Advances in Bioresearch
Adv. Biores., Vol 10 (1) January 2019: 40-45
©2019 Society of Education, India
Print ISSN 0976-4585; Online ISSN 2277-1573
Journal's URL:http://www.soeagra.com/abr.html
CODEN: ABRDC3
DOI: 10.15515/abr.0976-4585.10.1.4045

Advances in Bioresearch

ORIGINAL ARTICLE

Bioprocess conditions of Acetic Acid production by using *Acetobacter cerevisiae* in shake flask fermentation

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ABSTRACT
The current research designed the improve acetic acid production by using Acetobacter cerevisiae through enhancement of the generation medium. Through this research, medium contain of [g/L]:Yeast extract 6, peptone 5, glucose 20, ethanol 25,acetic acid 15, $MgSO_4.7H_2O$ 0.57, K_2HPO_4 1.5, $FeSO_4.7H_2O$ 0.03, and $MnSO_4.4H_2O$ 0.12. The procedure improvement was begun by enhancement of medium synthesis utilizing one factor at time and affected on carbon and nitrogen sources, also affect the cell dry weight (CDW) on acetic acid yield and screening compering between developments through un-optimized and optimize medium in the shake flask stage. Result demonstrated that, the cell dry weight through optimized medium and un-optimized medium was9.10 [g/L] and 4.38 [g/L], individually and the acetic acid through optimized medium and un-optimized medium was 13.5[g/L], 5.0[g/L], respectively. All the results derived from this research have been found to be optimization medium was developed Acetic acid production.
Keywords*: Acetic acid, Acetobacter cerevisiae, optimization medium, fermentation process.*

Received 21.10.2018 Revised 21.11.2018 Accepted 31.12.2018

How to cite this article:
R A. Abd Alsaheb, A A Mustafa, J K Abdullah. Bioprocess conditions of Acetic Acid production by using *Acetobacter cerevisiae* in shake flask fermentation. Adv. Biores., Vol 10 [1] January 2019.40-45.

INTRODUCTION

Acetic acid is one of important organic acids extensive variety for modern implementations. Acetic acid (CH3COOH) is one of the least difficult natural carboxylic acid[1]. This color less feeble acid is described by particular acrid taste and impact smell. these days ,this acid is considered as one of the key intermediate for many industries including detergent, wood and food industries[2].For a long time, acetic acid have been delivered by various types from bacteria and broadly connected in chemical industries[3]. All the more as of late, numerous modern applications have been accounted for this acid in nourishment, cosmetics products, wood, and pharmaceutical field [4].

For manufacturing production, there are numerous types of *Acetobacter* that can be described as the essential vinegar producer such as, *A. pateurianus, A. peroxydans, A. aceti, A. orleaniensis, A. estuniensis, A. malorum, A. lovaniensis and A. cerevisiae*[5]. The best temperature for cells *Acetobacter* strains between 28 °C and 34 °C in culture medium [6]. *Acetobacter cerevisiae ,Acetobacte riumwoodii, and Acetobacter iumcarbinolicum* have the optimal growth temperature between 30°C and 37°C. generality of homoacetogens can utilize a enormous variety of carbon sources, including one carbon compounds, such as CO_2, CO and methanol, sugars such as glucose and fructose, other compounds such as lactate and pyruvate for cell growth and acetate production [7].Acetic acid is produced naturally as the sole or major fermentation product by anaerobic homoacetogens and aerobic acetic acid bacteria. Acetic acid bacteria contain 12 genera belonging to the family of *Acetobacter aceae*. They are gram-negative, aerobic and gram-variable, ellipsoidal to rod-shaped microbes which oxidize Ethanol to acetate in aerobic vinegar fermentation process. [8]. about 75% of acetic acid in the chemical industry is produced by carboxylation of methanol with carbon monoxide [9]. Only about 10 -20% of acetic acid is produced by fermentation, mainly as vinegar. The tremendous extension of Acetic acid request in worldwide market is paying

extraordinarily by advancement of all the more financially essential scale of fermentation process [10].Also acetic acid is considered the generality possibility feedstock monomer for chemical conversions [11].Among various concoction strategies utilized, methanol carboxylation is the overwhelming creation innovation and representing more than 60-80% of worldwide limit took after by alkane oxidation forms and ethylene oxidation [12].Today, acetic acid is an indispensable as moderate compound for the manufacturing production of different chemicals, for example, cellulose acetic acid derivation, dimethyl terephthalate, calcium magnesium acetic acid derivation, vinyl acetic acid derivation polymer, acetic acid esters/acidic anhydride [13]. Every one of these items is produced using oil determined acidic acid [14].
This production procedure is exceptionally delicate for development conditions connected and the compound synthesis of the creation medium. Carbon source utilized assumes critical part for bacterial development and acetic acid production [15].In addition to all this we have a great deal of speculations and applications about the acetic acid to comprehend and ingest its highlights and properties and its significance part in the substance business and biotechnology a significant agent in the progress for these industries.The current research is focused on the optimization of acetic acid production for high acetate production using glucose based cultivation medium. The main study was focused on the effect of Nemours medium components on acetic acid production and the kinetics of cell growth in shake flask stage.

