Research Article

Maximization of Bio-Ethanol Production by Yeasts using Sugar Cane and Sugar Beet Molasses

Zaghloul, R.A.[1], Solafa A. Ismail[2], Gamal Enan[3*], Rasha M. El-Meihy[1], Abdel-Rahman H.M.[1]

[1]Agricultural Microbiology Department, Faculty of Agriculture, Benha University, Moshtohor, Kaluybia, 13736, Egypt; [2]Faculty of Technology and Development, Zagazig University, Egypt; [3]Faculty of Science, Zagazig University, Egypt.

Abstract | This research was carried out to evaluate the bio-ethanol production by yeasts. Out of the 7 tested yeast isolates, only 5 isolates were able to produce bio-ethanol efficiently during the fermentation of sugarcane molasses. It is worthily to mention that the highest bio-ethanol producers from sugarcane and sugar beet molasses were Saccharomyces cerevisiae F15 (*S. cerevisiae*) followed by Candida kefyr EMCC77 (*C. kefyr*). Therefore, they were selected for further experiments. Regarding the effect of environmental factors on bio-ethanol yield, the optimum pH values for bio-ethanol yield were 4.5 and 5 for *S. cerevisiae* and *C. kefyr* respectively. Whereas the optimum incubation temperature for bio-ethanol production for both *S. cerevisiae* and *C. kefyr* was 32oC. Maximum production of bio-ethanol by *S. cerevisiae* and *C. kefyr* was optimally obtained after 48hours incubation period. Under all optimum conditions, *S. cerevisiae* gave bio-ethanol yield from sugar cane molasses higher than that obtained by *C. kefyr* and vice versa in case of sugar beet molasses. Finally, immobilized cell technique showed better results in bio-ethanol production than free cells. In view of the obtained results, it could be recommended that the *S. cerevisiae* and *C. kefyr* can be used for industrial production of bio-ethanol.

Keywords | Bio-ethanol, Biofuels, Immobilization, Fermentation, Sugarcane molasses, Sugar beet molasses, Saccharomyces cerevisiae, Candida kefyr EMCC77

INTRODUCTION

Due to the environmental pollution caused throughout by fossil fuels, it was necessary to find out other eco-friendly renewable and sustainable sources of energy (Demirbas, 2009; Kiran et al., 2014; El-Gazzar et al., 2021). As liquid biofuels represent 40% of renewable energy sources, they are prioritized worldwide (Tan et al., 2008). The use of liquid biofuels helps in reduction of greenhouse gas emissions, creation of job opportunities, regional development and supply security (Demirbas, 2009; Enan et al., 2018; El-Bahr et al., 2021). Biofuels are of certain interest world wide as they donot pollute the environmental. Bio-ethanol is one of the most widely used biofuel for transportation worldwide, since it is a renewable, nontoxic, biodegradable resource and it is oxygenated (Hernández ant Kafarov, 2009; Enan et al., 2020; Osman et al., 2021a). Worldwide, bio-ethanol fuel share about 10–20% of the gasoline consumption by 2030 (Walter et al., 2008).

As reported previously, growth and bio-ethanol production of yeasts are influenced by nutritional and environmental conditions (Saini et al., 2018; Abdel-Shafi et al., 2020; El-Gazzar and Ismail, 2020). Temperature and pH value effect membrane turgidity, enzymatic activity and metabolism

of yeast cells (Chohan et al., 2020; El-Sayed et al., 2020). Yeasts prefer acidic pH values that enhance growth at the industrial level (El-Gazzar and Enan, 2020; Osman et al., 2021b). Since, yeasts tolerate high temperature and low pH, they are ideal for bio-ethanol production. Yeasts require different nutrients of which sugar mostly limit their growth and activity. Sugar cane and beet molasses are the most utilized sucrose-containing sources for bio-ethanol production. Molasses and other intermediates from sugar beet processing are very good raw materials for bio-ethanol production as they are used for fermentation without modification (Rodríguez et al., 2010). Molasses is a traditional raw material for distilleries in Egypt, and about 90% of bio-ethanol production is provided by raw material nowadays (Rasmey et al., 2018).

Saccharomyces cerevisiae strain is the most widely used bio-ethanol producer organism. To enhance bioethanol production by yeasts with promising cost, different immobilized carriers are used (Laopaiboon et al., 2009). Production of bio-ethanol by immobilization has many technical and economic advantages compared to free cell system (Verbelen et al., 2006). The most widely used immobilization methods are based on cell entrapment in natural or polymers gels (Pan et al., 2010). The main drawbacks that control the use of polymer beads as cells carrier is problem of gel degradation, low physical strength, considerable substrate and product mass transfer limitation and gel particles disruption due to intensive CO2 evolution (Phisalophong et al, 2007; El-Gazzar et al., 2020). Other alternatives widely used method are based on passive adhesion of cells onto the surfaces of insoluble carrier such as glass beads, stainless steel wire spheres, sorghum bagasse (Yu et al., 2010; Razmovski ant Pejin, 1996; Tsakiris et al., 2004), sugar cane bagasse (Santos et al., 2008). However, in order to use this technology in bio-ethanol production, the immobilization

carrier must be very cheap and cell immobilization should be achieved with minimal additional cost (Ogbonna et al., 2001). It was mentioned that immobilization of the yeast cells can avoid inhibition caused by high concentration of substrate and product, and also enhance bio-ethanol yield and productivity (Nikolić et al., 2009).

