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Factors Affecting the Ethanol Production from Sugar Beet Molasses

Attia EL-Makhzangy*, Abd EL-Gawad EL-Shawaf, Awatif Lotfy Mohamed

Food and Dairy Science Department, Faculty of Technology & Development, Zagazig University, Zagazig, Egypt

Email address:
attiamakhzangy@yahoo.com (Attia EL-Makhzangy)
*Corresponding author

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Abstract: Sugar beet molasses is one of the important by products in sugar industry, it in dark brown to black colored and rich source of various polysaccharides. So, it can be used in the production of ethanol by *Saccharomyces cerevisiae*. Data showed that 2% inoculums size was the best size of all tested for molasses media. Where the alcohol production was 4.6 ml /100 media with economic coefficient 53.7% and yield coefficient 46.1%. Also, incubation periods were tested from 2 to 14 days. Maximum alcohol production was noticed at the sixth day of fermentation, it was 4.71% ml/100ml medium. The economic and yield coefficient were 53.7% and 46.1%; respectively. Results showed at 30°C incubation temperature the yeast of Saccharomyces cerevisiae produced high value of ethanol production. 4.61 ml /100ml medium with high value of economic coefficient 53.7% and 46.1% of yield coefficient. While PH 5 was the optimal value for ethanol production from molasses, where alcohol production was 4.61%, economic coefficient and yield coefficient were 53.7% and 46.1%, respectively. Increasing the PH value up to 5.5 did not increase the ethanol production. but ethanol production was recorded decrease value. Data showed that at 20°C incubation temperature and 14.0% initial, sugar the ethanol production was 5.16% with 40% economic coefficient, while at 30°C incubation temperature the maximum ethanol production was 6.3% with economic coefficient 49.0% where the initial sugar was 14.0%.

Keywords: Agricultural & Food Wastes, Ethanol Alcohol, Ethanol Production, Sugar Beet Molasses, *Saccharomyces cerevisia*

1. Introduction

Food wastes are the final wastes of the industrial processes of crops vegetables and fruits that cannot be retained to processed foods and can not be used for other purposes, and the costs paid to gather them are higher than their economic value [11]. Therefore discarded as waste since ours is an agricultural country where agricultural wastes are dumped in large volumes, ethanol derived from biomass may serve as a viable option. Molasses, the non crystallizable residue remaining after sucrose purication, has additional advantages: it is a relatively inexpensive raw material, readily available, and already used for industrial ethanol production [13]. Sugar beet (Beta vulgaris) is grown commercially or as an industrial crop for its main constituent, which is sucrose present in the root part. Beetroot is not only useful for sugar production but also gives various by-products such as pulp residues, molasses, and greens as animal feed or for the production of fibers or alcohol production [3]. One of the important by-products from sugar extraction is molasses; it is a dark brown to black colored, viscous running syrup which is the leftover after extraction and crystallization of sugar from the raw pulp [4]. Molasses was the first form of sugar or say sweetener used for consumption by humans, used by the poor population because of its cheap price as compared to honey or refined sugars. It is mainly used for the production of ethanol (recently for biofuel production) by fermentation technology [2]. Sugar beet and cane molasses are abundant liquid by-products from the sugar industry,which are generally found at high amount of total sugars (50.6–71.0% w/w) and traces of micronutrients such as minerals (Ca, Mg, Na and K), phosphate and nitrogen compounds These sugar-rich solutions does not require any major physical or chemical pretreatment (such as hydrolysis, filtration, sterilization, etc.) before fermentation, making them very appropriate for

ethanol production [12]. Some countries without known petroleum reserves have started to develop their fermentation industries for producing ethanol. However, signicant improvements in alcohol production technology are necessary in order to reduce production costs and make ethanol a competitive resource material [6]. In addition, beet molasses has many industrial uses in different countries. In the USA, beet molasses are added to chloride salt used for de-icing roads, making the process more environmentally friendly [7] and is also used in Germany in the production of synthetic rubbers [8]. The *Saccharomyces cerevisiae* is widely used as a biocatalyst in bioconversion processes and is suitable for production of ethanol from molasses under certain conditions [13]. *Saccharomyces cerevisiae* is the most popular organism used for ethanol production due to its high ethanol yield and high tolerance [9, 13, 17].

