**Optamization ethanol production by two Local Yeast Strains using static batch fermentation**

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**ABSTRACT**

Two isolates of ethanol producing yeasts were isolated and identified as *Clavispora lusitaniae* Gr45 and *Saccharomyces cerevisiae* B1, then tested for improving its ethanol productivity by changing some nutritional and environmental conditions using YFM medium by static culture technique. Among different sources of nitrogen, ammonium sulfate give slight increase in many of measured factors than other sources with both strains, and it was found that 0.1% of ammonium sulfate is the best concentration. Determine the best carbon source, glucose was the obvious choice as the most appropriate sugar substrate for ethanol production by both strains, and by examining the effect of its concentration, increasing glucose concentration more than 5 % resulted in decreasing the ethanol concentration (gl-1) and ethanol yield (%) by both strains , record the lowest value at 30% glucose. There are significant effect on the growth and ethanol production by *Cl. lusitaniae* Gr 45 and *Sacch. cerevisiae* B1 on glucose concentrations ranged from 5 to 30 % and from 5 % to 25 %, respectively. The optimum environmental conditions which gave the highest ethanol productivity were: initial pH values 6.2, incubation temperature 30oC, inoculum size 10% and fermentation period 72h.

**Key words**: Bioethanol production, *Saccharomyces cerevisiae, Clavispora lusitaniae,* static batch culture, ammonium sulfate, glucose,environmental conditions

**INRTODUCTION**

Bioethanol is considered as one of the most promising renewable bioenergy resource in the future. It is nontoxic and biodegradable and has many economical and environmental benefits. For instance, contribution as an alternative to fossil fuels in reducing emissions of greenhouse gases and environmental pollution that contributes in global warming and climate chane **(Balat & Balat, 2009 and Matsakas & Christakopoulos, 2013a, 2013b)**.Worldwide, ethanol share could reach 10–20% of the gasoline consumption by 2030 **(Walter *et al.,* 2008)**.

The majority of the yeast species described so far is capable to ferment sugars to ethanol and carbon dioxide **(Barnett *et al.,* 1990). Walker (1998)** mentioned that if a yeast does not ferment glucose, it will not ferment other sugars and if a yeast ferment glucose, it will also ferment fructose and mannose. He mentioned also, that some yeasts respire pentoses better than glucose and large number of yeasts ferments disaccharides. **Rao *et al.* (2008)** isolated a total of 374 yeasts from a variety of rotten fruits and barks of trees. Out of these, 27 yeast strains were able to assimilate xylose and produce 0·12–0·38 g of ethanol per gram of xylose. **Miranda *et al.,* (2012)** studied five industrial *Saccharomyces cerevisiae* strains and found that maltose and glucose fermentations are strongly affected by the structural complexity of the nitrogen source where peptone and ammonium sulfate induced improved fermentation. several investigators have observed that yeast extract **(Casey *et al.,* 1984; Thomas & Ingledew, 1990 and Bafrncova *et al.,* 1999)**, ammonium **(Jones *et al.,* 1994)**, urea **(Jones &Ingledew, 1994a)**, calcium and magnesium **(Dornbek & Ingram, 1986*)*** have protective effects either on growth and fer­mentation or viability, which stimulate the fermentation rate and ethanol production **(Laopaiboon *et al.,* 2009).**

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**Fikret and Serpil** **(2006)** found that the rate extent of ethanol formation by *K. marxianus* NRRL-1995 did not increase by supplementing cheese whey powder (CWP) with external nutrient nitrogen and phosphate sources. Indicating that, the nitrogen and phosphorous content of CWP was sufficient for ethanol fermentation. **Ronghou *et al.* (2008)** stated that the inorganic salts, including N, P, K and Mg salt as nutrition materials, could improve the ethanol fermentation and enhance ethanol productivity due to the reasons that the inorganic salts are necessary for the growth and metabolism of yeast cells. The order of influence on improving ethanol yield was (NH4)2 So4<MgSO4<K2HPO4. The optimal inorganic salts supplement dose was determined as follows: K2HPO4 0.125%, (NH4)2 SO4 0.20% and MgSO4 0.05%. **Nikolić**[**a**](http://www.sciencedirect.com.dlib.eul.edu.eg/science/article/pii/S0961953410001352#aff1#aff1) ***et al.* (2010)** tested four different initial glucose concentrations (98, 125, 150 and 176 g l−1) as a carbon source in corn meal hydrolyzates enzymatically obtained free and immobilized *S. cerevisiae* var. *ellipsoideus* yeast, while the initial inoculum concentration was constant at 2% (v v−1). A maximum ethanol concentration (over 9% w w−1) was achieved during the fermentation with the highest initial glucose concentration of 176 g l−1 at 50 h of fermentation time. **Kiran sree *et al.* (2000)** mentioned that *Sacch. cerevisiae* optimum temperature ranges between 25°C and 30°C and the ethanol concentrations were highest at 30°C and 35°C and decreased at higher temperatures. Maximum ethanol yields obtained from 150 g/l glucose were 75 g/l at 30°C, respectively. They also stated that, various factors might have contributed to the decreased ethanol fermentation, such as the reduction of the pH in the fermentation media and loss of sugar transport activity at high initial sugar concentration as suggested by **Salmon and Maurico (1994). Fikret and Serpil (2006)** investigated the effects of initial pH, (initial pH was varied between 3 and 7) on the ethanol yield produced from fermentation of cheese whey powder solution by *K. marxianus* NRRL-1995 in batch experiments. They found that the most suitable initial pH maximizing the final ethanol yield is 5, whereas, **Sujit *et al.* (2009)** found that pH of 6.0 and temperature of 30°C were optimum for maximum ethanol concentration (225.0 4.0 g/kg flower) obtained from mahula flowers (*Madhuca latifolia* L.) after 72 h of fermentation in solid-state fermentation. **Nikolić**[**a**](http://www.sciencedirect.com.dlib.eul.edu.eg/science/article/pii/S0961953410001352#aff1#aff1) ***et al.*(2010)** investigated the effect of initial inoculum concentration on the ethanol fermentation by free and immobilized yeast using different initial inoculum concentrations: 2 and 5% in a free system, and 2, 10 and 20% in an immobilized system (w v−1) at constant initial glucose concentration of 176 g/l. The maximum final ethanol concentration, ethanol yield and volumetric productivity were achieved at initial inoculum concentration of 2%.

The present study was designed to evaluate the potential of using local yeast isolates for ethanol production. The study focused on isolation of ethanol producing yeasts, selection and identification of the most efficient producing isolates. The optimum nutritional and environmental conditions for maximum ethanol production were detected.

**MATERIAL AND METHODS**

**Yeast culture**

Two yeast isolates, Gr45 & B1 were isolated from grapes and banana juice respectively then selected as most efficient ethanol producing yeasts (**unpublished data**) ,were used in this study.

**Media used**

* Yeast extract peptone medium (YEPM) **(Liang *et al.,* 2008)**, which has the following composition (g/l): glucose (20), yeast extract (10) peptone (20) agar (20) and distilled water 1000 ml (pH 6.0). This media was used for culture maintainance as preservation media at 5°c.
* Yeast fermentation mediaum (YFM) **(Banat and Marchant, 1995)** which has the following composition (g/l) : KH2PO4 (2.0), MgSO4.7H2O(1.0),Amm.sulphate(1.0), Yeast extract (0.5), MnSO4(0.1), glucose (50) and distilled water 1000ml (pH 6.0). This media was used in all experiments with amount of 100 ml in 250 ml Erlenmeyer flasks.