The objective of the current study was to maximize bio-ethanol production by optimizing the various factors governing its bio-synthesis while using suitable strains and raw materials.

MATERIALS AND METHODS

MICROORGANISMS, FEEDSTOCK AND MATERIALS
About 6 yeasts isolates and one bacterial strain listed in (Table 1) were used for bio-ethanol production. The yeasts strains, bacterial strain were subcultured onto Sabouraud's agar; nitrate agar (Oxoid) respectively (Enan et al., 2013, 2020; Osman et al., 2021a).

Two types of molasses (Table 2) were used as carbon source for bio-ethanol production by the given microbial strains. Egyptian sugar-cane molasses was provided by El Hawamdia factory (no. 1) and Cairo Factory (no. 2) for integrated sugar industry, Giza Governorate (20 Km West Cairo). While Egyptian sugar beet molasses was provided by El-Hamool sugar Factory, Kafr El-sheikh Governorate (120 Km, North Cairo).

Five different immobilizing carriers were used such as sodium alginate, agar–agar, carboxymethyl cellulose, sodium salt (Na–CMC) All of them obtained from HI-Media Co., Germany and artificial sponges, of loofa and fibers that were collected from mature dried fruit of Luffa cylindrica (Zagazig, Sharkia Governorate, Egypt).

Table 1: Experimental microbial strains used in this study.

Strains	Source	
Saccharomyces cerevisiae	F514	National Research Centre
Saccharomyces cerevisiae	ATCC4095	American Type culture collection, Ain Shams University, Egypt
Candida kefyr	EMCC77	
Candida tropicalis	EMCC2	Culture collection, Ain Shams University, Egypt
Kluyveromyces marxianus	NRRL1109	
Pichia stipites	DSM3651	
Zymomonas mobilis	ATCC31823	

Table 2: Composition of sugarcane and beet molasses used in this study.

Molasses type	Total protein %	Total sugar (mg. ml⁻¹)	Organic carbon %	Total P %	Ashes %	Mineral substances mg .kg⁻¹								
								P					C	
						C	O	b	Mn	Cr	Cu	Ni	d	Fe
Sugarcane molasses 1	6.01	3.57	47.56	0.12	12.74	Nil	Nil		13.13	0.76	8.8	1.91	Nil	345.3
Sugarcane molasses 2	11.44	4.46	42.98	0.012	-	Nil	Nil		15.86	3.38	7.93	7.52	Nil	366.46

MOLASSES PRETREATMENT

About 1 liter of molasses was added to 0.5 L distilled water and then 0.001% concentrated sulfuric acid were added to remove the sludge and colloids, and pH value was adjusted to 4.0 using 0.1 N HCL by Automatic pH –meter (New Brunswek Science-Co.) (Enan et al., 2013). Later on, this mixture was heated in water bath to boiling and allowed to cool, then stand in refrigerator overnight and centrifuged at 3000 rpm for 15 min and the supernatant was used for further experiments (Sadik and Halema, 2014).

SUGAR AND BIO-ETHANOL ESTIMATION

Dinitrosalycilic acid (DNS) method was used for estimation reducing sugar (Miller, 1959). Bio-ethanol production in the fermentable broth was measured by method (Nasir et al., 2017).

IMMOBILIZATION OF YEAST STRAINS

Sodium alginate, agar–agar, carboxymethyl cellulose sodium salt, artificial fiber sponges, and loofa sponges were used for immobilization of both S.cerevisiae and *C. kefyr* Ismaiel et al. (2015).

FERMENTATION PROCESS

For preparation of fermentation medium, about 250 mL, pretreated molasses; 3 g yeast extract; 0.1 g urea in 750 mL distilled water (Enan et al., 2013). About 50 ml of fermentation medium was controlled to pH 6 with 1M NaOH and sterilized at 121°C for 15 min. Then, inoculated with 5 ml of growing microbial culture containing 108. mL-1 CFU at 25°C and (150 rpm) for 3 days (Ismail et al., 2015).

STATISTICAL ANALYSIS

Analysis of variance (ANOVA) was carried out using CoStat version 6.400 (CoHort software, Monterey, CA, 93940, USA). Mean values among treatments were compared by the Duncan test at 5% level (p. value < 0.05) of significance and presented as the mean values ± standard deviation (SD).

