JPAM/8422/23 - Request to please peer review the article

13 messages

Journal of Pure and Applied Microbiology <editor@microbiologyjournal.org> Sat, Jan 28, 2023 at 1:
To: astrilia.damayanti@mail.unnes.ac.id

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It is our immense pleasure to request you to review one of the research article submitted in our journal for publishing. You are kindly requested to please go through the Abstract of the article titled "**Medium Optimization and Bioprocess Development for Bioethanol Production from Molasses Using *Saccharomyces cerevisiae* MTCC 173**"

Your approval to review this article is welcome; please do let us know at your earliest convenience so that we can send you the Full-text article along with Crossref Similarity Check report Powered by iThenticate.
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TITLE

Medium Optimization and Bioprocess Development for Bioethanol Production from Molasses Using *Saccharomyces cerevisiae* MTCC 173

ABSTRACT

Bioethanol demand accelerated through the last years. Thus, the need to improve acid production economically is aggravated. The fermentation process by *Saccharomyces cerevisiae* showed promising production by using molasses as a main substrate for the fermentation process. The present study designed for developing bioethanol production by *Saccharomyces cerevisiae* MTCC-170 in optimized medium through survey the bioprocess parameters. The medium used contains g/L^{-1} : Molasses 20, Yeast Extracts 4, peptone 5, Sodium acetate 1.5, K2HPO4 1.5, MnSO4.4H2O 0.12, MgSO4.7H2O 0.57, FeSO4.7H2O 0.03, KCl 0.3. The optimization process begin by medium optimization composition using one factor at time (OFAT) method for all bioprocess parameters included studies: incubation temperatures, culture medium, pH and inoculum sizes to reach the optimal parameters for bioethanol production, after that statistical compare with growth in the optimum medium in the shake flask phase and bioreactor 4-litter scale. The result showed that, the bioethanol production through optimized medium in shake flask stage reached to 4.50 g/L. while in bioreactor scale reach bioethanol produced the maximum yield at 16.50 g/L with rate production 0.170 g. $L^{-1}.h^{-1}$. On the other hand, Finally, the main goals of research have been efficiently achieved where the process parameters optimized and validated.

Keywords: Bioethanol, Saccharomyces cerevisiae, Medium optimization, Molasses, Bioreactor.

We sincerely hope that you will accept our invitation to review the article.
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Astrilia Damayanti <astrilia.damayanti@mail.unnes.ac.id> Sat, Jan 28, 2023 at 5:12 PM
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Astrilia Damayanti <astrilia.damayanti@mail.unnes.ac.id> Thu, Feb 9, 2023 at 5:50 PM
To: Journal of Pure and Applied Microbiology <editor@microbiologyjournal.org>

Dear editor,
I hereby submit the results of my review.

Best regards,

Dr. Ir. Astrilia Damayanti, S.T., M.T.

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Astrilia Damayanti <astrilia.damayanti@mail.unnes.ac.id> Fri, Feb 10, 2023 at 4:03 AM
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Astrilia Damayanti <astrilia.damayanti@mail.unnes.ac.id> Sun, Feb 12, 2023 at 2:51 AM
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Dr. Ir. Astrilia Damayanti, S.T., M.T.

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Journal of Pure and Applied Microbiology <editor@microbiologyjournal.org> Mon, Feb 13, 2023 at 4:20 PM
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Astrilia Damayanti <astrilia.damayanti@mail.unnes.ac.id> Mon, Feb 13, 2023 at 4:25 PM
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Best regards,

Dr. Ir. Astrilia Damayanti, S.T., M.T.