**Stander inoculum**

Standard inoculum was prepared by inoculation of conical flask (250 ml in volume) containing 100 ml of YEPM with a loop of the tested culture. Then, inoculated flasks were incubated at 30oC for 24 hrs. The content of this flask was used as standard inoculum (0.D.1.3 and 0.9 for *Sacch. cerevisiae* and *Cl. lusitaniae*, respectively) for static flask culture.

**Identification of yeast isolates**

* **Sequence analysis of 26 S r RNA gene**

Used yeasts were completely identified using 26 S rDNA sequences analysis**.** Where,pure yeast cultures were grown overnight on nutrient broth, then cellular DNA was isolated using Cell Lysis method. 26S rRNA was amplified by Thermocycler (PTC – 100 TM Programmable Thermal Controller) using the primers, Forward: 5- AGAGTTTGATCCTGGCTCAG-3¢ Reverse: 5¢- TACCTTGTTACGACTT 3. The amplified 26S rRNA PCR product was sequenced using automated sequencer (Synergy scientific, Chennai). The Sequence similarity search was done for the 16S rDNA sequence using online search tool called BLAST (http://www.ncbi.nlm. nih.gov/ blast/). The unknown species was identified using the maximum aligned sequence through BLAST search **(Altschul *et al.,*1997**).

* **phylogenetic relationships**

The acquired sequences were used for a gene homology search, with the 26S rRNA sequences available in the public databases from BLAST http://www.ncbi.nlm.nih.gov/ BLAST/, NCBI, Bethesda, MD, USA), and were identified to the generic level. Using the CLUSTAL-X Multiple Sequence Alignment Program (Strasburg, France), the 16S rRNA sequences of the isolated strains were (aligned with sequences of related organisms obtained from GenBank. Phylogenetic analysis was performed with PHYLIP, and a phylogenetic tree was constructed via the neighbor-joining method using the TreeView program. To validate the reproducibility of the branching pattern, a bootstrap analysis was performed.

**Fermention process**

The fermentation was carried out in 250 ml Erlenmeyer flasks containing 100 ml of YFM. The flasks were inoculated with 10 ml stander inoculum and incubated at 30°C for 3 days as static batch culture. At the end of incubation the growth denisty as optical denisty, ethanol and glucose concentration were determined. Ethanol parameters were also calculated.

**Nutritional requirement**

Different organic and inorganic nitrogen and carbon sources were studied. Nitrogen sources applied were trypton, peptone, malt extract, beef extract, ammonium chloride, sodium nitrate and ammonium nitrate, i.e., seven trials were done to replace present source in the original medium (ammonium sulfate) with an amounts equal to that the previous sources of nitrogen. Also, Five trial with different ammonium sulphate [(NH4)2 SO4] concentrations (from 0.05 % to 0.4%) were used to study their effect on ethanol production by used yeast strains.

six different carbon sources were tested for ethanol production by tested strains in YFM medium in amounts equal to that present in the original medium. Carbon sources applied were fructose, sucrose, maltose, , mannitol, sodium acetate and sodium citrate. Also, the effects of different concentrations of glucose ranged from 5 to 30 % on ethanol production were studied.

**Environmental conditions**

Six levels of initial pH of the productive medium ranged from 3.0 to 8.0.

Different incubation temperatures ranged from 20°C to 45°C were chosen for studying their effects on ethanol production by tested strains.

In order to detect the optimum fermentation period, ethanol production were determined periodically every 24 hrs during the fermentation period (144 hrs).

Different volumes of standard inoculum ranging from 2 to 14 ml were used to inoculate 100 ml of productive medium to determine the best inoculum size.

**Chemical determination**

* Residual glucose in fermented culture was determined with glucose kits according to the methods of **Young DS (2001).**
* Ethanolin fermented culture was determined using the method of **Lau and Luk (1994).**
* Yield of ethanol and productivity were calculated according to **Gamal *et al.* (1991**) using the fallowing equations:



* Conversion coefficient of ethanol was calculated according to **Ramadan et al, (1985)** as following:



**Statistical analysis**

The collected data were statistically analyzed using SPSS computer analysis program **(Forster, 2001).**

**RESULTS AND DISCUSSION**

**Identification of yeast isolates by amplification and sequencing of 26S rRNA gene**

From sequencing the 26S rRNA gene, the isolate B1 was recognized as *Saccharomyces cerevisiae*, while the isolate Gr45 was recognized as *Clavispora lusitaniae*. The 26S rRNA sequence of the isolate revealed a close relatedness to Bacillus amyloliquifaciens with 100 % and 96 % similarity for *Saccharomyces cerevisiae* and *Clavispora lusitaniae*, respectively. Upon the amplification of 26S rRNA sequence using specific primer, an amplified product of 1500 bp was obtained which was then sequenced and compared with the Gen Bank data bases using nucletoid BLASTN by the Finch TV program (<http://www.geospiza.com/> Products/finchtv.shtml). Amplified product was subjected to DNA sequencing with automatic sequencer. The following were the sequences of *Saccharomyces cerevisiae* and *Clavispora* *lusitaniae* 26S rRNA complete sequence.



*Saccharomyces cerevisiae* 26S ribosomal RNA gene, partial sequence



*Clavispora lusitaniae* 26S ribosomal RNA gene, partial sequence

**Effect of Different Nitrogen Sources**

Data presented in table (1) show that on all tested nitrogen sources, *Cl. lusitaniae* Gr45 attained a recordable growth. The highest growth were obtained on tryptone (O.D = 1.65). While the control treatment (ammonium sulfate) achieved growth of 1.06. The highest concentration of ethanol, ethanol productivity and ethanol yield (11.5 gl-1, 0.16gl-1h-1, 23.04 % respectively) were obtained by using tryptone, malt extract, beef extract or ammonium sulfate as sole nitrogen source on YFM medium. Glucose was nearly consumed completely in all treatments except malt extract treatment. The lowest consumed sugar and highest conversion coefficient being 48.14gl-1 and 23.89% were recorded at malt extract treatment. In case of *Sacch. cerevisiae* B1 (Table 2), the highest growth was obtained on ammonium sulfate (O.D = 1.88), followed on ammonium nitrate that achieved growth of 1.73. The highest concentration of ethanol, ethanol productivity, ethanol yield and conversion coefficient (11. 5 gl-1, 0.16 gl-1h-1, 23.0 % and 23.0%, respectively) were obtained by using malt extract or ammonium sulfate (control). Glucose was nearly consumed completely by all treatments which ranged from 48.96 to 50 gl-1.At the end of fermentation period, pH values of 6.2 and ranged from 3.6 to 4.9 were attained by *Cl. lusitaniae* Gr45 and *Sacch. cerevisiae* B1, respectively. In similar studies **Nahvi *et al.* (2002)** noticed that addition of amm. sulfate can improve ethanol production about 10%. In contrast, **Duhan *et al.* (2013**) found that peptone at 1.5 g/l gave higher ethanol yield, than yeast extract or ammonium sulphate for ethanol production by *Sacch. cerevisiae* MTCC-170 in YEPD media.

From the forgoing results, it could be concluded that the production of ethanol by *S. cerevisiae* B1and *Cl. lusitaniae* Gr45 was preferable on ammonium sulfate as nitrogen source.