RESULTS AND DISCUSSION

SCREENING OF BIO-ETHANOL PRODUCTION BY YEAST AND BACTERIAL STRAINS

Six yeast strains and one bacterial strain were surveyed for their ability to produce bio-ethanol from sugarcane and sugar beet molasses. The results given in Table 3 showed that all the tested yeast strains gave lower bio-ethanol production from beet molasses compared to that obtained from sugarcane molasses except for K. marxianus. On the other hand, these yeast strains were significantly (P. value 0.01) able to produce considerable amounts of bio-ethanol from sugarcane molasses. Out of the 7 yeast strains tested, 5 strains were able to produce more than or equal 5% bio-ethanol during the fermentation of sugarcane molasses. However, 2 strains only produced more than or equal 5% bio-ethanol during the fermentation of sugar beet molasses; the bio-ethanol amounts ranged between 50.6 and 62.4 g. L-1. It is worth to mention that the highest bio-ethanol producer strain on sugarcane and sugar beet molasses was *S. cerevisiae* followed by *C. kefyr*. These results conform to the findings of Singth et al. (2013). Al-Dulaimi et al. (2018) studied the ability of some yeasts to produce ethanol using dates, apricot and grapes, they found that alcoholic production obtained by *S. cerevisiae* was significantly higher than that obtained by *C. kefyr*. This is might be due to that *S. cerevisiae* is known to tolerate the growth in media rich with molasses and this could increase its production capability of bio-ethanol. However, *C. kefyr* is more preferable in kefyr fermentation (Al-Mohammadi et al., 2020).

EFFECT OF INITIAL pH ON BIO-ETHANOL YIELD

Initial pH value of fermentation broth had an influence on bio-ethanol production by yeast strains using two types of molasses as carbon source. Optimal pH value is necessary for the activity of plasma membrane-based proteins, including enzymes and transport proteins (Enan et al., 2013).

Table 3: Reducing sugar and bio-ethanol yield using two molasses as substrate.

Strains	Sugar cane molasses		Sugar beet molasses	
	Reducing sugar	Bio-ethanol yield (g/L)	Reducing sugar	Bio-ethanol yield (g/L)
S. Cerevisiae F514	40.8 ± 2.2b	94.9 ± 0.8a	8.74 ± 1.9c	62.4 ±1.5a
S. cerevisiae ATTCC4098	38.0 ± 0.4b	52.4 ± 1.5c	11.5 ± 0.5ab	50.6 ± 0.7c
C. kefyr	47.8 ± 2.3a	55.7 ± 0.7b	13.7 ± 2.3a	56.1 ± 0.4b
C. tropicalis	34.6 ± 3.2c	54.6 ± 1.3b	9.4 ± 0.6 bc	29.4 ± 2.6 f
K. marxianus	37.7 ± 1.8bc	9.87 ± 1.1e	12.7 ± 2.4a	34.7 ± 3.3e
P. stipites	34.9 ± 0.3c	51.2 ± 0.2c	4.58 ± 0.5d	4.67 ± 0.3g
Zymomonas mobilis	31.6±2.5d	45.8±0.96d	12.2±0.21a	44.2±3.5d

Means ± standard deviation within a column followed by the same letter are not significantly different at P= 0.05 when compared by Duncan test.

Fermentation using the strain F514, was carried out at pH 4, 4.5, 5, 5.5 and 6.0 at 30°C, to determine the effect of pH on bio-ethanol production. Data given Figure 1 showed that bio-ethanol yield by *S. cerevisiae* from sugarcane molasses was higher at pH 4.5 (176.9 g L-1) than PH 5 (143 g L-1) and 6 (95.5 g L- 1). On the other hand, bio-ethanol yield by *C. kefyr* from the sugarcane molasses was higher at pH 5.5 (119.1 g L-1). From the data given in Figure 1 pH 4.5 and 5.5 were optimal for bio-ethanol production by *S. cerevisiae* and *C. kefyr* from sugarcane molasses respectively. However, pH 4 was the optimal pH value for bio-ethanol production by *S. cerevisiae* and *C. kefyr* from sugar beet molasses respectively. This result also in contradiction with that given in the study of El-Sayed et al. (2020) who reported that optimal pH for bio-ethanol production was around 4.5. The optimum pH value that induces the production of certain metabolite depends on the producer strain itself; different strains within certain one species or different species within one Genus could show different growth conditions (Enan et al., 1996; El-Sayed et al., 2020).