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Journal of Pure and Applied Microbiology <editor@microbiologyjournal.org> Mon, Feb 13, 2023 at 4:40 PM
To: Astrilia Damayanti <astrilia.damayanti@mail.unnes.ac.id>

Medium Optimization and Bioprocess Development for Bioethanol Production from Molasses Using Saccharomyces cerevisiae MTCC 173

By JPAM- 8422

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Medium Optimization and Bioprocess Development for Bioethanol Production from Molasses Using *Saccharomyces cerevisiae* MTCC 173

ABSTRACT

Bioethanol demand accelerated through the last years. Thus, the need to improve acid production economically is aggravated. The fermentation process by *Saccharomyces cerevisiae* showed promising production by using molasses as a main substrate for the fermentation process. The present study designed for developing bioethanol production by *Saccharomyces cerevisiae* MTCC-170 in optimized medium through survey the bioprocess parameters. The medium used contains g/L^{-1} : Molasses 20, Yeast Extracts 4, peptone 5, Sodium acetate 1.5, K2HPO4 1.5, MnSO4.4H2O 0.12, MgSO4.7H2O 0.57, FeSO4.7H2O 0.03, KCl 0.3. The optimization process begin by medium optimization composition using one factor at time (OFAT) method for all bioprocess parameters included studies: incubation temperatures, culture medium, pH and inoculum sizes to reach the optimal parameters for bioethanol production, after that statistical compare with growth in the optimum medium in the shake flask phase and bioreactor 4-litter scale. The result showed that, the bioethanol production through optimized medium in shake flask stage reached to 4.50 g/L. while in bioreactor scale reach bioethanol produced the maximum yield at 16.50 g/L with rate production 0.170 $g. L^{-1}.h^{-1}$. On the other hand, Finally, the main goals of research have been efficiently achieved where the process parameters optimized and validated.

***Keywords**: Bioethanol, Saccharomyces cerevisiae, Medium optimization, Molasses, Bioreactor.*

Introduction

Bioethanol regard an alternative source of energy has received distinctive attention in industrial over due to depletion in fossil fuels [1]. The bioethanol industrial of is very widespread and it is feasible both through chemical synthesis method or through a fermentation method [2]. Bioethanol is made using sugars as a feedstock, either by fermentation or through petrochemical and chemical processes [3]. For centuries, Bioethanol has been production of several types of microorganisms and broadly used in chemical and food industry [4]. Nowadays, several studies have been reported for bioethanol in pharmaceutical, food, fuel and grow to be one of the main organic acids in medical industries [5]. The global implementations of bioethanol in the food industry are based totally its protection category, according to the drug and food policies [6].

One of the important reasons for using bioethanol to use as an octane promotional in unleaded gasoline instead of triethyl butyl (C5H12O) and emissions from volatilization. in addition to decrease environmental risks [7]. Bioethanol produced in fermenter used a molasses as the main carbon source in the bioethanol manufacturing technology [8]. Furthermore, Bioethanol may also be made from other sources by fermenting lactic acid bacteria, which have two lines of bacterial fermentation pathways, the first of which produces lactic acid as the primary result and ethanol as a secondary product at different rates [9].

Recently there is growing global concern for finding best feedstock and economically inexpensive sources for the production of bioethanol [10]. The large expansion the of bioethanol request in the universal market related to improvement of more economically large scale fermentation process techniques and diverse sources of suitable raw materials [11]. In an endeavor to maximize byproducts for beneficial materials, molasses use in bioethanol production. Molasses are cheap, abundant and environmentally friendly [12].

A high quality manufacturing of bioethanol depends on the availability to produce high bioethanol concentrations and support high quality yield at the end of the process. pH, Temperature additionally inoculum sizes are significant bioprocess parameters for bioethanol production as they have their own role and function in order to ensure an appropriate quality eco-friendly bioethanol production. Optimal parameters for bioethanol production from molasses are fundamental to reduce capital cost and to obtain a high quality production of bioethanol [13].

Also, because the increasing charges of substrates such as sugarcane [14], sweet sorghum [15], sugar beet [16], wheat starch [17] and banana waste showed significant improvements in cultivation process parameters in shake flask fermentation process [18]. They are used in the food industry, All of these factors necessitate the search for a less expensive and plentiful substrate, as well as the development of a more efficient and less expensive technique, so that the product may be made more widely available at a lower cost. Bioethanol, which is created from natural, replaceable resources, is at the forefront of this transformation.