**Effect of Different Ammonium Sulphate Concentrations**

Data given in **Tables (3&4)** show that , there was a slight increase in ethanol production by *Cl. Lusitaniae* Gr 45 and *Sacch. Cerevisiae* B1 with increase of amm. sulfate from 0.05 to 0.1 % recording the maximum value of ethanol production being 11.4 and 11.5 gl-1, respectively. The corresponding figures of ethanol yield and productivity were 22.8 % & 0.16 g l-1h-1 and 23 % & 0.16 g l-1h-1 for *Cl. lusitaniae* Gr 45 and Sacch*. Cerevisiae* B1, respectively. Increasing the amm. sulfate concentration than 0.1 % led to decrease the ethanol production to record the lowest value by *Cl. lusitaniae* Gr 45 and *Sacch. cerevisiae* B1 being 10.05 and 11.02 g l-1, respectively. The cell densities (O.D) gave the same trend and recorded the highest value at 0.1 % amm. sulfate .Moreover , significant effect on the growth and ethanol production by *Cl. lusitaniae* Gr 45 was observed at all amm. sulfate concentrations, whereas no significant change between amm. sulfate at 0.1 & 0.2 % or between amm. sulfate 0.3 & 0.4 % on the growth and ethanol production by *Sacch. cerevisiae* B1.Also ,it could be noticed that there are little change in the final PH values for all treatments was obtaind by tested strains and ranged from 3.9 to 4.0 and from 3.4 to 3.7 by *Cl. lusitaniae* Gr 45 and *Sacch. cerevisiae* B1,respectively.

Generally, it could be concluded that increasing ammonium sulphate from 0.1 to 0.4%is recommended when *Cl.lusitaniae* G*r*45 or *Sacch. cerevisiae* B1 was used for ethanol production. Therefore, further studies will be carried out using 0.1% (NH4)2SO4 as nitrogen source.

Obtained result is relatively different from results obtained by **Ronghou *et al.* (2008)** and **Banat and Marchant (1995),** wherefound that0.2 % ammonium sulfate is best percentage for highest ethanol production. **Liu *et al.* (2008**) indicated that the influence order on improving ethanol yield was (NH4)2 SO4>MgSO4>K2HPO4 and the optimal concentration of amm. sulfate was 0.2%.

**Effect of Different Carbon Sources**

Data in Tables (5&6) clearly show that *Cl. lusitaniae* Gr45and *Sacch.cerevisiae* B1 grew perfectly on most tested carbon sources, except sodium acetate and sodium citrate*.* At all different carbon sources, *Cl. lusitaniae* Gr45 gave higher growth than *Sacch.cerevisiae*B1 and recorded the highest values of optical density being 2.28, 2.27 and 2.27 on glucose, maltose, and mannitol, respectively. Whereas, the highest ethanol concentration produced by *Cl. lusitaniae*Gr45 and *Sacch. cerevisiae* B1 being 12.3&11.5 gl-1 were obtained in glucose medium (control). At this treatment, *Cl. lusitaniae.*Gr45 recorded the highest values of ethanol productivity and ethanol yield being 0.17 gl-1h-1 and 24.6 %, respectively. For *Sacch. cerevisiae* B1, the highest values of ethanol productivity and ethanol yield were 0.16 gl-1h-1, 23.0 %, respectively. At different carbon sources, the highest value of final pH were recorded at sodium acetate and sodium citrate treatments by both strains where ranged from 3.5 to 4.6 and from 3.5 to 4.1 by *Cl. lusitaniae* Gr 45 and *Sacch. cerevisiae* B1 respectively. Generally, it could be concluded that *Cl. lusitaniae* Gr45 gave higher ethanol production than *Sacch. cerevisiae* B1in glucose as sole carbon source whereas the vice versa was true for sucrose and maltose. So, glucose was the obvious choice as the most appropriate sugar substrate for ethanol production by both strains and will be added as sole source of carbon in YFM medium in further studies.

It is worthy to mention that the fermentable sugars in the hydrolysate of lignocelluloses are mainly composed form glucose and xylose. However, few microorganisms can efficiently convert both of these sugars to ethanol. Thus, isolation of new strains of yeasts have the ability to utilize glucose efficiently as single source of carbon is required for economical production of ethanol from lignocellulosic materials **(Galbe and Zacchi, 2002 and Li *et al.,* 2009).**

**Effect of Different Glucose Concentrations**

Data presented in Tables (7 & 8) reveal that *Cl .lusitaniae* Gr 45 and *Sacch. cerevisiae* B1 grew well on media containing glucose concentrations ranged from 5 to 30 %. The growth being 2.28 and 2.17 was observed in medium containing 10 % glucose by *Cl. lusitaniae* Gr 45 and *Sacch. cerevisiae* B1, respectively. Whereas, the highest values of ethanol concentration being 12.0 and 11.5 gl-1 were recorded at glucose concentration of 5% by *Cl.lusitaniae*Gr45 and *Sacch. cerevisiae* B1,respectively. Increasing the glucose concentration more than 5 % resulted in decreasing the ethanol concentration (gl-1) and ethanol yield (%) by both strains recorded the lowest value at 30% glucose. It means that, 5%glucose was the best carbon source concentration for ethanol production. The results also, indicated that there are significant effect on the growth and ethanol production by *Cl. lusitaniae* Gr 45 and *Sacch.cerevisiae* B1 on glucose concentrations ranged from 5 to 30 % and from 5 % to 25 %, respectively. Similar results are obtained by **Srivastava *et al.,* (1997)** noticed that the ethanol production by *Sacch. cerevisiae* MTCC 1972 was decreased from 1.5 % to 0.1 % with increasing the initial glucose concentration from 10% to 20 %. While **Yu and Zhang (2004)** used a strain of *S. cerevisiae* VS can produce 4.02% (w/v) bioethanol in the presence of about 9.6 % glucose.

**Effect of Different Initial pH Values**

Data tabulated in **Tables (9 &10)** indicated that pH 6.2 was the most favorable value for ethanol production by both tested strains. At this value, the highest values for ethanol productivity, concentration, yield and conversion coefficient were 0.17 gl-1h-1, 12.25 gl-1, 24.5 % and 25.16 % for *Cl. lusitaniae* Gr 45 and were 0.16 gl-1h-1, 11.52 gl-1, 23.04 %, 31.7% for *Sacch.cerevisiae*B1, respectively. Also, the final pH was 4.6 and 4.2 at this treatment. The highest value of growth for both strains (1.8) was obtained at pH 7.0. Decreasing the initial pH value from 7.0 to 6.2 led to decrease the growth of *Cl. Lusitaniae* Gr 45 and *Sacch.cerevisiae*B1 about 38 % and 11 % and increased the ethanol production about 17 % and 15 %, respectively.

In this respect to our results, **Fikret and Serpil**  **(2006)** found that most suitable initial pH maximizing the final ethanol yield is 5, when investigated the effects of initial pH, (initial pH was varied between 3 and 7) on the ethanol yield produced from fermentation of cheese whey powder solution by *K. marxianus* NRRL-1995 in batch experiments. Also results obtained by **Banat and Marchant (1995)** and **Yu *et al* (2009**), found that the optimum initial pH value for ethanol production were 6.0 and 6.39, respectively. While **Sujit *et al* (2009)** found that pH 6.0 and temperature 30°C were optimum for maximum ethanol concentration (225.0 4.0 g/kg flower) obtained from mahula flowers after 72 h of fermentation by *Sacch. cerevisiae* in solid-state fermentation.

