kinetics Production*		
			g/100ml	*Yield (%)*	*Efficiency (%)*			*g/100ml*	*Yield (%)*	*Efficiency (%)*			*g/100ml*	*Yield (%)*	*Efficiency (%)*
0	**0.13**	**0**	**0**	**0**	**0**	**0.1**	**0**	**0**	**0**	**0**	**0.11**	**0**	**0**	**0**	**0**
24	**0.8**	**2.12**	**0.95**	**44.78**	**87.63**	**0.5**	**2.53**	**1.16**	**45.9**	**89.81**	**0.22**	**4.28**	**1.62**	**37.85**	**74.07**
48	**1.43**	**3.35**	**1.57**	**46.71**	**91.51**	**1.03**	**3.3**	**1.58**	**47.9**	**93.74**	**0.29**	**4.5**	**2**	**44.44**	**86.98**

Values are means of 3 replicates, LSD value = 0.3589 at P = (05)

Table.9 Ethanol production from the mixture of molasses and whey permeate by mixed culture

Tested strains	Incubation Time (hr)	D.W.(g/100ml)	Consumed sugar (g/100ml)	Ethanol Kinetics Production		
				g/100ml	Yield (%)	Efficiency (%)
S+Z	**0**	**0.5**	**0**	**0**	**0**	**0**
	24	**0.95**	**1.81**	**0.77**	**42.68**	**83.52**
	48	**1.23**	**3.26**	**1.48**	**45.61**	**89.25**
K+S	**0**	**0.6**	**0**	**0**	**0**	**0**
	24	**0.94**	**2.17**	**0.87**	**39.98**	**78.24**
	48	**1.33**	**3.98**	**1.7**	**42.87**	**83.88**
K+Z	**0**	**0.7**	**0**	**0**	**0**	**0**
	24	**0.88**	**2.11**	**0.93**	**43.52**	**85.17**
	48	**1.13**	**5.1**	**1.66**	**42.34**	**82.85**
K+S+Z	**0**	**0.5**	**0**	**0**	**0**	**0**
	24	**0.87**	**2.58**	**1.12**	**43.02**	**84.19**
	48	**1.63**	**4.33**	**2.06**	**47.72**	**92.71**

Values are means of 3 replicates, LSD value = 0.2142 at P = (05)

Table.10 Comparison between different agro- industrial wastes in ethanol production

Incubation Time (hr)	Whey permeate					Mixture of molasses & whey permeate					Molasses with 10% sugar conc.				
	K.marxianus NRRL 85.54					*mixed culture*					*K.marxianus NRRL 85.54*				
	D.W.(g/100ml)	*Consumed sugar (g/100ml)*	*Ethanol Kinetics Production*			*D.W.(g/100ml)*	*(g/100ml)*	*Ethanol Kinetics Production*			*D.W.(g/100ml)*	*Consumed sugar (g/100ml)*	*Ethanol Kinetics Production*		
			g/100ml	*Yield (%)*	*Efficiency (%)*			*g/100ml*	*Yield (%)*	*Efficiency (%)*			*g/100ml*	*Yield (%)*	*Efficiency (%)*
0	**0.09**	**0**	**0**	**0**	**0**	**0.5**	**0**	**0**	**0**	**0**	**0.09**	**0**	**0**	**0**	**0**
24	**0.53**	**45.32**	**0.98**	**45.32**	**88.68**	**0.87**	**2.58**	**1.12**	**43.02**	**84.19**	**0.43**	**7.74**	**2.63**	**33.98**	**66.5**
48	**0.8**	**48.81**	**1.74**	**48.81**	**95.53**	**1.63**	**4.33**	**2.06**	**47.72**	**92.71**	**0.46**	**8.25**	**3.14**	**38.12**	**74.6**

Values are means of 3 replicates, LSD value = 0.3096 at P = (05)

Ethanol production from the mixture of molasses and whey permeate by mixed culture

This experiment was carried out to evaluate ethanol production by mixed culture from either (*S.cerevisiae* & *Z.mobilis*), (*K.marixuanus* & *S.cerevisiae*),(*K.marixuanus* & *Z.mobilis*) or (*K.marixuanus*,*S.cerevisiae* & *Z.mobilis*) grown on medium No.(2) contained 10% sugar from sugar cane molasses and whey permeate.

Results in table (9) show ethanol production from the mixture of molasses and whey permeate by different mixed cultures. It could be resulted that ethanol production from mixed culture of three strains is the best one; the productivity of ethanol was 2.06 g /100 ml with efficiency 92.71 % from 4.33 g/100 ml consumed sugar, followed by (*S.cerevisiae* & *Z.mobilis)* then *(K.marixuanus* & *S.cerevisiae)* & *(K.marixuanus* & *Z.mobilis*).

It could be stated that_the best strain is *K.marixuanus*. As shown in table (10) when comparing between all previous data. Brady et al.(1994) stated that K.marixuanus fermented lactose more rapidly than others sugars. Singh et al.(1998) found that K.marixuanus was capable of producing ethanol when grown on medium molasses. Also it could be mentioned that The best agro industrial waste for ethanol production is whey permeate with *K.marixuanus* followed by the mixture of molasses and whey permeate with mixed culture of three strains then molasses with *K.marixuanus* .

K.marixuanus is the most efficient ethanol producer microorganisms from whey permeate and molasses.The optimum conditions of ethanol production from agro industrial wastes (Whey permeate and molasses) were 10% sugar concentration obtained using the mixed culture from three strains *(K.marixuanus, S.cerevisiae & Z.mobilis)* with ratio of (1:1:1).This was verified in both small and large scale experiments.

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