During this research different bioprocess parameters included studies: incubation temperatures, culture medium, pH and inoculum sizes to reach the optimal parameters for bioethanol production. The method of improving the media started by improving the medium optimization with OFAT strategy , then statistical comparison between growth in the optimum medium in the shake flask and the 4-liter bioreactor. Improving the medium components increases the bioethanol production. Also, validate that the semi specific medium formula being developed in this research can be applied for large scale bioethanol production in terms of cost, yield and quality.

Material and Methods

2.1. Working Cell Culture Preparation

Saccharomyces cerevisiae MTCC-170 is suitable strain for producing bioethanol since it shows its feasibility in former studies. This isolate preserved in freezer at -80°C.Were selected for investigation based on the bioethanol production.

Before sterilization, the strain was transported in a frozen glycerol, and the cells were cultured in medium and adjusted to pH 7.0. strain incubated for 48 h at 35°C. The colonies were collected and aspirated into a succession of 2 ml sterile cryovials tubes using a 50 % glycerol solution. These tubes were then frozen for 48 h at 4.0°C before being stored as a functioning cell bank at -80 °C ultra-deep freezer for future use.

2.2. Raw Material

Molasses is used as a main substrate for the fermentation process in this project. it was obtain from Baghdad company for the sugar industry, Baghdad City, Iraq. and stocked at 25°C for further use.

2.3. Medium of Fermentation

The selected medium for bioethanol production optimized in shake flask study. Medium composition consist from [gL^{-1}]: Molasses 20, Yeast Extracts 4.0, peptone 5.0, K_2HPO_4 1.5, Sodium acetate1.5, $MnSO_4.4H_2O$ 0.12, $MgSO_4.7H_2O$ 0.57, $FeSO_4.7H2O$ 0.03, KCL 0.3. pH set at 7.0.

2.4. Cell Dry Weight Determination

During cultivations, two flasks (each of 50 ml) were used for sample in shaking flask studies and 25 ml of broth was collected in sterile falcon tubes in bioreactor experiments. To remove cells from the fermentation broth, the samples were centrifuged at 5oC right away. For Bioethanol analysis,

the supernatants were promptly kept at -20 ºC, The precipitate was rinsed three times with distilled water, centrifuged again, and dried in an oven at 80 oC for cell dry weight [19].

2.5. pH Determination

Calculation of pH values in the study by pH Meter (Mettler Toledo Delta 320).

2.6. Determination of Bioethanol

The supernatant separated from the cell, when the sampling centrifuged for 10 min and 6000 rpm at 4 ºC. Bioethanol analysis will be used the supernatant, and by using HPLC (High Performance Liquid Chromatography), for bioethanol determine.

2.7. Bioreactor Systems

Bioreactor working volume of 5-L (Bioflo III, New Brunswick, USA) with a working volume of 3-L.

3. Statistical Analysis

All complete experiments were repeated two times to confirm reproducibility of results. Results are presented as Mean ± SD. Data SPPS 9.0 was used to analyze gained data. (ANOVA) one-way analysis applied to compare between estimated parameters. Statistical significance defined when $p < 0.03$.

Result and Discussion

4.1. Influence of Various Molasses Concentrations on Bioethanol Production

For Choose the optimal concentration feedstock for bioethanol production by *Saccharomyces cerevisiae* using medium containing 0-20 g/L molasses. The bioethanol concentration grew as the molasses content increased up to 15.00 g/L, according to the results. Table: 1 show the maximum bioethanol 7.50 g/L, 6.45 g/L and cell dry weight respectively obtained after 48 h fermentation with an initial molasses concentration and pH stopped at 4.00 in the end of cultivation.

Table 1. Shows the Influence of various molasses concentrations on bioethanol production.

Molasses g/L	pH	Bioethanol g/L	CDW g/L
0	7	1.98	1.25
5	5.7	4.4	3.45
10	4.68	5.5	5.00
15	4.30	7.50	6.45
20	4	7	6.22

When the molasses content was more than 15.00 g/L, the bioethanol concentration rise. This is due to increased substrate concentrations inhibiting the enzyme. As a result, 15.00 g/L of molasses concentration was chosen as the main carbon source in the medium for the isolate *Saccharomyces*

cerevisiae to produce bioethanol. This ratio is close to what was mentioned by [20] as the optimum production conditions molasses concentration 18%wt.%.