**Effect of Different Incubation Temperature**

The results, seen in **Tables (11 and 12)**, obvious that ethanol concentration produced by *Cl. lusitaniae* Gr 45*.* and *Sacch. cerevisiae* B1 was increased as incubation temperature increased to reach the maximum values being 12.5 &11.5 gl-1, respectively, at 30°C in YFM medium. Also, it could be noticed that increasing the temperature from 20 to 30°C led to consume all the glucose concentration added (5%) and recorded the highest values of productivity, ethanol yield and conversion coefficient being 0.17 gl-1h-1, 25.0 % and 25.0 % by *Cl. lusitaniae* Gr 45 and 0.16 gl-1h-1,23.0% and 23.0% by *Sacch.cerevisiae*B1 respectively. Moreover, significant effect on yeast growth and ethanol production by tested strains was observed at all tested incubation temperature. These results are agreement with that obtained by **Kiran Sree *et al* (2000),** they stated that the ethanol production were highest at 30oC and 35oC and decreased at higher temperature.

**Effect of Different Fermentation Period**

Data presented in **Tables (13 and 14)** show the growth behavior and ethanol production by *Cl. lusitaniae* Gr45and *Sacch. cerevisiae* B1 grown on YFM medium during 144 h fermentation period at 30°C.There are significant effect on the growth and ethanol production by both strains during the fermentation period ranged from 24 to 144h. Data also revealed that *Cl. lusitaniae* Gr45*.* And *Sacch. cerevisiae* B1 mostly grew during the first 24 h and gave the highest biomass being 2.06 after 144 h for incubation period for *Cl. lusitaniae* Gr45*.* Whereas the growth of *Sacch. cerevisiae* B1 was stable after 24 h till 144 h of incubation period. During the first 24 h of fermentation, the glucose was approximately consumed then slight increase to record the highest figures of consumed sugar after 120 &72 h fermentation period by *Cl. lusitaniae* Gr45 and *S*. *cerevisiae* B1, respectively resulting the highest values of ethanol concentration, productivity, ethanol yield, conversion coefficient, being12.55gl-1, 0.17 gl-1h-1, 25.1 % , 25.1 % and 11.8 gl-1,0.16 gl-1h-1,23.6% and 23.6% for *Cl. lusitaniae* Gr45 and *Sacch. cerevisiae* B1 respectively after 72 h fermentation period. Also, **Nikolića *et al*, (2010)** found that glucose concentration in corn meal hydrolyzates enzymatically obtained by free and immobilized *Sacch. cerevisiae* var. *ellipsoideus* yeast, reached the maximum level after 72 h of the fermentation when the initial glucose concentration was 15.0%. Also, glucose concentrations of 17.6 % give maximum ethanol concentration (over 9% w w-1) after 50 h of fermentation.

**Effect of Different Inoculum Size**

Results recorded in **Tables (15 and 16)** indicated to significant effect of tested inoculum sizes on the growth of *Cl. lusitaniae* Gr 45 and on the production of ethanol by both tested strains. Whereas no significant effect was noticed on the growth of *Sacch.cerevisiae*B1.Also, the results revealed that the inoculation with 10% seed culture gave the highest figure of ethanol concentration being 12.5and 11.9 gl-1 by *Cl. lusitaniae* Gr45*.* and *Sacch. cerevisiae* B1, respectively. The corresponding figures for ethanol parameters recorded by *Cl. lusitaniae* Gr 45 were 0.17 gl-1h-1, 25.0 % and 29.5 % for productivity, ethanol yield and conversion coefficient, respectively. The equivalent parameters recorded by *Sacch. cerevisiae* B1 were 0.15 gl-1h-1, 22.2 % and 22.2 % for productivity, ethanol yield and conversion coefficient, respectively. Increasing the inoculum size than 10 % had the drastic effect on ethanol production by both tested strains. The final pH values obtained by *Cl. lusitaniae*Gr 45 were higher than *Sacch.cerevisiae*B1 and ranged from 3.5 to 4.2, whereas it ranged from 3.2 to 3.8 by *Sacch.cerevisiae*B1.

In similar studies, **Nikolić**[**a**](http://www.sciencedirect.com.dlib.eul.edu.eg/science/article/pii/S0961953410001352#aff1#aff1) ***et al.* (2010)** recorded that no significant differences in fermentation parameters after 38 and 74 h were realized by increasing the initial inoculum concentration from 2 to 5 % in a free system or from 2 to 20% in an immobilized system. The maximum final ethanol concentration, ethanol yield and volumetric productivity were achieved at initial inoculum concentration of 2% (w v−1).

On the light of the foregoing results, dealing with the effect of some factors on the production of ethanol by *Cl. lusitaniae* Gr45 and *Sacch.cerevisiae*B1,it could be stated that the highest ethanol concentration (12.55 &11.8 gl-1) as well as productivity ( 0.17 & 0.16 gl-1h-1) of ethanol could be obtained in YFM medium containing glucose 50 gl-1 as carbon source, amm. sulfate 1.0 gl-1 as nitrogen source,KH2PO4 2.0,MgSO4.7H2o 1.0,Yeast extract 0.5,MnSo4 0.1 gl-1 and 1000ml distilled water, at initial pH of 6.5,inoculation size 10 % of fermentation medium and incubated at 30oC for 72 h as a static batch culture.

**CONCLUSION**

By using YFM medium as a base medium to study different factors affecting ethanol production and the growth of *Sacch. cerevisiae* B1 and *Cl. lusitaniae* it could be concluded that ammonium sulfate was the best nitrogen source for the growth and ethanol production by both tested strains recorded the highest value at 0.1 % of amm. sulfate was the best concentration. Using 5%. Of glucose enhanced the growth and ethanol production by *Cl*. *lusitaniae* Gr45 and *Sacch. cerevisiae* B1 than other tested carbon sources (6 sources ). pH 6.2 found to be the most favorable for ethanol production and productivity by *Cl. lusitaniae* Gr45 (12.25 gl-1& 0.17 gl-1h-1) and *Sacch. cerevisiae* B1 ( 11.52 gl-1 & 0.16 gl-1h-1 ).Whereas the highest figure of growth for both strains (O.D= 1.8 ) was obtained at pH 7.0. The optimum fermentation temperature was 30oC, where recorded the highest values of ethanol concentration, productivity, yield and conversion coefficient being 12.5 gl-1, 0.16 gl-1 h-1, 25% & 25% for *Cl. lusitaniae* Gr45 and being 11.5 gl-1, 0.16 gl-1h-1,23 % & 23 % for *Sacch*. *cerevisiae* B1,respectively. The optimum fermentation period was found to be after 72 h, as it gave the highest figures of ethanol parameters by both tested strains, while there are significant effect on the growth and ethanol production by both strains during the fermentation period ranged from 24 to 144h. Inoculation with 10 % cell suspension gave the highest ethanol production being 12.5 and 11.9 gl-1 by *Cl. lusitaniae* Gr45 and *Sacch. cerevisiae* B1, respectively. Finally, there is significant effect of tested inoculum sizes on the growth of *Cl.* *lusitaniae* Gr 45 and on the production of ethanol by both tested strains. Whereas no significant effect was noticed on the growth of *Sacch. cerevisiae* B1.