MATERIAL AND METHODS

Preparation of Working Cell Culture and Microorganisms

The isolated*Acetobacter cerevisiae* were kept at -80°C in freezer. Bacteria were transfer in frozen glycerol. Cells were first increase in Medium. Medium was regulating to 7.0 prior sterilization. Put in incubator at 30 °C for 72 h. The colonies of bacteria colonies created were harvested by 50% from glycerol solution and aspirated to be set in group of 2 ml sterilized vials tubes.

Medium of Fermentation

For this stage in research medium was selection for acid production was optimized in shaking flask study. The compounds for production media were as follows [g/L]: Yeast extract 6, peptone 5, K_2HPO_4 1.5, glucose 20, Ethanol 25, acetic acid, 15, $MnSO_4.4H_2O$ 0.12, $MgSO_4.7H_2O$ 0.57,$FeSO_4.7H_2O$ 0.03.pH of medium was set at 7.0 before sterilize.

Growth kinetic comparison between optimized and un-optimized medium

The next part of research study the kinetics of growth for *Acetobacter cerevisiae* will be studied in 25 ml working volume from125 Erlemenyer flask cultures utilize for optimum medium and non- optimized medium. Samples were taken in every 6 hours. Cell dry weight (CDW) and the effect of pH and Acetic acid production were calculated for growth kinetics.

Analytical process

Cell dry weight (CDW) calculation

Specimens in form of 5 flask of 50 ml broth per shake flask were possessed at several time duration through cultivation conditions. 1 ml of specimens were aspirated and added to 9 ml filtered water, which is done in bottles. Dilution reduplicate to 1000 dilution. The optical density (OD) was calculated by using spectrophotometer .Absorbance was possessed for each dilution and its increase at (600) nm. Dilution that describes correspondence in results is taken as results while cell dry weight (CDW) calculated according to the standard curve.

RESULTS AND DISCUSSION

Study effect of various glucose concentrations on Acetic acid production

The appropriate concentration of glucose to Acetic acid production by the chosen isolate *Acetobacter cerevisiae* was determined using medium containing 0 - 20[g/L] of glucose. Ethanol or Glucose can act as a major carbon source. It is significant to get the pure products of acetic acid after fermentation by utilize a glucose which is from pure sugar. Sugars and Carbohydrates like: xylose, ribose, mannose, arabinose and glucose. Can be using and support also acetic acid production Outcome showed the maximum Acetic acid yield was 8 [g/L] was acquired at 72 h fermentation process with an elementary glucose concentration. On the other hand maximum cell dry weight (CDW) was 6.6 [g/L]. Therefore, 20 [g/L] concentration of glucose was chosen for used as source for carbon to production acetic acid by *Acetobacter cerevisiae*.