So, the aim of the present study were carried out to study the possibilities to use agricultural and food wastes "sugar beet molasses " to produce ethanol alcohol under laboratory scale. To study the economic coefficient and predict alcohol fermentation by *S. cerevisiae* as affected by factors such as, initial sugars concentration, Inoculum size, Incubation period, Incubation temperature and pH.

2. Materials and Methods

2.1. Saccharomyces Cerevisiae

It was obtained in the form of pellets from Microbiological Resource Center, Cairo "MERCEN", Faculty of Agriculture, Ain-Shams University, Egypt.

2.2. Media

2.2.1. Yeast Mold Broth (BD 271120) as Manufacturer, s Instructions

Dextrose 10.0g; Peptones 5.0g; yeast extract 3.0g; malt extract 3.0g Agar 15.0g and Distilled water 1.0 L.

2.2.2. Potato Dextrose Agar (PDA)

Potato dextrose agar medium was prepared as following: 200 gm. Potato tuber was boiled in water and then filtered 20 gm Dextrose and 17 gm Agar-agar were added to 200 ml of the above filtrate. The final volume of the medium was adjusted to 1000 ml. by adding tap water. pH was adjusted at 6.5.

2.3. Sugar-Beet Molasses

It was obtained from Egyptian sugar and Distillery company Belgass Factory, EL- Dakahlia Governorate., Egypt.

2.4. Chemical Analysis

Total sugars, reducing and non-reducing sugars, were determined as [1].

2.5. Preparation of Inoculums

Yeast appeared on (P.D.A) Potato dextrose agar media scrapped off using 5 ml sterilized distilled water, and dispensed in a flask containing 30 ml. sterilized distilled water. colony forming unit (C F U) were counted by using plate count.

2.6. Fermentation

The molasses was dissolved in distilled water to give different sugar concentration (8, 10, 12, and 14%) and then the pH value was adjusted to (4.0, 4.5, 5.0, and 5.5). Dispensing 200 ml fermenting solution in every one liter Erlenmeyer flask were added. Sufficient sterilization by autoclaving at 1.5 atmosphere pressure for 20 min. The inoculation was carried out using suitable size of yeast suspension for each flask (200 ml.) the fermenting flasks were incubated at different temperatures for different time to indicate the optimal temperature and the optimal time for ethanol production.

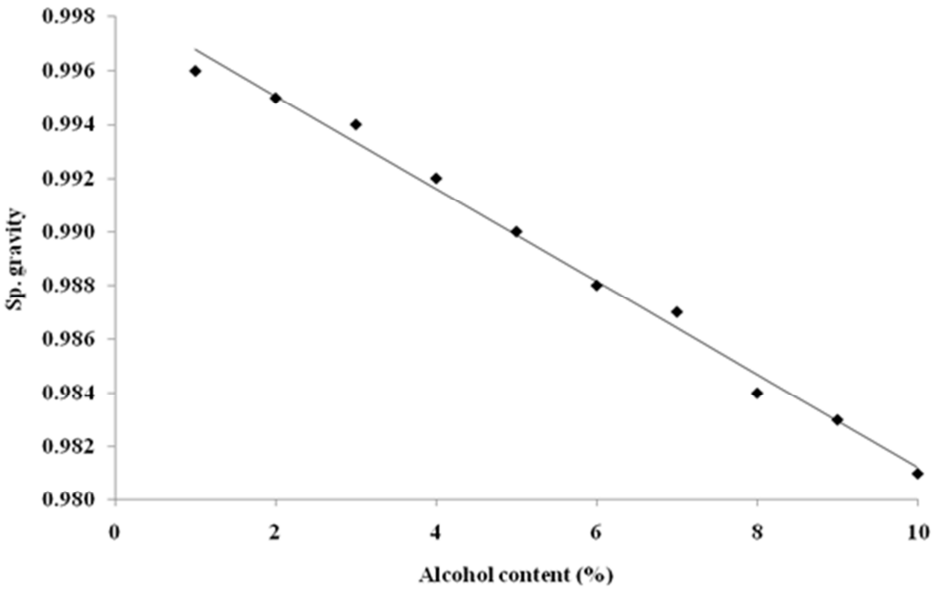

Figure 1. *Standard curve for ethanol estimation.*

2.7. Determination of Ethyl Alcohol Content

According to [9] using pyknometer method for determining the specific gravity of the distillate as follows: take a clean and dry pyknometer and weigh it empty along with the stopper at 20°C (W). Fill it with liquor sample to the brim and insert the stopper gently wipe the liquid that spills out using water absorbing filter paper and weigh at 20°C (W_1). Next remove the liquor sample and wash it with distilled water. Fill the pyknometer with distilled water in the same manner as described above and take the weight (W_2).

$$\text{Sp.Gravity} = W_1 - W/W_2\text{-}W$$

Find out the corresponding alcohol percent by volume from the standard curve prepared previously using absolute Ethanol and distilled water.