**Table (1): Effect of different nitrogen sources on ethanol production by *Cl.lusitaniae* Gr45after 3 days incubation at 30oC as a static batch culture.**

|  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- |
| Nitrogen source | Growth  (O.D) | Residual glucose  (gl-1) | Consumed glucose  (gl-1) | Ethanol concentration  (gl-1) | Ethanol productivity  (gl-1h-1) | Ethanol yield  (%) | Conversion coefficient  (%) | Final pH |
| Tryptone | 1.65 | 0 | 50 | 11.5 | 0.16 | 23.0 | 23.0 | 6.2 |
| Peptone | 1.52 | 0.004 | 49.99 | 11.0 | 0.15 | 22.0 | 22.0 | 6.2 |
| Malt extract | 0.98 | 1.86 | 48.14 | 11.5 | 0.16 | 23.0 | 23.89 | 6.2 |
| Beef extract | 1.23 | 0.03 | 49.97 | 11.5 | 0.16 | 23.0 | 23.0 | 6.2 |
| (NH4)2SO4  (control) | 1.06 | 0 | 50 | 11.52 | 0.16 | 23.04 | 23.04 | 6.2 |
| NH4Cl | 1.1 | 0.009 | 49.99 | 11.05 | 0.15 | 22.1 | 22.1 | 6.2 |
| NaNO3 | 1.61 | 0 | 50 | 11.2 | 0.15 | 22.4 | 22.4 | 6.2 |
| NH4NO3 | 1.22 | 0 | 50 | 11.25 | 0.16 | 22.5 | 22.5 | 6.2 |

Productivity = Ethanol concentration (gl-1) / fermentation time (h) = gl-1h-1 **(Gamal *et al*., 1991).**

Ethanol yield (%) = [Ethanol concentration (gl-1) ÷ initial sugars (gl-1)]x100 **(Gamal *et al*., 1991).**

Conversion coefficient (%) = [Ethanol concentration (gl-1) ÷ consumed sugars (gl-1)] x100 **(Gamal *et al*., 1991).**

The values are mean of three replicates.

**Table (2): Effect of different nitrogen sources on ethanol production by *Saccharomyces cerevisiae* B1 after 3 days incubation at 30oC as a static batch culture.**

|  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- |
| Nitrogen source | Growth  (O.D) | Residual glucose  (gl-1) | Consumed glucose  (gl-1) | Ethanol concentration  (gl-1) | Ethanol productivity  (gl-1h-1) | Ethanol yield  (%) | Conversion coefficient  (%) | Final pH |
| Tryptone | 0.98 | 0 | 50 | 11.3 | 0.156 | 22.6 | 22.6 | 4.9 |
| Peptone | 1.58 | 0.106 | 49.89 | 11.2 | 0.155 | 22.4 | 22.4 | 4.8 |
| Malt extract | 1.6 | 0.035 | 48.96 | 11.5 | 0.16 | 23.0 | 23.0 | 4.9 |
| Beef extract | 1.64 | 0.01 | 49.99 | 10.8 | 0.15 | 21.6 | 21.6 | 4.6 |
| (NH4)2SO4  (control) | 1.88 | 0.13 | 49.87 | 11.52 | 0.16 | 23.04 | 23.1 | 3.6 |
| NH4Cl | 1.63 | 0.01 | 49.87 | 11.4 | 0.158 | 22.8 | 22.8 | 3.6 |
| NaNO3 | 1.3 | 0.022 | 49.97 | 11.03 | 0.15 | 22.06 | 22.07 | 4.4 |
| NH4NO3 | 1.73 | 0.015 | 49.98 | 11.4 | 0.158 | 22.8 | 22.8 | 3.6 |

Productivity = Ethanol concentration (gl-1) / fermentation time (h) = gl-1h-1 **(Gamal *et al*., 1991).**

Ethanol yield (%) = [Ethanol concentration (gl-1) ÷ initial sugars (gl-1)]x100 **(Gamal *et al*., 1991).**

Conversion coefficient (%) = [Ethanol concentration (gl-1) ÷ consumed sugars (gl-1)] x100 **(Gamal *et al*., 1991).**

The values are mean of three replicates.

**Table (3): Effect of different ammonium sulphate concentrations on ethanol production by *Cl. lusitaniae* Gr45after 3 days incubation at 30oC as a static batch culture.**

|  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- |
| Nitrogen source concentration  (%) | Growth  (O.D) | Residual glucose  (gl-1) | Consumed glucose  (gl-1) | Ethanol concentration  (gl-1) | Ethanol productivity  (gl-1h-1) | Ethanol yield  (%) | Conversion coefficient  (%) | Final pH |
| 0.05 | 1.928 e | 0.02 | 49.98 | 10.5 a | 0.15 | 21.0 | 21.0 | 4.0 |
| 0.1 (control) | 2.0 d | 0.02 | 49.98 | 11.4 b | 0.16 | 22.8 | 22.8 | 4.0 |
| 0.2 | 2.01 b | 0 | 50 | 10.4 c | 0.141 | 20.8 | 20.8 | 3.9 |
| 0.3 | 2.03 a | 0 | 50 | 10.2 d | 0.141 | 20.4 | 20.4 | 4.0 |
| 0.4 | 2.002 c | 0 | 50 | 10.05 e | 0.14 | 20.1 | 20.1 | 3.9 |

Productivity = Ethanol concentration (gl-1) / fermentation time (h) = gl-1h-1 **(Gamal *et al*., 1991).**

Ethanol yield (%) = [Ethanol concentration (gl-1) ÷ initial sugars (gl-1)]x100 **(Gamal *et al*., 1991).**

Conversion coefficient (%) = [Ethanol concentration (gl-1) ÷ consumed sugars (gl-1)] x100 **(Gamal *et al*., 1991).**

The values are mean of three replicates.

values in the same parameter followed by the same latter do not significantly differ from each other , according to **Duncon,s** at 5% level.

**Table (4): Effect of different ammonium sulphate concentrations on ethanol production by *Saccharomyces cerevisiae* B1 after 3 days incubation at 30oC as a static batch culture.**

|  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- |
| Nitrogen source concentration  (%) | Growth  (O.D) | Residual glucose  (gl-1) | Consumed glucose  (gl-1) | Ethanol concentration  (gl-1) | Ethanol productivity  (gl-1h-1) | Ethanol yield  (%) | Conversion coefficient  (%) | Final pH |
| 0.05 | 1.83 d | 0 | 50 | 10.8 c | 0.15 | 21.6 | 21.6 | 3.5 |
| 0.1 (control) | 1.92 a | 0 | 50 | 11.5 a | 0.16 | 23.0 | 23.0 | 3.4 |
| 0.2 | 1.92 a | 0 | 50 | 11.52 a | 0.16 | 23.04 | 23.04 | 3.5 |
| 0.3 | 1.89 c | 0 | 50 | 11.05 b | 0.15 | 22.1 | 22.1 | 3.7 |
| 0.4 | 1.91 b | 0.03 | 49.97 | 11.02 b | 0.15 | 22.04 | 22.05 | 3.6 |

Productivity = Ethanol concentration (gl-1) / fermentation time (h) = gl-1h-1 **(Gamal *et al*., 1991).**

Ethanol yield (%) = [Ethanol concentration (gl-1) ÷ initial sugars (gl-1)]x100 **(Gamal *et al*., 1991).**

Conversion coefficient (%) = [Ethanol concentration (gl-1) ÷ consumed sugars (gl-1)] x100 **(Gamal *et al*., 1991).**

The values are mean of three replicates.