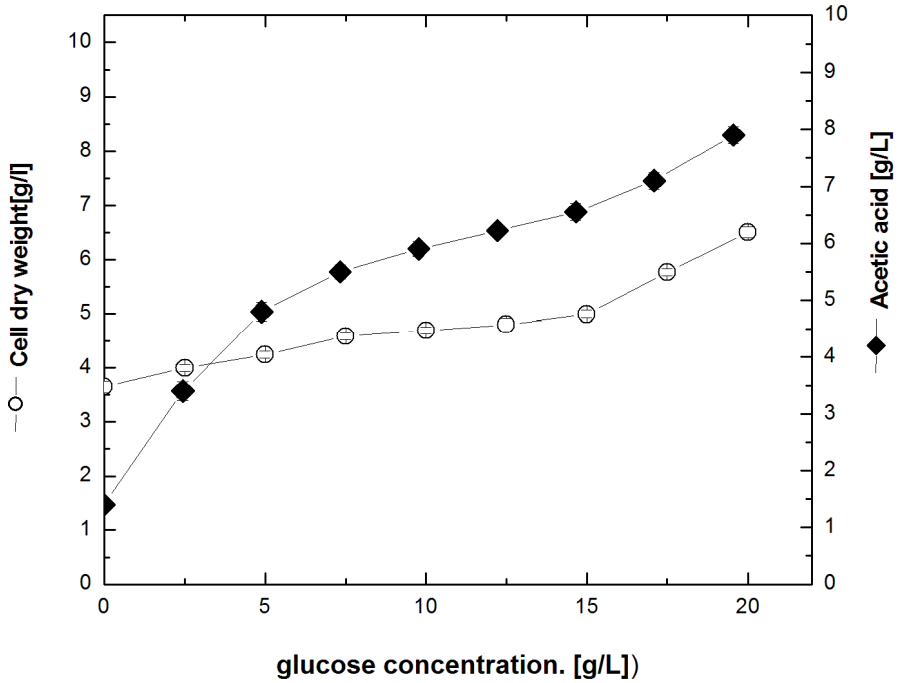


Figure 1: Acetic acid production, Cell dry weight at various concentrations of glucose in shake flasks fermentation.

Study effect of various peptone concentrations on Acetic acid production

Through *Acetobacter cerevisiae* cultivation Nitrogen sources cannot be neglected as it is very crucial and strongly influence on the *Acetobacte rcerevisiae* metabolism and growth through fermentation process. Absorption of nitrogen is the most important composition in the fermentation medium aside from sources of carbon sources. Also, identical to carbon source the extent of cell growth is hugely dependent on the type and concentration of the nitrogen sources. effect of various concentrations of peptone 0-5[g/L] after cultivating *Acetobacter cerevisiae* for 72 h at 30°C , the maximum production of 7.5 [g/L] of acetic acid and maximum cell dry weight (CDW) was 8.3 [g/L].Thus, 5 [g/L] peptone has been selected as the ideal concentration for production.

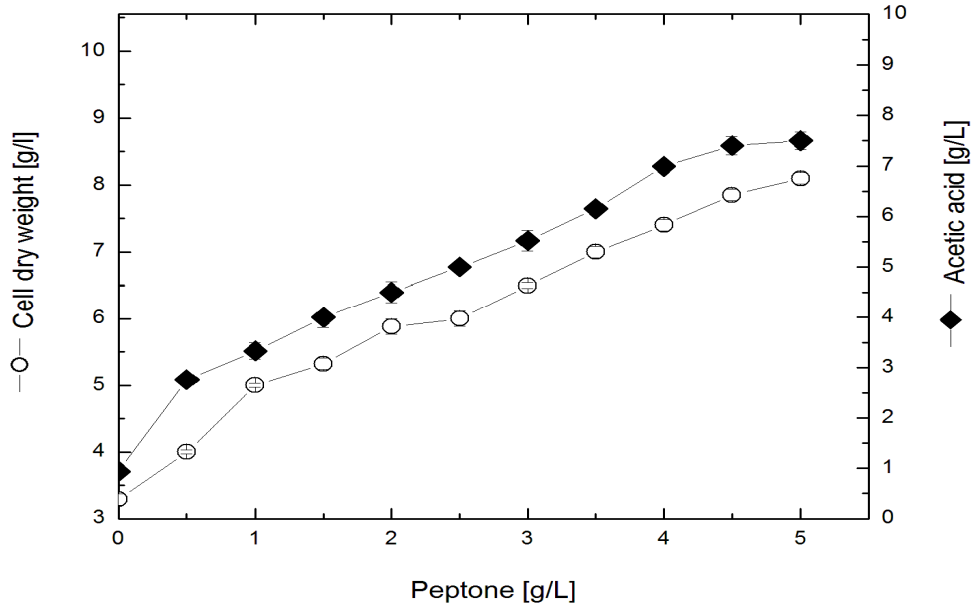


Figure 2: Acetic acid production, Cell dry weight at various concentrations of peptone in shake flasks fermentation.

Study effect of various Yeast extract concentrations on Acetic acid production

Yeast extract is generally usually utilized as factors for growth which provided compound nutrients. Various concentrations of yeast extract 0-6 [g/L] were added for media. After cultivating isolate *Acetobacter cerevisiae* for 72 h at 30°C under anaerobic conditions, research showed that the maximum cell dry weight (CDW) was 3.5 [g/L] and the maximum yield of 4 [g/L] of acetic acid was produced. The results indicated that yeast extract at the concentration of 4.5 [g/L] was portable to acetic acid yield using *Acetobacter cerevisiae*.