of bio-ethanol at various temperatures indicated that the fermentation temperature 30°C was the optimum incubation temperature for production of bio-ethanol by two yeast strains on medium containing sugarcane molasses as carbon source. While, it was 30 and 30 and 32 °C for *S. cerevisiae* and *C. kefyr*, respectively. Bio-ethanol production by S.cerevisiae increased with the increase in temperature reaching the maximum value at 30°C (Figure 2). Further increase in temperature reduced the percentage of bio-ethanol production. These results contradict with the study of Yah et al. (2010) who found that the optimal temperature of bio-ethanol production was 25°C as *S. cerevisiae* IFST -072011 can produce bio-ethanol at higher temperature, it will be more suitable for industrial production of bio-ethanol. Hashem et al. (2021), studied the optimization of fermentation conditions for enhanced bioethanol yields by some yeasts. They found that the temperature of incubation has certain effect on bioethanol production; optimum temperature of incubation appeared to be in the range 30-35 °C. This indicated on the mesophilic nature of the yeasts.
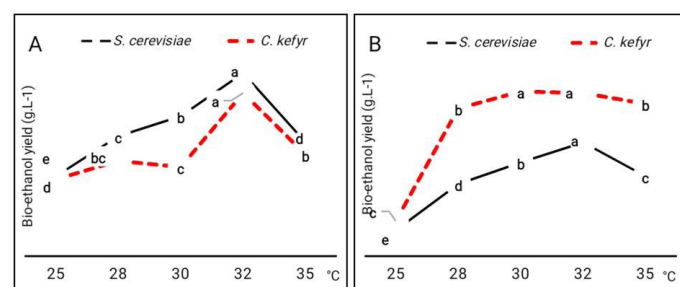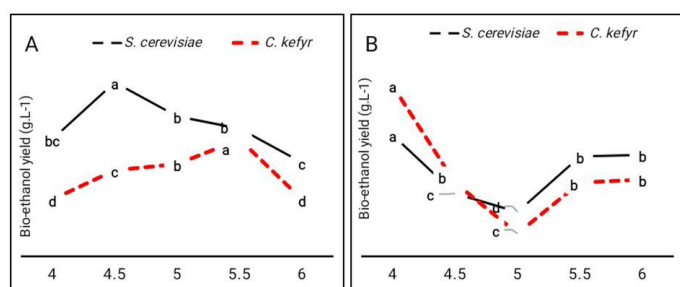
Figure 1: Effect of initial pH values on bio-ethanol production (g.L-1) by *S. cerevisiae* and *Ckefyr* using two kinds of molasses. (A) Sugarcane molasses (B) Sugar beet molasses Number followed by the same letter are not significantly different at P= 0.05 when compared by Duncantest.

EFFECT OF DIFFERENT TEMPERATURE INCUBATION ON BIO-ETHANOL YIELD

The incubation temperatures showed marked effect on bio-ethanol production by the two strains using molasses as carbon source. To determine the effect of incubation temperature on bio-ethanol production of yeast strains, fermentation experiments were carried out at 25, 28, 30, 32 and 35°C using reducing sugar of sugarcane and sugar beet molasses. Bio- ethanol production gradually increased during fermentation temperature of 25 to 30°C and then sharply decreased with higher fermentation temperatures (Figure 2). At 32°C, *S. cerevisiae* and *C. kefyr* produced the highest yield of bio-ethanol using sugarcane molasses with superiority of *S. cerevisiae* in bio-ethanol production.

Moreover, growing of yeasts on sugar beet molasses showed maximum bio-ethanol production at 30 and 32 °C for *S. cerevisiae* and *C. kefyr* respectively. The yield

Figure 2: Effect of incubation temperature on bio-ethanol production (g.L-1) by *S. cerevisiae* and *C. kefyr* using two kinds of molasses. (A) Sugarcane molasses (B) Sugar beet molasses Number followed by the same letter are not significantly different at P= 0.05 when compared by Duncan test at P= 0.05 when compared by Duncan test.

EFFECT OF INCUBATION PERIODS ON BIO-ETHANOL PRODUCTION

Incubation periods showed significant distinctive on bio-ethanol production (P<0.05) by the investigated yeast strains using two types of molasses as carbon source. To assess the effect of incubation periods on bio-ethanol (Figure 3) production, fermentation processes were carried out for 1, 2, 3, 4 and 5 days using both sugarcane sugar beet molasses. The fermentation was done under optimal pH and temperature for two yeast strains that were obtained from the previous trials. Data in Table 4 observed that the bio- ethanol production was increased during incubation periods up to two days and then decreased again to reach the minimum levels at the fifth days of incubation. These results are real in fermentation process using two kinds of molasses by two yeast strains. The highest production of bio-ethanol was showed at the second day of fermentation.

The production of bio-ethanol from sugarcane molasses reached 137.3 and 120.5 using *S. cerevisiae* and *C. kefyr* respectively. Moreover, the bio-ethanol production from sugarcane molasses was higher than its production from sugar beet molasses. (Table 5) Previous published results have indicated that the fermentation time greatly influence the biotechnol production by yeasts (Enan et al., 2018). It was approved that yeasts produce optimal values of bioethanol by fermentation of sugary substrates using actively growing yeast cells in the early to mid exponential phase of growth after 2 days of growth (El-Sayed et al., 2015) (Table 5).
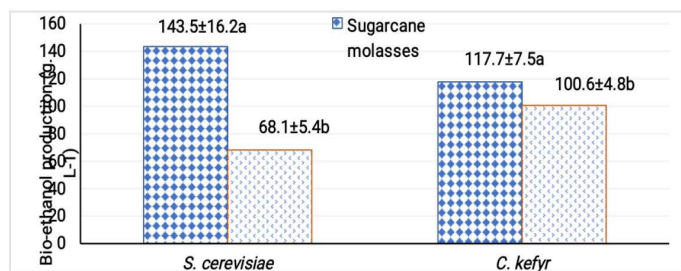
Figure 3: Effect of optimal conditions on bio-ethanol production by *S. cerevisiae* and *C. kefyr* using two kinds of molasses. Number followed by the same letter are not significantly different at P= 0.05 when compared by Duncan test.