Values in the same parameter followed by the same latter do not significantly differ from each other , according to **Duncon,s** at 5% level.

**Table (5): Effect of different carbon sources on ethanol production by *Cl. lusitaniae* Gr45after 3 days incubation period in YFM medium at 30°C as a static batch culture.**

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| Carbon  source | Growth  (O.D) | Ethanol concentration  (gl-1) | Ethanol productivity  (gl-1h-1) | Ethanol yield  (%) | Final pH |
| Fructose | 2.14 | 11.0 | 0.15 | 22.0 | 4.6 |
| Glucose  (control) | 2.28 | 12.3 | 0.17 | 24.6 | 3.5 |
| Sucrose | 2.09 | 9.7 | 0.13 | 19.4 | 4.3 |
| Maltose | 2.27 | 4.8 | 0.06 | 9.6 | 4.6 |
| Mannitol | 2.27 | 6.9 | 0.09 | 13.8 | 3.9 |
| Sodium acetate | 1.41 | 0.1 | 0.001 | 0.2 | 6.9 |
| Sodium citrate | 1.25 | 0.034 | 0.0004 | 0.068 | 6.9 |

Productivity = Ethanol concentration (gl-1) / fermentation time (h) = gl-1h-1 **(Gamal *et al*., 1991).**

Ethanol yield (%) = [Ethanol concentration (gl-1) ÷ initial sugars (gl-1)]x100 **(Gamal *et al*., 1991).**

The values are mean of three replicates.

**Table (6): Effect of different carbon sources on ethanol production by *Saccharomyces cerevisiae B*1 after 3 days incubation period in YFM medium at 30°C as a static batch culture.**

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| Carbon  source | Growth  (O.D) | Ethanol concentration  (gl-1) | Ethanol productivity  (gl-1h-1) | Ethanol yield  (%) | Final pH |
| Fructose | 2.03 | 10.3 | 0.14 | 20.6 | 4.1 |
| Glucose  (control) | 2.07 | 11.5 | 0.16 | 23.0 | 3.5 |
| Sucrose | 2.09 | 10.5 | 0.14 | 21.0 | 4.0 |
| Maltose | 2.09 | 10.02 | 0.14 | 20.04 | 4.1 |
| Mannitol | 0.88 | 1.4 | 0.019 | 2.8 | 5.5 |
| Sodium acetate | 0.8 | 0.034 | 0.0004 | 0.068 | 6.6 |
| Sodium citrate | 0.58 | 0.1 | 0.001 | 0.2 | 6.9 |

Productivity = Ethanol concentration (gl-1) / fermentation time (h) = gl-1h-1 **(Gamal *et al*., 1991).**

Ethanol yield (%) = [Ethanol concentration (gl-1) ÷ initial sugars (gl-1)]x100 **(Gamal *et al*., 1991).**

The values are mean of three replicates

**Table (7): Effect of different glucose concentrations on ethanol production by *Cl. lusitaniae* Gr 45 after 3 days incubation at 30oC in YFM medium as a static batch culture.**

|  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- |
| Glucose concentration  (%) | Growth  (O.D) | Residual glucose  (gl-1) | Consumed glucose  (gl-1) | Ethanol concentration  (gl-1) | Ethanol productivity  (gl-1h-1) | Ethanol yield  (%) | Conversion coefficient  (%) | Final pH |
| 5 (control) | 2.2 b | 0 | 50 | 12.0 a | 0.17 | 24.0 | 24.0 | 4.5 |
| 10 | 2.28 a | 14.5 | 35.5 | 11.5 b | 0.16 | 23.0 | 32.0 | 4.2 |
| 15 | 1.91 c | 13 | 37 | 11.01 c | 0.15 | 22.02 | 29.7 | 4.4 |
| 20 | 1.76 e | 11.8 | 38.2 | 10.9 d | 0.15 | 21.8 | 28.5 | 4.4 |
| 25 | 1.81 de | 14.8 | 35.2 | 10.6 e | 0.15 | 21.2 | 30.0 | 4.5 |
| 30 | 1.88 cd | 17.8 | 32.5 | 6.8 f | 0.09 | 13.6 | 20.0 | 4.5 |

Productivity = Ethanol concentration (gl-1) / fermentation time (h) = gl-1h-1 **(Gamal *et al*., 1991).**

Ethanol yield (%) = [Ethanol concentration (gl-1) ÷ initial sugars (gl-1)]x100 **(Gamal *et al*., 1991).**

Conversion coefficient (%) = [Ethanol concentration (gl-1) ÷ consumed sugars (gl-1)] x100 **(Gamal *et al*., 1991).**

The values are mean of three replicates.

Values in the same parameter followed by the same latter do not significantly differ from each other , according to **Duncon,s** at 5% level.

**Table (8): Effect of different glucose concentrations on ethanol production by *Saccharomyces cerevisiae B* 1 after 3 days incubation at 30oC in YFM medium as a static batch culture.**

|  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- |
| Glucose concentration  (%) | Growth  (O.D) | Residual glucose  (gl-1) | Consumed glucose  (gl-1) | Ethanol concentration  (gl-1) | Ethanol productivity  (gl-1h-1) | Ethanol yield  (%) | Conversion coefficient  (%) | Final pH |
| 5 (control) | 2.05 c | 0.07 | 49.93 | 11.5 a | 0.16 | 23.0 | 23.0 | 4.1 |
| 10 | 2.17 a | 0.09 | 49.91 | 11.0 b | 0.15 | 22.0 | 22.03 | 3.8 |
| 15 | 2.17 a | 0.13 | 49.87 | 10.8 c | 0.15 | 20.6 | 21.65 | 3.9 |
| 20 | 2.09 b | 0.83 | 49.17 | 10.5 d | 0.14 | 21.0 | 21.3 | 3.9 |
| 25 | 1.79 e | 17.8 | 32.2 | 9.5 e | 0.13 | 19.0 | 29.5 | 3.9 |
| 30 | 1.84 d | 17.8 | 32.2 | 9.5 e | 0.13 | 19.0 | 29.5 | 4.0 |

Productivity = Ethanol concentration (gl-1) / fermentation time (h) = gl-1h-1 **(Gamal *et al*., 1991).**

Ethanol yield (%) = [Ethanol concentration (gl-1) ÷ initial sugars (gl-1)]x100 **(Gamal *et al*., 1991).**

Conversion coefficient (%) = [Ethanol concentration (gl-1) ÷ consumed sugars (gl-1)] x100 **(Gamal *et al*., 1991).**

The values are mean of three replicates.

Values in the same parameter followed by the same latter do not significantly differ from each other , according to **Duncon,s** at 5% level.