4.2. Influence of Various Peptone Concentrations on Bioethanol Production

Several concentrations of peptone g/L:0-5. use to examine the effect of peptone on bioethanol production, The maximal bioethanol production was 4.25 g/L and the cell dry weight 4.15 g/L. respectively. The results indicated that peptone in concentration 3.00 g/L convenient for production bioethanol by *Saccharomyces Cerevisiae*. Table: 2. show how the optimization method result.

Table 2. Influence of various peptone concentrations on bioethanol production by *Saccharomyces cerevisiae*

Peptone g/L	pH	Bioethanol g/L	CDW g/L
0	7.00	0.48	0.30
1	6.00	2.00	1.20
2	5.55	2.82	2.00
3	4.50	4.75	4.15
4	4.34	4.40	3.70
5	4.12	3.70	3.50

The influence of various Peptone concentrations on the production of bioethanol was tested during *Saccharomyces cerevisiae* cultivation. Nitrogen sources cannot be overlooked because they are regarded as key factors in the growth of sugars and metabolism during fermentation, as recommended in the literature [21]. Table 2 shows peptone concentration 4.00 g/L convenient for *Saccharomyces cerevisiae* bioethanol production. The size of cells rises in general depending on the concentration and type of nitrogen sources. This totally agrees with [22] and his colleagues, who detailed the effect of nitrogen sources on *Saccharomyces cerevisiae* in detail.

4.3. Influence of Various Yeast Extract Concentrations on Bioethanol Production

Yeast extract is the most essential elements in growth especially in *Saccharomyces cerevisiae* is which provide complex nutrients as nitrogen source [23]. Table: 3 Show that various concentrations of yeast extract g/L: 0-4 survey in the medium. the maximum bioethanol production 4.50 g/L and 3.65 g/L and cell dry weight respectively. at pH 4.80. This result recommended that yeast extract at the concentration 3.00 g/L best concentration for bioethanol production by *Saccharomyces cerevisiae*.

Table 3. Influence of various yeast extract concentrations on bioethanol production by *Saccharomyces cerevisiae*.

Yeast extract g/L	pH	Bioethanol g/L	CDW g/L
0	7.00	0.28	0.20
1	6.30	2.30	1.20
2	5.45	2.70	2.30
3	4.80	4.50	3.65
4	4.22	3.77	3.20
5	4.00	3.40	2.43

The effects of several yeast extract concentrations on bioethanol production were studied, and the findings indicated yeast extract at a concentration 3.00 g/L is the best concentration for *Saccharomyces cerevisiae* bioethanol production. Therefore, when sett yeast extract concentration at 3.00 g/L gradually increased the concentration of the obtained cell biomass. Further experiments were

performed at an initial yeast extract concentration of 3.00 g/L for bioethanol production, This value correspond with [24].

4.4.Influence of pH on Bioethanol Production

The effect of pH for the bioethanol production of fermentation medium estimated by using the optimized medium. The pH 4.0 was discovered to be the optimal condition when compared to other pH values. The bioethanol yield reached a high of 5.75 g/L, as shown in Fig: 1. While the yields of bioethanol production stabilized at the other pH values.

In technical point of view, cultivation of cells under controlled pH conditions obtained cells will be more adapted to acidity (as pH dropped to about 4.0 during the growth phase) [25]. From result, The pH 4.0 was shown to be the optimum condition among the other pH values, as shown in Fig. 1.

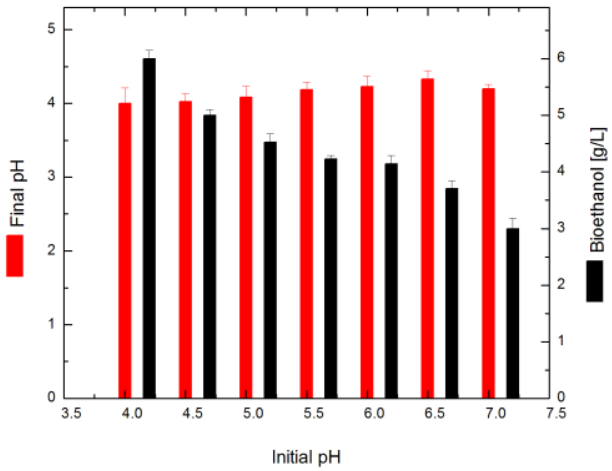

Figure 1. Influence of pH in bioethanol production by *Saccharomyces cerevisiae*.