Table 4: Effect of incubation period on bio-ethanol production by *S. cerevisiae* and *C. kefyr* using two kinds of molasses.

Incuba-tion period (day)	Sugarcane molasses		Sugar beet molasses	
	S. cerevisiae	*C. kefyr*	*S. cerevisiae*	*C. kefyr*
1	110±7.1b	110.3±4.3a	43.9±4.2d	67.6±1.2c
2	137.3±14.4a	120.5±6.1a	69.1±2.8a	99.6.2±4.9a
3	96.6±11.7b	77.6±5.4b	63.9±4.1ab	76.8±2.6b
4	70.7±3.6c	64.2±8.6c	61.9±1.2b	62.2±0.7d
5	54± 5.8c	40.1±9.0d	53.9±2.5c	31.6±2.5e

Means ± standard deviation within a column followed by the same letter are not significantly different at P= 0.05 when compared by Duncan test.

EFFECT OF OPTIMAL CONDITIONS ON BIO-ETHANOL PRODUCTION

At the optimal fermentation temperature and initial pH, free cells of *S. cerevisiae* and *C. kefyr* were able to produce 143.5 and 68.1 g L-1 from sugarcane molasses, bio- ethanol after 48 h while gave 117.7 and 100.6 g L-1 from sugar beet molasses. Under all optimum condition *S. cerevisiae* gave bio-ethanol yield from sugarcane molasses higher than *C. kefyr* and vice versa in case of sugar beet molasses (El-sayed et al., 2015). Values of bioethanol produced were more or less similar to other published results as different

factors could prevent the absolute similarly of bioethanaole produced such as nature of the yeast strains used, media, growth conditions and nutritional factors (Enan et al., 2018; Abdel Shafi et al., 2020).

Table 5: Effect of different carriers on bio-ethanol production by *S. cerevisiae* and *C. kefyr* using two kinds of molasses.

Carriers	Sugarcane molasses		Sugar beet molasses	
	S. cerevisiae	*C. kefyr*	*S. cerevisiae*	*C. kefyr*
Loofa sponge	164.5±4.7bc	147.3±5.9c	134.2±4.3c	150.5±0.5c
Artificial fiber sponge	159.7±1.6c	139.0±6.1c	145.5±1.8b	162.5±3.2b
Agar agar	161.2±5.2c	150.6±5.1c	142.6±2.3bc	129.6±2.0d
Na-CMC	168.6±1.2b	164.5±1.4b	150.8±5.4b	158±2.7b
Sodium alginate	200.8±3.4a	198.1±10.3a	178.9±9.0a	196.1±5.4a

IMMOBILIZATION EFFICIENCY ON BIO-ETHANOL PRODUCTION

Immobilizations are of various advantages in industrial applications of yeasts. The most significant advantages of immobilized yeast cell systems is the ability to operate with high production yeild at different dilution rates that exceed the maximal specific growth rate; the increase of bio-ethanol yield and cellular stability and the decrease of process expenses due to the cell recovery and reutilization are which existed by this technique (El-Sayed et al., 2020). Immobilization of cells showed significantly effect on bio-ethanol production, as bio-ethanol production increased with immobilized cells. At optimum condition, maximal bio-ethanol production by *S. cerevisiae* was 143.5 and 117.7 g L-1 by free cells whereas 200.8 and 198.1 g L-1 bio-ethanol produced by immobilized cells using sodium alginate in the same condition at 48 hrs. regarding *C. kefyr*, the same trend of results was obtained. The data obtained herein have showed that the maximum production or bio-ethanol was obtained by immobilizing the cell on sodium alginate follow by Na-CMC. A study by Enan et al. (2020) supported this finding, where they concluded that immobilized *S. cerevisiae* produced higher concentration of bio-ethanol than free cells and within 24 h, as the immobilized *S. cerevisiae* strain could consume all the available sugar (Sing et al., 2013).

CONCLUSIONS AND RECOMMENDATIONS

From the obtained results, it can be concluded that a successful fermentation process depends on sugar concentration of the medium and nutritional parameters. The maximal production of bio-ethanol by *S. cerevisiae* was

obtained after 48 h of incubation with Sugarcane molasses adjusted at pH 4.5 and incubated at 32°C. At these conditions, 143.5 g L-1 bio-ethanol was produced by free cells against 200.8 g L-1 of bio-ethanol by immobilized cells. Concerning *C. kefyr*, the maximal production of bio-ethanol was obtained after 48 h of incubation with Sugarcane molasses, at pH 5.5 and 32°C by immobilized cells technique which was better for bio-ethanol production than free cells. These strains used (*S. cerevisiae* F514 and *C. kefyr* EMCC77) can be used for industrial production of bio-ethanol from different kinds of molasses.

ACKNOWLEDGEMENTS

NOVELTY STATEMENT

The maximal production of bio-ethanol by *S. cerevisiae*.

AUTHOR'S CONTRIBUTION

All authors have made asubstantial and intellectual contribution to the work, and confirmed it for publication.

Conflict of interest
The authors have declared no conflict of interest.