**Table (9): Effect of initial pH on ethanol production by *Cl. lusitaniae* Gr45after 3 days incubation at 30oC in YFM medium as a static batch culture.**

|  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- |
| Initial pH | Growth  (O.D) | Residual glucose  (gl-1) | Consumed glucose  (gl-1) | Ethanol concentration  (gl-1) | Ethanol productivity  (gl-1h-1) | Ethanol yield  (%) | Conversion coefficient  (%) | Final pH |
| 3 | 0.84 d | 21.1 | 28.9 | 3.4 c | 0.047 | 6.8 | 11.7 | 3.4 |
| 4 | 1.0 c | 0.43 | 48.57 | 3.1 d | 0.043 | 6.2 | 6.2 | 4.0 |
| 5 | 1.04 d | 8.1 | 41.9 | 10.4 b | 0.144 | 20.8 | 24.8 | 4.3 |
| 6.2 (control) | 1.10 b | 1.32 | 48.68 | 12.25 a | 0.17 | 24.5 | 25.16 | 4.6 |
| 7 | 1.8 a | 0.03 | 49.97 | 10.4 d | 0.144 | 20.8 | 20.8 | 4.9 |
| 8 | 0.7 e | 0.04 | 49.96 | 2.8 e | 0.038 | 5.6 | 5.6 | 5.1 |

Productivity = Ethanol concentration (gl-1) / fermentation time (h) = gl-1h-1 (Gamal et al., 1991).

Ethanol yield (%) = [Ethanol concentration (gl-1) ÷ initial sugars (gl-1)]x100 (Gamal et al., 1991).

Conversion coefficient (%) = [Ethanol concentration (gl-1) ÷ consumed sugars (gl-1)] x100 (Gamal et al., 1991).

The values are mean of three replicates

Values in the same parameter followed by the same latter do not significantly differ from each other , according to Duncon,s at 5% level.

**Table (10): Effect of initial pH on ethanol production by *Saccharomyces cerevisiae* B1 after 3 days incubation at 30oC in YFM medium as a static batch culture.**

|  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- |
| Initial pH | Growth  (O.D) | Residual glucose  (gl-1) | Consumed glucose  (gl-1) | Ethanol concentration  (gl-1) | Ethanol productivity  (gl-1h-1) | Ethanol yield  (%) | Conversion coefficient  (%) | Final pH |
| 3 | 1.1 c | 16.6 | 33.4 | 4.2 f | 0.06 | 8.4 | 12.5 | 3.4 |
| 4 | 1.2 e | 17.7 | 23.3 | 5.4 e | 0.07 | 10.8 | 16.7 | 3.9 |
| 5 | 1.4 d | 16.6 | 33.4 | 10.8 b | 0.15 | 21.6 | 32.3 | 4.0 |
| 6.2 (control) | 1.6 f | 13.7 | 36.3 | 11.52 a | 0.16 | 23.04 | 31.7 | 4.2 |
| 7 | 1.8 b | 1.5 | 48.5 | 10.0 c | 0.14 | 20.0 | 20.6 | 4.6 |
| 8 | 1.7 a | 1.4 | 48.6 | 7.3 d | 0.10 | 14.6 | 15.0 | 4.6 |

Productivity = Ethanol concentration (gl-1) / fermentation time (h) = gl-1h-1 (Gamal et al., 1991).

Ethanol yield (%) = [Ethanol concentration (gl-1) ÷ initial sugars (gl-1)]x100 (Gamal et al., 1991).

Conversion coefficient (%) = [Ethanol concentration (gl-1) ÷ consumed sugars (gl-1)] x100 (Gamal et al., 1991).

The values are mean of three replicates.

Values in the same parameter followed by the same latter do not significantly differ from each other , according to Duncon,s at 5% level.

**Table (11): Effect of incubation temperature on ethanol production by *Cl. lusitaniae* Gr45 after 3 days incubation at 30oC in YFM medium as a static batch culture.**

|  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- |
| Incubation temperature  (°C) | Growth  (O.D) | Residual glucose  (gl-1) | Consumed glucose  (gl-1) | Ethanol concentration  (gl-1) | Ethanol productivity  (gl-1h-1) | Ethanol yield  (%) | Conversion coefficient  (%) | Final pH |
| 20 | 1.15 c | 18.8 | 31.2 | 0.8 f | 0.011 | 1.6 | 2.5 | 5.0 |
| 25 | 2.02 b | 6.4 | 43.6 | 11.2 b | 0.16 | 22.4 | 25.6 | 4.2 |
| 30 (control) | 2.06 g | 0 | 50 | 12.5 a | 0.17 | 25.0 | 25.0 | 4.8 |
| 35 | 2.2 a | 1.4 | 48.6 | 10.0 c | 0.14 | 20.0 | 20.5 | 4.3 |
| 40 | 1.11 d | 17.7 | 32.3 | 6.5 d | 0.09 | 13.0 | 20.1 | 4.6 |
| 45 | 0.42 e | 16.6 | 33.4 | 1.7 e | 0.02 | 3.4 | 5.0 | 5.3 |

Productivity = Ethanol concentration (gl-1) / fermentation time (h) = gl-1h-1 (Gamal et al., 1991).

Ethanol yield (%) = [Ethanol concentration (gl-1) ÷ initial sugars (gl-1)]x100 (Gamal et al., 1991).

Conversion coefficient (%) = [Ethanol concentration (gl-1) ÷ consumed sugars (gl-1)] x100 (Gamal et al., 1991).

The values are mean of three replicates.

values in the same parameter followed by the same latter do not significantly differ from each other , according to Duncon,s at 5% level.

**Table (12): Effect of incubation temperature on ethanol production by *Saccharomyces cerevisiae* B1 after 3 days incubation at 30oC in YFM medium as a static batch culture.**

|  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- |
| Incubation temperature  (°C) | Growth  (O.D) | Residual glucose  (gl-1) | Consumed glucose  (gl-1) | Ethanol concentration  (gl-1) | Ethanol productivity  (gl-1h-1) | Ethanol yield  (%) | Conversion coefficient  (%) | Final pH |
| 20 | 1.1 c | 16.6 | 33.4 | 2.7 f | 0.03 | 5.4 | 8.0 | 4.2 |
| 25 | 1.9 b | 5.4 | 44.6 | 9.6 c | 0.13 | 19.2 | 21.5 | 4.0 |
| 30 (control) | 1.88 b | 0.13 | 49.87 | 11.5 a | 0.16 | 23.0 | 23.0 | 3.6 |
| 35 | 2.2 a | 16.6 | 33.4 | 11.2 b | 0.16 | 22.4 | 33.5 | 4.1 |
| 40 | 0.7 d | 17.7 | 32.3 | 7.7 d | 0.1 | 15.4 | 23.8 | 4.5 |
| 45 | 0.41 e | 17.7 | 32.3 | 3.8 e | 0.05 | 7.6 | 11.3 | 4.9 |

Productivity = Ethanol concentration (gl-1) / fermentation time (h) = gl-1h-1 (Gamal et al., 1991).

Ethanol yield (%) = [Ethanol concentration (gl-1) ÷ initial sugars (gl-1)]x100 (Gamal et al., 1991).

Conversion coefficient (%) = [Ethanol concentration (gl-1) ÷ consumed sugars (gl-1)] x100 (Gamal et al., 1991).

The values are mean of three replicates.

Values in the same parameter followed by the same latter do not significantly differ from each other , according to Duncon,s at 5% level.