4.5.Temperature Influence on Bioethanol Production

For select suitable cultivation Temperature for bioethanol during cultivation *Saccharomyces cerevisiae* in the optimization medium. The incubation temperatures between 25-45°C based on the growth of the cell according to the temperatures range. As show in Table 5 result reported that the maximum bioethanol production 5.80 g/L when the temperature was 35°C. As shown in Figure 5, the maximum bioethanol production was 5.50 g/L, making 35°C the optimal temperature for *Saccharomyces cerevisiae* bioethanol production. this result conforms to bioethanol production study as mentioned by [26].

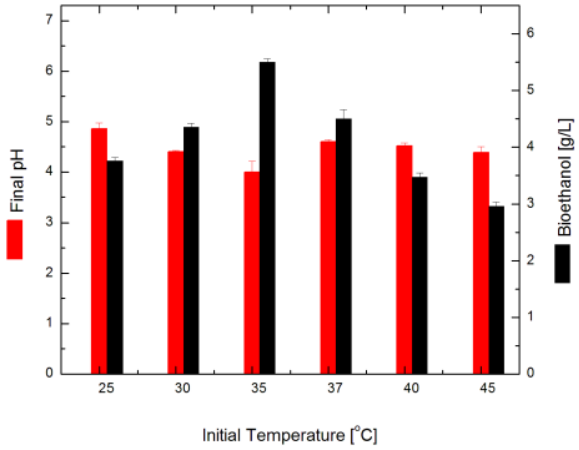

Figure 2. Influence of different temperatures in bioethanol production by *Saccharomyces cerevisiae*.

4.6. Influence Sizes Influence on Bioethanol Production

The inoculum size Influence evaluate via different inoculum sizes from 1-5% (v/v) added to the medium separately. Bioethanol production increased when inoculum size settled at 3% (v/v). From other side, inoculum size at 1-5% were fiddling difference in the bioethanol yields. 3% (v/v) of inoculum size would be considered to be optimum size for acquire maximal production 6.00 g/L. Influence inoculum sizes tested on bioethanol production, many of relevant research reported that increasing the *Saccharomyces cerevisiae* yeast inoculum size at 3% (v/v) showed positive effects on production from 20%-30% bioethanol and reduced the fermentation time. Thus, 3% (v/v) the best result chosen for bioethanol production by *Saccharomyces cerevisiae* [27].

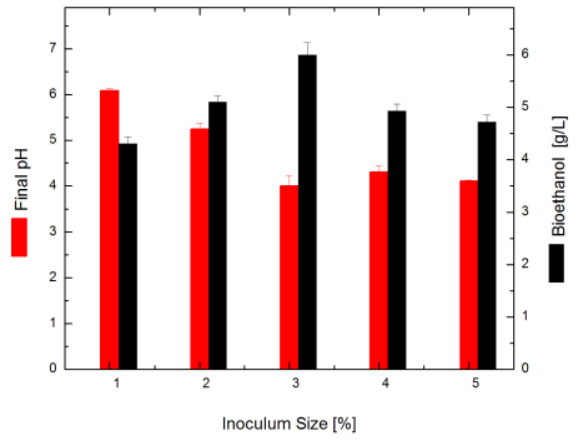

Figure 3. Influence of inoculum sizes in bioethanol production by *Saccharomyces cerevisiae*.

4.7. Bioethanol Production and Kinetics Cell Growth in Shake Flask Scale

In this stage of work, Cell growth, Bioethanol production, Total sugar and The pH was studied as a time function in the culture of a shake flask at 35 °C for 48h. The cells went through a lag phase for the first 6 h of culture, following which they started to develop gradually, achieving a maximum cell dry weight of 6.50 g/L before entering the stationary phase.