REFERENCES

• Abdel-Shafi S, Al-Mohammadi AR, Almanaa TN, Moustafa AH, Saad TMM, Ghonemy A, Anacarso I, Enan G, El-Gazzar N (2020). Identification and testing antidermatophytic oxaborole-6-benzene sulphonoamide derivative (OXBS) from Streptomyces atrovirens KM192347 isolated from soil. Antibiotics, 9: 176. https://doi.org/10.3390/antibiotics9040176

• Al-Dulaimi FKY, Abd-Alwahab WIA, Jasim ME (2018). Study ability of some yeasts to ethanol production by using dates, apricot and graps. Int. J. Adv. Res. Sci. Eng., 7(11): 5-10.

• Al-Mohammadi AR, Osman A, Enan G, Abdel Shafi S, El-Nemer M, Sitohy M, Taha MA (2020). Powerful antibacterial peptides from egg albumin hydrolysates., 13:9(12). 901. https://doi.org/10.3390/antibiotics9120901

• Balat M, Balat H (2009). Recent trends in global production and utilization of bio- ethanol fuel. Appl. Energy, 86(11): 2273-2282. https://doi.org/10.1016/j.apenergy.2009.03.015

• Behera S, Mohanty RC, Ray RC (2011). Ethanol production from mahula (*Madhuca latifolia* L.) flowers with immobilized cells of Saccharomyces cerevisiae in Luffa cylindrica L.

• sponge discs, Appl. Energy, 88(1): 212–215. https://doi.org/10.1016/j.apenergy.2010.07.035

• Chohan NA, Aruwajoye GS, Sewsynker-Sukai Y, Gueguim Kana EB (2020). Valorisation of potato peel wastes for bio-ethanol production using simultaneous accharification and fermentation: process optimization and kinetic assessment. Renew. Energy, 146: 1031-1040. https://doi.org/10.1016/j.renene.2019.07.042

• Demirbas A (2009). Biofuels securing the planet's future energy needs, Energy Conver. Manage., 50: 2239-2249. https://doi.org/10.1016/j.enconman.2009.05.010

• Demirbas MF, Balat M (2006). Recent advances on the production and utilization trends of bio-fuels: A global perspective. Energy Convers. Manage., 47: 2371-2381. https://doi.org/10.1016/j.enconman.2005.11.014

• El-Bahr MS, Elbakery MA, El-Gazzar N, Amin AA, Al-Sultan S, Alfattah M, Shousha S, Alhojaily S, Shathele M, Sabeq I, Hamouda A (2021). Biosynthesized iron oxide nanoparticles from petroselinum crispum leaf extract lead-acetate- induced anemia in male albino rats: Hematological, biochemical and histopathological features. Toxics, 9(6): 123. https://doi.org/10.3390/toxics9060123

• El-Gazzar N, Almaary KH, Ismail A, Polizzi G (2020). Influence of Funneliformis mosseae enhanced with titanium dioxide nanoparticles (TiO2NPs) on Phaseolus vulgaris L. under salinity stress. PLoS One, 15(8): e0235355. https://doi.org/10.1371/journal.pone.0235355

• El-Gazzar N, Almanaa NT, Reda MR, El Gaafary NM, Rashwan AA, Mahsoub F (2021). Assessment the using of silica nanoparticles (SiO2NPs) biosynthesized from rice husks by Trichoderma harzianum MF 780864 as water lead absorbent for immune status of Nile tilapia (Oreochromis niloticus). Saudi J. Biol. Sci., S1319-562X(21): 00395-8. https://doi.org/10.1016/j.sjbs.2021.05.027

• El-Gazzar N, Ismail AM (2020). The potential use of Titanium, Silver and Selenium nanoparticles in controlling leaf blight of tomato caused by Alternaria alternata. Biocatal. Agric. Biotechnol., 27: 101708. https://doi.org/10.1016/j.bcab.2020.101708

• El-Gazzar NS, Enan G (2020). Advances in phage inspired nanoscience-based therapy. Nanobioscience, Chapter 10, pp: 237-257 eds. Saxena S.K., Khurana S.P. Springer Nature Singapore. Pte Ltd. https://doi.org/10.1007/978-981-32-9898-9_10

• El-Sayed A, Enan G, Al-Mohammadi AR, Moustafa A, El-Gazzar N (2020). Detection, purification, elucidation of chemical structure and antiproliferative activity of taxol produced by Penicillium chrysogenum. Molecules, 25: 4822. https://doi.org/10.3390/molecules25204822

• El-Sayed TI, Atef D, Amer M, Mahdy A, Enan G (2015). Molecular characterization and inhibition by natural agents of multidrug resistant Candida strains causing vaginal candidiasis. Res. J. Med. Sci., 9(1): 1-7.

• Enan G, Abdel-Shafi S, Ouda S, Negm (2013). Characterization of probiotic lactic acid bacteria to be used as starter culture and protective cultures for diary fermentations. Int. J. Probiot. Prebiot., 8(4): 157-163.