3. Results and Discussion

From the data in Table 1, it could be observed that 2% inoculum size was the best size of all tested size; where the alcohol production was 4.6 ml/100 ml media with economic coefficient 53.7% and yield coefficient 46.1%. When use 2.5, 3 and 3.5% inoculums size the percentage of ethanol production were decreased till 4.1% and the economic coefficient also decreased to 47%.

Table 1. *Effect of inoculums size on ethanol production efficiency after 4 days of fermentation from molasses media using Saccharomyces cerevisiae.**

Inoculums Size %	Initial sugars %	Residual sugars %	Consumed sugars %	Alcohol production %	Economic coefficient %	Yield coefficient %
1.0	10.0	1.98	8.02	4. 60	50.6	40.6
1.5	10.0	1.71	8.29	4.20	50.6	42.0
2.0	10.0	1.42	8.58	4.61	53.7	46.1
2.5	10.0	1.40	8.60	4.10	47.0	41.0
3.0	10.0	1.37	8.63	4.10	47.0	41.0
3.5	10.0	1.37	8.63	4.10	47.0	41.0

*at pH 5, and incubation temperature 30°C.

Generally, above or under this size of inoculums the economic and yield coefficient were decreased as the large number of yeast in the anaerobic medium compete to use sugar available is the production on non-alcohol materials. On the contrary, small number of yeast (low inoculum size) consume the sugar available in there reproduction and build themselves [11]. Singh, A. et al. [16] who reported that 3% yeast concentration was the optimal for completion of fermentation and the maximum ethanol production was completely achieved in 7 days. Also, [17] reported that maximum ethanol concentration, ethanol productivity and ethanol yield were obtained with an initial inoculum of 3% when carob extract as a substrate by using *S. cerevisiae*.

Table 2. *Effect of Incubation periods (days) on ethanol production from sugar beet molasses media using Saccharomyces cerevisiae.**

Incubation Period (day)	Initial sugars %	Residual sugars %	Consumed sugars %	Alcohol Production %	Economic coefficient %	Yield coefficient %
2	10.0	1.53	8.47	4.10	50.0	41.0
4	10.0	1.42	8.58	4.61	48.4	41.6
6	10.0	1.39	8.61	4. 71	53.7	46.1
8	10.0	1.10	8.90	4.31	48.4	43.1
10	10.0	0.92	9.08	4.40	48.4	44.0
12	10.0	0.91	9.09	4.35	47.8	43.5
14	10.0	0.91	9.09	4.33	47.8	43.5

* at pH 5, incubation temperature 30°C and inoculum volume was 2%.

Table 3. *Effect of incubation temperature (°C) on ethanol production from molasses media using Saccharomyces Cerevisiae. **

Incubation Temperature °C	Initial sugars %	Residual sugars %	Consumed sugars %	Alcohol Production %	Economic coefficient %	Yield coefficient %
20	10.0	1.74	8.53	4.03	47.2	40.3
25	10.0	1.52	8.48	4.13	48.7	41.3
30	10.0	1.39	8.61	4. 61	53.7	46.1
35	10.0	0.73	9.72	4.52	48.7	45.2

*at pH 5, incubation period 6 days and inoculum volume 2%.

Data in this Table 2 shows that incubation periods were tested at 2, 4, 6, 8, 10, 12 and 14 days. Maximum alcohol production was noticed at the sixth day of fermentation, it was 4.71% (ml/100ml medium). The same observation were found with the economic and yield coefficient; it were 53.7 and 46.1%; respectively. Long period of incubation after the sixth days showed gradually decreased in both alcohol, economic and yield coefficient. There may be attributed to

consumed the produced alcohol as a carbon source to feed the yeast. On the other hand, the fermentation periods before the sixth days 2 and 4 days showed decrement amounts is ethanol production and economic coefficient this may be to the need of yeast to achieve the necessary enzyme for saccharification [14].