On the other hand, production of bioethanol reaching a maximal value 4.50 g/L. Also, the medium pH decreasing progressively and extent a minimum at 4.50. The bioethanol formation caused a drop in pH in the cultures during the incubation phase, whereas the total sugar value dropped from 12.50 g/l to 0.00 after 54 h of fermentation. Furthermore, *Saccharomyces cerevisiae* has a specific growth rate of 0.120 h^{-1}, with a maximum specific bioethanol production of 1.040 g. g^{-1} and a production rate of 0.124 g. $L^{-1}.h^{-1}$.

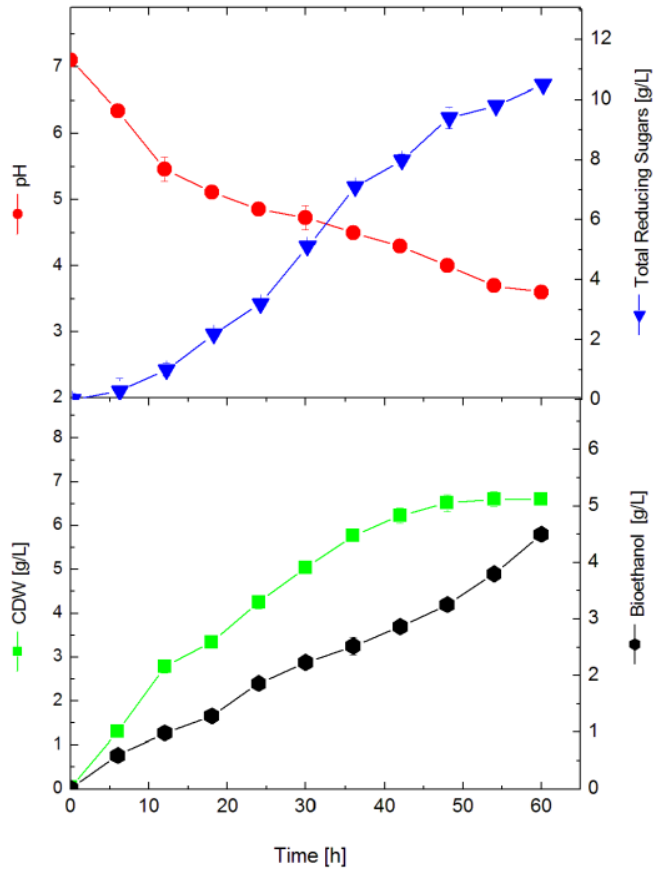

Figure 4. Bioethanol production and Kinetics Cell growth in Shake flask scale.

4.8. Bioethanol Production and Kinetics Cell Growth in A 4-Litter Bioreactor

The technique was scaled up for bioethanol production using bioreactor 4-L based on the developing medium in the shake flask phase. Scaling up a process from a shake flask to a bioreactor is a critical step in the industrialization of bioprocesses [27].

Cell growth, production of bioethanol and pH culture evaluate each 6 h. while, the dissolved oxygen decreased rapidly after 3 h of Incubation. pH set on 7.0 as initial value cultivation the dissolved oxygen (DO) level started uncontrolled with 100% during cell cultivation process at 200 rpm.

The cells grew slowly for the first 3 h before entering an exponential phase with a specific growth rate of 0.260 h^{-1} and a maximum cell mass of 18.00 $g.L^{-1}$ after 48 h of incubation. At 51 h after the

fermentation period began, the total sugar began to gradually and steadily decrease from 14.20 g/L to 0.00 g/L.

On the other hand, pH decreased from 7.0 to 4.0. in addition, Dissolved oxygen (DO) decrease gradually from 100 [%] to 27 [%] in end of fermentation process. Finally, the bioethanol production as a primary metabolite is strictly dependent on cell growth, Therefore, after 48 h of cultivation bioethanol reached to 16.50 g/L at maximum rate production 0.170 g. $L^{-1}.h^{-1}$.