**Table (13): Effect of incubation period on ethanol production by *Cl. lusitaniae* Gr45grown in YFM medium at 30°C as a static batch culture.**

|  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- |
| Incubation period  (h) | Growth  (O.D) | Residual glucose  (gl-1) | Consumed glucose  (gl-1) | Ethanol concentration  (gl-1) | Ethanol productivity  (gl-1h-1) | Ethanol yield  (%) | Conversion coefficient  (%) | Final pH |
| 24 | 1.58 f | 1.348 | 48.65 | 5.2 e | 0.072 | 10.4 | 10.6 | 3.95 |
| 48 | 1.68 e | 0.31 | 49.69 | 9.9 d | 0.137 | 19.8 | 19.8 | 4.47 |
| 72  (control) | 1.76 d | 0.026 | 49.94 | 12.55 a | 0.17 | 25.1 | 25.1 | 4.03 |
| 96 | 1.82 c | 0.0224 | 49.97 | 11.5 b | 0.16 | 23.0 | 23.0 | 4.26 |
| 120 | 1.9 b | 0.0112 | 49.98 | 11.0 c | 0.15 | 22.0 | 22.0 | 3.21 |
| 144 | 2.06 a | 0.11 | 49.98 | 11.0 c | 0.15 | 22.0 | 22.0 | 4.25 |

Productivity = Ethanol concentration (gl-1) / fermentation time (h) = gl-1h-1 (Gamal et al., 1991).

Ethanol yield (%) = [Ethanol concentration (gl-1) ÷ initial sugars (gl-1)]x100 (Gamal et al., 1991).

Conversion coefficient (%) = [Ethanol concentration (gl-1) ÷ consumed sugars (gl-1)] x100 (Gamal et al., 1991).

The values are mean of three replicates.

Values in the same parameter followed by the same latter do not significantly differ from each other , according to Duncon,s at 5% level.

**Table (14): Effect of incubation period on ethanol production by *Saccharomyces cerevisiae* B1 grown in YFM medium at 30°C as a static batch culture.**

|  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- |
| Incubation period  (h) | Growth  (O.D) | Residual glucose  (gl-1) | Consumed glucose  (gl-1) | Ethanol concentration  (gl-1) | Ethanol productivity  (gl-1h-1) | Ethanol yield  (%) | Conversion coefficient  (%) | Final pH |
| 24 | 1.85 d | 0.05 | 49.95 | 10.2 e | 0.141 | 20.4 | 20.4 | 3.48 |
| 48 | 1.88 a | 0.025 | 49.97 | 10.4 d | 0.144 | 20.8 | 20.8 | 3.6 |
| 72  (control) | 1.86 c | 0.0134 | 49.99 | 11.8 a | 0.16 | 23.6 | 23,6 | 3.56 |
| 96 | 1.88 a | 0.0078 | 49.99 | 11.2 c | 0.155 | 22.4 | 22.4 | 3.61 |
| 120 | 1.87 b | 0.0134 | 49.99 | 11.3 b | 0.156 | 22.6 | 22.6 | 3.66 |
| 144 | 1.88 a | 0.0033 | 49.99 | 11.3 b | 0.156 | 22.6 | 22.6 | 3.66 |

Productivity = Ethanol concentration (gl-1) / fermentation time (h) = gl-1h-1 **(Gamal *et al*., 1991).**

Ethanol yield (%) = [Ethanol concentration (gl-1) ÷ initial sugars (gl-1)]x100 **(Gamal *et al*., 1991).**

Conversion coefficient (%) = [Ethanol concentration (gl-1) ÷ consumed sugars (gl-1)] x100 **(Gamal *et al*., 1991).**

The values are mean of three replicates.

Values in the same parameter followed by the same latter do not significantly differ from each other , according to **Duncon,s** at 5% level.

**Table (15): Effect of inoculum size on growth and ethanol production by *Cl. lusitaniae* Gr 45after 3 days incubation at 30°C in YFM medium as a static batch culture.**

|  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- |
| Inoculum size  (%) | Growth  (O.D) | Residual glucose  (gl-1) | Consumed glucose  (gl-1) | Ethanol concentration  (gl-1) | Ethanol productivity  (gl-1h-1) | Ethanol yield  (%) | Conversion coefficient  (%) | Final pH |
| 2 | 1.74f | 16.11 | 33.9 | 8.9 e | 0.123 | 17.8 | 26.2 | 3.7 |
| 4 | 1.93 a | 13.8 | 36.2 | 10.3 c | 0.14 | 20.6 | 28.4 | 3.5 |
| 6 | 1.8 b | 12.2 | 37.8 | 11.8 b | 0.16 | 23.6 | 31.2 | 3.9 |
| 8 | 1.77 e | 11.7 | 38.3 | 11.8 b | 0.16 | 23.6 | 30.8 | 4.12 |
| 10  (control) | 1.78 d | 7.7 | 42.3 | 12.5 a | 0.17 | 25.0 | 29.5 | 4.0 |
| 12 | 1.79 c | 3.9 | 46.1 | 9.8 d | 0.136 | 19.6 | 21.2 | 4.1 |
| 14 | 1.78 d | 0.2 | 49.8 | 6.0 f | 0.083 | 12.0 | 12.0 | 4.2 |

Productivity = Ethanol concentration (gl-1) / fermentation time (h) = gl-1h-1 **(Gamal *et al*., 1991).**

Ethanol yield (%) = [Ethanol concentration (gl-1) ÷ initial sugars (gl-1)]x100 **(Gamal *et al*., 1991).**

Conversion coefficient (%) = [Ethanol concentration (gl-1) ÷ consumed sugars (gl-1)] x100 **(Gamal *et al*., 1991).**

The values are mean of three replicates.

Values in the same parameter followed by the same latter do not significantly differ from each other , according to **Duncon,s** at 5% level.

**Table (16): Effect of inoculum size on growth and ethanol production by *Saccharomyces cerevisiae B*1 after 3 days incubation at 30°C in YFM medium as a static batch culture.**

|  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- |
| Inoculum size  (%) | Growth  (O.D) | Residual glucose  (gl-1) | Consumed glucose  (gl-1) | Ethanol concentration  (gl-1) | Ethanol productivity  (gl-1h-1) | Ethanol yield  (%) | Conversion coefficient  (%) | Final pH |
| 2 | 1.8 a | 0.258 | 49.74 | 10.2 e | 0.141 | 20.4 | 20.5 | 2.9 |
| 4 | 1.89 a | 0.2 | 49.8 | 10.6 d | 0.147 | 21.2 | 21.2 | 3.2 |
| 6 | 1.83 a | 0.11 | 49.89 | 10.7 c | 0.148 | 21.4 | 21.4 | 3.3 |
| 8 | 1.8 a | 0.08 | 49.94 | 10.9 f | 0.15 | 21.8 | 21.8 | 3.3 |
| 10  (control) | 1.86 a | 0.05 | 49.94 | 11.9 a | 0.15 | 22.2 | 22.2 | 3.6 |
| 12 | 1.82 a | 0.06 | 49.94 | 10.79 b | 0.15 | 21.6 | 21.6 | 3.6 |
| 14 | 1.88 a | 0.022 | 49.97 | 10.8 b | 0.15 | 21.6 | 21.6 | 3.8 |

Productivity = Ethanol concentration (gl-1) / fermentation time (h) = gl-1h-1 (Gamal et al., 1991).

Ethanol yield (%) = [Ethanol concentration (gl-1) ÷ initial sugars (gl-1)]x100 (Gamal et al., 1991).

Conversion coefficient (%) = [Ethanol concentration (gl-1) ÷ consumed sugars (gl-1)] x100 (Gamal et al., 1991).

The values are mean of three replicates.

Values in the same parameter followed by the same latter do not significantly differ from each other , according to Duncon,s at 5% level.

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