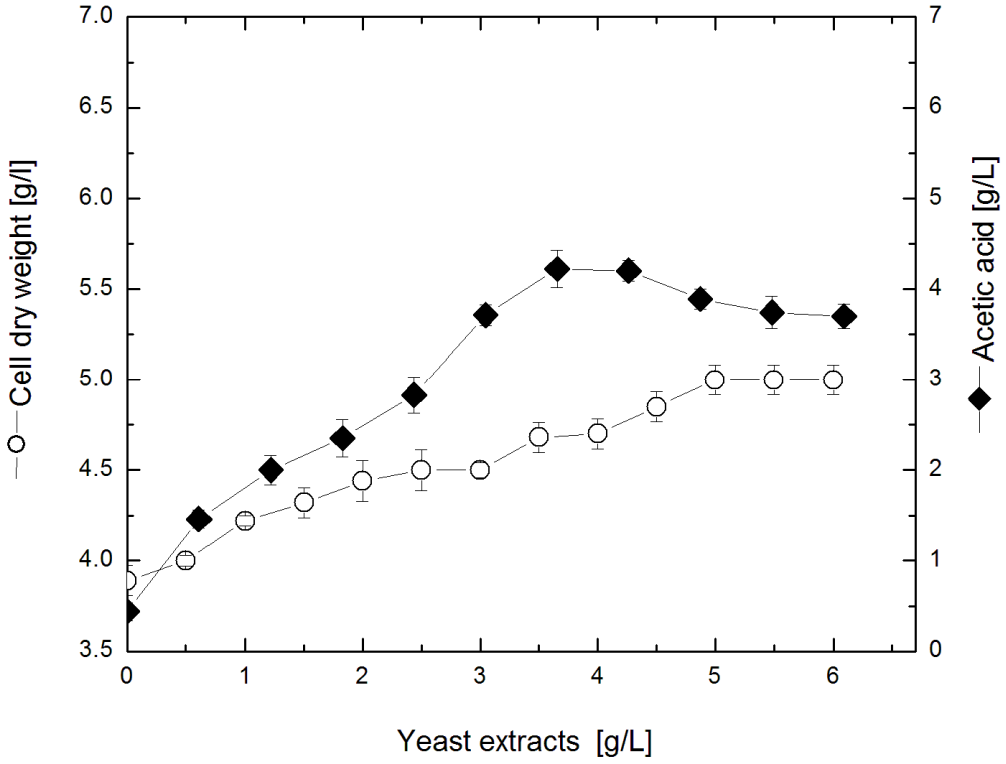


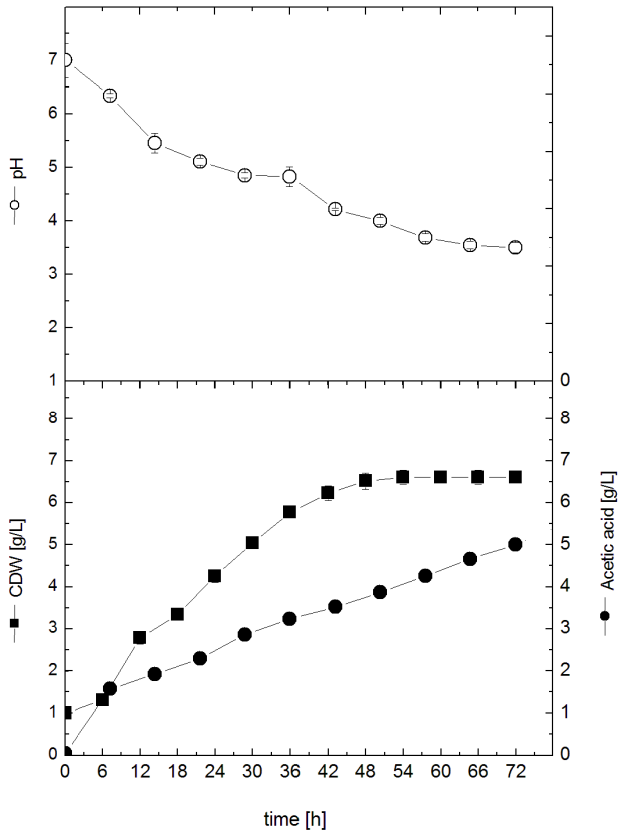
Figure: 3Acetic acid production, Cell dry weight at various concentrations of yeast extract in shake flasks fermentation.