• Enan G, Al-Mohammadi AR, Mahgoub S, Abdel SS, Eman A, Ghaly M, Mohamed T, El-Gazzar N (2020). Inhibition of Staphylococcus aureus LC554891 by Moringa oleifera seed extract either singly or in combination with antibiotic. Molecules, pp. 20-29. https://doi.org/10.3390/molecules25194583

- Enan G, El-Essawy AA, Uyttendael M, Debevere J (1996). Antibacterial activity of Lactobacillus planetarium UG1 isolated from dry sausage: characterization, production and bactericidal action of plantaricin UG1. Int. J. Fd. Microbiol., 30: 189-215. https://doi.org/10.1016/0168-1605(96)00947-6

- Enan G, Osman ME, Abdel-Haliem MEF, Abdel-Ghany S (2018). Advances in microbial and nucleic acids biotechnology. Biomed. Res. Int., pp. 1-2. https://doi.org/10.1155/2018/3102374

- Farmakis L, Kapolos J, Koliadima A, Karaiskakis G (2007). Study of the growth rate of Saccharomyces cerevisiae strains using wheat starch granules as support for yeast immobilization monitoring by sedimentation/sferic field-flow fractionation. Fd. Res. Int., 40: 717-724. https://doi.org/10.1016/j.foodres.2007.01.009

- Gokgoz M, Yigitoglu M (2011). Immobilization of Saccharomyces cerevisiae on to modified carboxymethylcellulose for production of ethanol. Bioproc. Biosyst. Eng., 34(7): 849-857. https://doi.org/10.1007/s00449-011-0535-x

- Hashem M, Alamri SA, Asseri TY, Mostafa YS, Lyberatos G, Ntaikou I (2021). On the optimization of fermentation conditions for enhanced bioethanol yields from starchy biowaste via yeast co-cultures. Sustainability, 13: 1890. https://doi.org/10.3390/su13041890

- Hernández L, Kafarov V (2009). Use of bio-ethanol for sustainable electrical energy production. Int. J. Hydrogen Energy, 34: 7041-7050. https://doi.org/10.1016/j.ijhydene.2008.07.089

- Hoekman SK (2009). Biofuels in the US-challenges and opportunities. Renew. Energy, 34: 14-22. https://doi.org/10.1016/j.renene.2008.04.030

- Inal M, Yigitoglu M (2011). Production of bio-ethanol by immobilized Saccharomyces cerevisiae onto modified sodium alginate gel. J. Chem. Technol. Biotechnol., 86(12): 1548-1554. https://doi.org/10.1002/jctb.2678

- Ismaiel AA, Ahmed AS, El-Sayed ER (2015). Immobilization technique for enhanced production of the immunosuppressant mycophenolic acid by ultraviolet and gamma-irradiated Penicillium roqueforti. J. Appl. Microbiol., 119(1): 112-126. https://doi.org/10.1111/jam.12828

- Kiran B, Kumar R, Deshmukh D (2014). Perspectives of microalgal biofuels as a renewable source of energy, Energy Conver. Manage., 88: 1228-1244. https://doi.org/10.1016/j.enconman.2014.06.022

- Kourkoutas Y, Kanellaki M, Koutinas AA (2006). Apple peaces as immobilization support of various micro, organisms. LWT, 39: 980-986. https://doi.org/10.1016/j.lwt.2006.02.024

- Laopaiboon L, Naunpeng S, Srinophakun P, Klarint P, Laopaiboon P (2009). Ethanol production from sweet sorghum juice using very high gravity technology: effects of carbon and nitrogen supplementations. Bioresour. Technol., 100: 4176-4182. https://doi.org/10.1016/j.biortech.2009.03.046

- Manyuchi M, Chiutsi MP, Mbohwa C, Muzenda E, Mutusva T (2018). Bio-ethanol from sewage sludge: A bio fuel alternative, S. Afr. J. Chem. Eng., 25: 123-127. https://doi.org/10.1016/j.sajce.2018.04.003

- Md F, Quayum M, Ahmed M, Chowdhury N (2012). Analysis of key factors affecting ethanol production by saccharomyces cerevisiae IFST-072011. Biotechnology, 11: 248-252. https://doi.org/10.3923/biotech.2012.248.252

- Miller GL (1959). Use of dinitrosalicylic acid reagent for determination of reducing sugar. Anal. Chem., 31(3): 426-428. https://doi.org/10.1021/ac60147a030

- Nasir A, Rahman SS, Hossain MM, Choudhury N (2017). Isolation of saccharomyces cerevisiae from pineapple and orange and study of metal's effectiveness on bio-ethanol production. Eur. J. Microbiol. Immunol., 7(1): 76-91. https://doi.org/10.1556/1886.2016.00035

- Nikolić S, Mojović L, Rakin M, Pejin D (2009). Bio-ethanol production from corn meal by simultaneous enzymatic saccharification and fermentation with immobilized cells of Saccharomyces cerevisiae var. Ellipsoideus. Fuel, 88(9): 1602-1607. https://doi.org/10.1016/j.fuel.2008.12.019