Data in Table 3 showed at 30°C incubation temperature the yeast of *Saccharomyces cerevisiae* produced a high value of ethanol production 4.60 ml alcohol per 100 ml medium with a high value of economic coefficient 53.7% and 46.1% of yield coefficient.

On the other hands, the incubation temperature under this value 20, 25 or obove at 35°C showed a decrease values of both ethanol production, economic and yield coefficient.

As generally, most references showed that in un aerated fermentation; maximum yeast metabolic activity takes place at about 30°C whereas the growth rate in aerated culture is highest at 35°C and greatly affected by increasing of temperature until 42°C [16]. Which stated that incubation temperature is an important factors that effect the fermentation process and product formation. It commonly believed that 20 – 35°C is the ideal range for fermentation.

Data in Table 4 clear that pH 5 was the optimal value of pH, where alcohol production was 4.60% while economic coefficient and yield coefficient were 53.7 and 46.1%; respectively. From this table also we can notice the decrease in alcohol production; economic coefficient and yield coefficient in case of increase or decrease above or under the optimum pH value. These results were in agreement with result at obtained by [11] who observed that pH 5 and temperature 30°C were the optimum conditions for production of bio ethanol from sugar refining molasses by the local isolate of *Saccharomyces cerevisiae*.

Table 4. *Effect of different pH values on ethanol production from molasses media using Saccharomyces Cerevisiae*.*

pH value	Initial sugars %	Residual sugars %	Consumed sugars %	Alcohol Production %	Economic coefficient %	Yield coefficient %
4.0	10.0	1.67	8.33	3.91	46.9	39.1
4.5	10.0	1.52	8.48	3.98	46.9	39.8
5.0	10.0	1.39	8.61	4.61	53.7	46.1
5.5	10.0	1.22	8.78	4.12	46.9	41.2
6.0	10.0	1.67	8.33	3.90	46.8	39.5
6.5	10.0	1.92	8.08	3.79	46.8	37.9

*Under optimum conditions tested before.

Data recorded in Table 5 show that at the end of 3^{ed} day of incubation period; ethanol production were 2.88, 3.76, 4.31, 5.86 and 6.3% with economic coefficient 48.0, 47.0, 45.9, 48.9 and 47.4% when the initial sugars were 6, 8, 10, 12, and 14%; respectively. Generally from this Table, it could be concluded that; at the end of 2nd day; maximum ethanol production was 6.3% at 14.0% initial sugars. The percentage of ethanol production didn't increase with increasing the incubation periods these results were agreeable with findings of [5, 10, 11].

Table 5. *Interaction between initial sugar concentrations and incubation period on ethanol production from molasses media using Saccharomyces cerevisiae.*

Incubation Period (days)	Initial sugars %	Residual sugars %	Consumed sugars %	Alcohol Production %	Economic coefficient %	Yield coefficient %
1St	6	1.47	4.53	2.03	44.8	33.8
	8	1.72	6.28	2.82	44.9	35.2
	10	2.82	7.13	3.20	44.8	32.0
	12	2.83	9.17	4.12	44.9	34.3
	14	3.73	10.63	4.78	44.9	34.1
2nd	6	0.04	5.96	2.80	46.9	46.6
	8	0.08	7.72	3.70	47.9	46.2
	10	1.39	8.61	4.31	44.0	43.1
	12	0.28	11.72	5.86	50.0	48.8
	14	1.13	12.87	6.30	48.9	45.0
3ed	6	0.00	6.00	2.88	46.9	46.6
	8	0.00	8.00	3.76	47.9	46.2
	10	0.62	9.38	4.31	44.0	43.1
	12	0.03	11.97	5.86	50.0	48.8
	14	0.64	13.36	6.30	48.9	45.0

From Table 6 we can notice that; at PH 4.5 the maximum alcohol production was 5.31% at 14.0% initial sugar used in the media with economic coefficient 43.9%. While, at 6.0, 8.0, 10.0, and 12.0% Initial sugar used the ethanol production were 2.71, 3.13, 3.85 and 4.57%; respectively. Whereas in case of pH at 5.0 the ethanol production was 6.3% when the initial sugar was 14.0%. Meanwhile; the ethanol production were 2.80, 3.75, 4.13, and 5.86% with economic coefficient 46.9, 47.9, 44.0 and 50% when initial sugars were 6, 8, 10 and 12%; respectively. Increasing the pH values up to 5.5 didn't increase the ethanol production but ethanol production were recorded a decrease value; 2.59, 2.98, 3.83, 4.46 and

5.1% when the initial sugars were 6.0, 8.0, 10.0, 12.0 and 14.0%; respectively [6, 11].