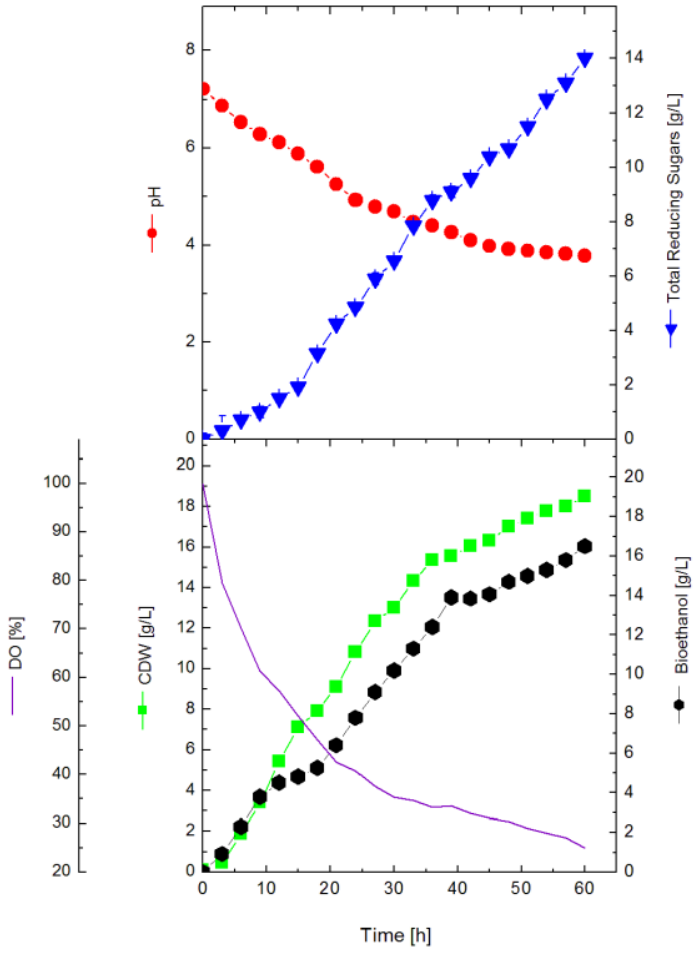

Figure 5. Bioethanol production and Kinetics Cell growth in Shake flask scale.

The main target for bioreactor cultivation in other studies was to improve the production process for primary metabolites for bioethanol production [29]. Finally, because bioethanol synthesis as a primary metabolite is largely dependent on cell growth, after 48 h of culture, bioethanol reached 16.50 g/L, with a maximum rate of production of 0.170 g. $L^{-1}.h^{-1}$.

Table 4. Different kinetic parameters obtained for bioethanol production by *Saccharomyces cerevisiae* in both shake flask and bioreactor cultivations.

Parameters	Shake flask Cultivation	Bioreactor cultivation
Maximal bioethanol production	4.50 g/L	16.50 g/L
Maximal cell dry weight	7.25 g/L	18.00 g/L
Specific growth rate	0.120 h^{-1}	0.260 h^{-1}
Bioethanol production rate	0.124 g/L/h	0.170 g/L/h
Total sugar	12.50 g/L	14.20 g/L
pH	4.00	4.00

Conclusion

According to the results, Molasses completely used in this research successfully as a main substance for bioethanol production because it is cheap and available throughout the year and eco-friendly. All Process parameters were studied successfully optimized in two stages for study shake flasks and bioreactor 4-litter scale as follows: at Cultivation temperature 35 °C, pH 4.0, Inoculum sizes 3% (v/v) and bioethanol production achieving the most possible value 4.50 g/L and 16.50 g/L in shake flask and bioreactor respectively quadruple in yield value. Therefore, all parameter conditions obtained from this research can be used to develop the bioethanol production process with estimate yield and quality for bioethanol.

Medium Optimization and Bioprocess Development for Bioethanol Production from Molasses Using Saccharomyces cerevisiae MTCC 173

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2	Tian, Kangming, Dandan Niu, Xiaoguang Liu, Bernard A. Prior, Li Zhou, Fuping Lu, Suren Singh, and Zhengxiang Wang. "Limitation of thiamine pyrophosphate supply to growing Escherichia coli switches metabolism to efficient D-lactate formation : Switchable cell growth improved D-lactate productivity", Biotechnology and Bioengineering, 2015. Crossref	13 words — < 1%
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