- Ogbonna JC, Mashima H, Tanaka H (2001). Scale up of fuel ethanol production from sugar beet juice using loofa sponge immobilized bioreactor. Bioresour. Technol., 76: 1-8. https://doi.org/10.1016/S0960-8524(00)00084-5

- Osman A, El-Gazzar N, Almanaa TN, El-Hadary A, Sitohy M (2021a). Lipolytic postbiotic from Lactobacillus paracasei manages metabolic syndrome in albino wistar rats. Molecules, 26(2): 472. https://doi.org/10.3390/molecules26020472

- Osman A, Enan G, Al-Mohammadi A, Abdel-Shafi S, Abdel-Hameid S, Sitohy M, El-Gazzar N (2021b). Antibacterial peptides produced by alcalase from cowpea seed proteins. Antibiotics, 10: 870. https://doi.org/10.3390/antibiotics10070870

- Pan H, Bao W, Xie Z, Zhang J, Li Y (2010). Immobilization of Escherichia coli cells with cis-epoxysuccinate hydrolase for D (-)-tartaric acid production. Biotechnol. Lett., 32: 235-241. https://doi.org/10.1007/s10529-009-0134-y

- Phisalophong M, Budiraharjo R, Bangrak P, Mongkolkajit J, Limtong S (2007). Alginate-Loofa as carrier matrix for ethanol production. J. Biosci. Bioeng., 104: 214-217. https://doi.org/10.1263/jbb.104.214

- Rasmey AHM, Hassan HH, Abdulwahid OA, Aboseidah AA (2018). Enhancing bio-ethanol production from sugarcane molasses by Saccharomyces cerevisiae Y17. Egypt. J. Bot., 58(3): 547-561. https://doi.org/10.21608/ejbo.2018.1820.1126

- Razmovski R, Pejin D (1996). Immobilization of saccharomyces diastaticus on wood chips for ethanol production. Folia Microbiol., 41: 201-207. https://doi.org/10.1007/BF02814700

- Rodríguez LA, Toro ME, Vazquez F, Cerrea-Daneri ML, Gouiric SC, Vallejo MD (2010). Bio-ethanol production from grape and sugar beet pomaces by solid- state fermentation. Int. J. Hydrogen Energy, 35: 5914-5917. https://doi.org/10.1016/j.ijhydene.2009.12.112

- Sadik MW, Halema AA (2014). Production of ethanol from molasses and whey permeate using yeasts and bacterial strains. Int. J. Curr. Microbiol. Appl. Sci., 3(3): 804-818.

- Saini P, Beniwal A, Kokkiligadda A, Vij S (2018). Response and tolerance of yeast to changing environmental stress during ethanol fermentation, Process Biochem., 72: 1-12. https://doi.org/10.1016/j.procbio.2018.07.001

- Sandven P, Lassen J (1999). Importance of selective media for recovery of yeasts from clinical specimens. J. Clin. Microbiol., 37: 3731-3732. https://doi.org/10.1128/JCM.37.11.3731-3732.1999

- Santos DT, Sarrouh BF, Rivaldi JD, Converti A, Silva SS (2008). Use of sugarcane bagasse as biomaterial for cell immobilization for xylitol production. J. Fd. Eng., 86: 542-548. https://doi.org/10.1016/j.jfoodeng.2007.11.004

• Shafiee S, Topal E (2009). When will fossil fuel reserves be diminished? Energy Policy, 37: 181-189. https://doi.org/10.1016/j.enpol.2008.08.016

• Singh AP, Sharma AK, Saran NS, Bishnoi NR (2013). Comparative study on ethanol production from pretreated sugarcane bagasse using immobilized Saccharomyces cerevisiae on various matrices. Renew. Energy, 50: 488-493. https://doi.org/10.1016/j.renene.2012.07.003

• Tan KT, Lee KT, AR (2008). Mohamed, role of energy policy in renewable energy accomplishment: The case of second-generation bio-ethanol. Energy Policy, 36: 3360-3365. https://doi.org/10.1016/j.enpol.2008.05.016

• Tsakiris A, Bekatrou A, Psarianos C, Koutinas AA, Marchant R, Banat LM (2004). Immobilization of yeast on dried raisin barriers for use in dry white winemaking. Fd. Chem., 87: 11–15. https://doi.org/10.1016/j.foodchem.2003.10.010

• Verbelen PJ, De Schuter DP, Delvaux F, Verstrepen KJ, Delvaux FR (2006). Immobilized yeast cell systems for continuous fermentation applications. Biotechnol. Lett., 28: 1515-1525. https://doi.org/10.1007/s10529-006-9132-5

• Walter A, Rosillo-Calle F, Dolzan P, Piacente E, da Chuna KB (2008). Perspectives on fuel ethanol consumption and trade. Biomass Bioenergy, 32: 730-748. https://doi.org/10.1016/j.biombioe.2008.01.026

• Yu J., Yue G, Zhong J, Zhang X, Tan T (2010). Immobilization of Saccharomyces cerevisiae to modified bagasse for ethanol production. Renew Energy, 35: 1130-1134. https://doi.org/10.1016/j.renene.2009.11.045