Table 6. *Interaction between different pH values and initial sugar concentrate on alcohol production from molasses media using Saccharomyces cerevisiae under optimum conditions.*

pH values	Initial sugars %	Residual Sugars %	Consumed sugars %	Alcohol production %	Economic coefficient %	Yield coefficient %
	6	0.09	5.91	2.71	46.0	45.1
4.5	8	1.03	6.97	3.13	44.9	39.1
	10	1.43	8.57	3.85	45.0	38.5
	12	1.83	10.17	4.57	45.0	38.0
	14	1.92	12.08	5.31	43.9	37.9
	6	0.04	5.6	2.80	46.9	46.6
5.0	8	0.08	7.72	3.75	47.9	46.2
	10	1.39	8.61	4.13	44.0	48.1
	12	0.28	11.72	5.86	50.0	48.8
	14	1.13	12.87	6.30	48.9	45.0
	6	0.10	5.90	2.59	44.9	43.1
5.5	8	1.21	6.79	2.98	43.8	37.25
	10	1.47	8.53	3.83	44.9	38.3
	12	1.85	10.15	4.46	43.9	37.1
	14	2.12	11.80	5.10	43.9	36.4
	6	0.10	5.90	2.59	44.9	43.1
6.0	8	1.21	6.79	2.98	43.8	37.25
	10	1.47	8.53	3.83	44.9	38.3
	12	1.85	10.15	4.46	43.9	37.1
	14	2.12	11.80	5.10	43.9	36.4

From data in Table 7, we can observe that at 25°C incubation temperature where the ethanol production was 6.19% with economic coefficient 46.7% when the initial sugar was 14.0%. Meanwhile, the, ethanol production were 2.58, 3.38, 3.94 and 5.13% when the initial sugars were 6.0, 8.0, 10.0 and 12.0%; respectively. From the same Table 7. It is clear that incubation temperature 30°C was the best where the ethanol production was 6.3% with economic coefficient 49.0% when the initial sugars was 14.0%. While the incubation temperature increased till 35°C the percentage of ethanol production decreased to 5.47% at 14.0% initial sugar this may be attributed to the ability of *Saccharomy cescerevisiae* to grow and active to produce ethanol under anaerobic conditions. These results are agreement with findings of [15].

Table 7. *Interaction between incubation temperature and initial sugars on ethanol production from molasses media using Saccharomyces Cerevisiae under optimum conditions.*

Incubation temperature °C	Initial sugars %	Residual sugars %	Consumed sugars %	Alcohol production %	Economic coefficient %	Yield coefficient %
	6	0.30	5.70	2.29	40.2	38.1
20	8	0.25	7.75	3.22	41.6	40.2
	10	1.15	8.85	3.57	40.4	35.7
	12	0.25	11.75	4.77	40.6	39.7
	14	1.10	12.90	5.16	40.0	36.8
	6	0.35	5.65	2.58	45.7	43.0
25	8	0.30	7.70	3.38	44.0	42.2
	10	0.97	9.03	3.94	43.7	39.4
	12	0.32	11.68	5.13	44.0	42.7
	14	0.37	13.27	6.19	46.7	44.2
	6	0.4	5.66	2.80	46.9	46.6
30	8	1.08	7.72	3.75	47.9	46.2
	10	1.39	8.61	4.13	44.0	48.1
	12	0.28	11.72	5.86	50.0	48.8
	14	1.13	12.87	6.30	48.9	45.0
	6	0.07	5.93	2.59	43.8	43.1
35	8	0.13	7.87	3.51	46.6	43.8
	10	0.80	9.20	4.04	44.0	40.4
	12	0.77	11.23	4.82	43.0	40.4
	14	0.97	13.03	5.47	42.0	39.0

4. Conclusion

*F*rom the result of this study, we can determine that under laboratory conditions, it was found that ethanol with 6.3% can be produced from diluted sugar beet molasses, which contains an initial sugar concentration 14%, PH 5.5 at 30°C after 2 nd days of fermentation with an economic coefficient 48.9%.

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