Acetic acid production and Cell growth Kinetics by *Acetobacter cerevisiae* for un-optimized medium

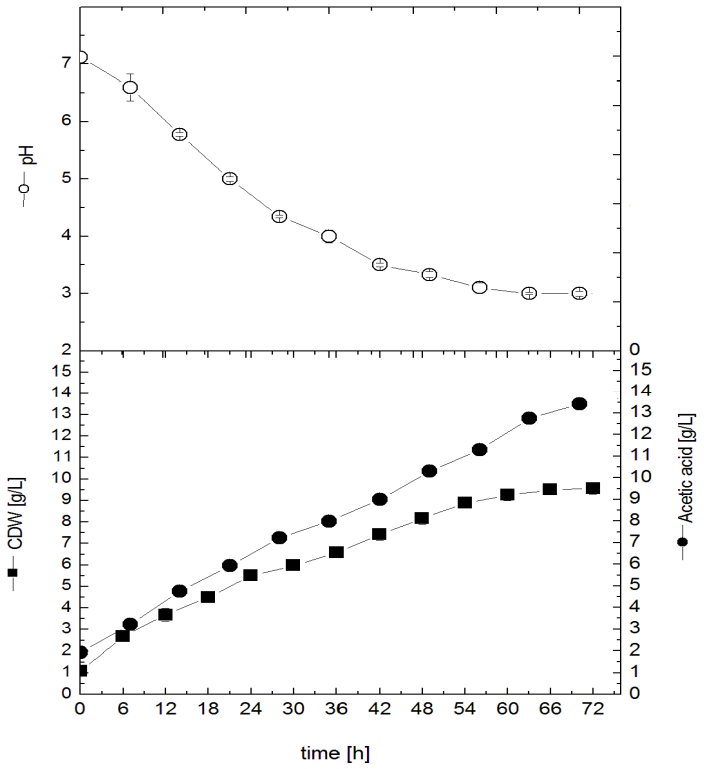
This part from research was designed to check the effect of cell growth in un-optimized medium and acetic acid production. Experiment showed the cell dry weight (CDW) reached to 4.38 [g/L] after 72 h of cultivation, value of pH change from 7.2 to 3.25, while after 72 h cultivation acetic acid attained to 5.00 [g/L] and rate of production reached to 0.188 [$g.L^{-1}.h^{-1}$] .figure:4 clarified the final results for this stage from research.

Cell growth and Acetic acid production Kinetics by *Acetobacter cerevisiae* or optimized medium

The next stage of work was designed to check the effects of cell growth in optimized medium and production acetic acid. The optimized medium contain the three main components yeast extract, glucose and peptone20 [g/L],4.5 [g/L], 5 [g/L] respectively, will be used as optimized medium for high acetic acid in this part from research. The development in acetic acid production and pH as an element of time were exact in shake flask cultivation under condition 30°C for 72 h. The cells came to in a slack stage until initial 6 long stretches of development, and after that the cells started to develop exponentially, where they add up to maximal cell dry weight of around 9.10 [g/L] preceding entering the stationary stage after 72 h of development. In this manner, after 72 h from development acetic acid reached to 13.5 [g/L] and rate of production reached to 0.258 [$g.L^{-1}.h^{-1}$].



Figures 4: Growth curve kinetic of *Acetobacter cerevisiae*in un-optimized media in shake flask fermentation.



Figures 5: Growth kinetic curve of *Acetobacter cerevisiae* in optimized media in shake flask fermentation.

From the other side the accurate rate growth for *Acetobacter cerevisiae* was 0.180 [h^{-1}], production rate of nearly 0.117 [$g.L^{-1}.h^{-1}$] and the specific acetic acid production 1.038 [$g.g^{-1}$]. These results detected the optimized production medium was able to backup best *Acetobacter cerevisiae growth* with an increase of about 17.50% when compared to the un-optimized medium. While the pH of the medium diminished dynamically and reached to 3.00. The following table shows statistics for all the research outputs.

Table 1: Summary of statistics for all the research outputs

Growth Parameters	Shake Flask	
	Optimized medium	Un-Optimized medium
Maximal cell dry weight	9.10	4.38
Specific growth rate	0.180	0.055
growth rate	0.258	0.188
Production Parameters		
Final pH	3.00	3.25
acetic acid production	13.5	5.00
production rate	0.117	0.079

CONCLUSION

In this study, effective information for all experimental in this work is outlined in last the table was specified previously. On contrasting these outcomes together, as a conclusion procedure for shake flaskstage, Optimized medium created more acetic acid yield which is 13.5 [g/L] contrasted with Un-optimized, 5.00 [g/L]. For development rate yield, Un-optimized delivers not as much as Optimized medium which is 0.188 [$g.L^{-1}.h^{-1}$] and streamlined which is 0.258 [$g.L^{-1}.h^{-1}$] while, the maximal cell dry weight achieved 4.38 [g/L] for the Un-Optimized medium which is 9.10 [g/L] for afflicted medium. Shake flask study into assurance of the ideal conditions for the fermentation procedure. Hence, the Optimized medium delivered more creation and high cell growth rate result.

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