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Optimization of production conditions for biomass and lipid production from *Debaryamyces*, a yeast isolated from Pome in Osun state, Nigeria

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Abstract

Lipid production formerly based on animal fats and plant oils competes with crops production for land, leading to the expansion of bioenergy crop cultures, posing crops scarcity to some extent. Having sustainable and economical alternative using oleaginous yeasts capable of utilizing low-cost substrates from renewable sources such as palm oil mill effluent (POME) with the ability to accumulate and produce oil which is not influenced by external factors like their origin, climatic changes or season and do not affect the ecological food chain is of great importance. This yeasts that are of high quality single cell oil producers with dense intracellular lipid droplets was isolated using Sudan Black B staining technique. *Debaryamyceshansenii* (Y14) isolated from palm oil mill effluent (POME) in Ile Ife, Nigeria, was investigated using Sudan Black B staining technique of Thakur *et al.* (2012). Optimal biomass and lipid production was assessed for twelve weeks, using Rotary incubator shaker. The optimum pH for the production of microbial lipid from the yeast was pH 6.0 with 0.79±0.03 g/15mL of biomass, 0.34±0.05 g/15mL of lipid and 43% of single cell oil (SCO) percentage productivity. The lowest SCO productivity of 25% was observed at pH 10.0. The optimum temperature for the maximum production of intracellular lipid from yeast was 35^{o}C with SCO production of 40%. Optimal carbon source for intracellular lipid accumulation of the yeast was glucose with SCO productivity of 50%. Optimal nitrogen source for production of yeast biomass and lipid was yeast extract with 0.67±0.02g/15mL of biomass and 0.28±0.03g/15 mL of lipid amounting to productivity of 41.8%. Micrbial Lipid produced from Y14 yeast has biodiesel potentials hence recommended for futher research for higher production quantity.

Keywords: Biodiesel, single cell oils, optimization, intracellular lipids, oleaginous microorganisms.
Subtheme: *Sustainability and Microbes in Bioeconomy (Industry)*

Introduction

Oleaginous microorganisms have the ability toaccumulate high amounts of lipids in separate lipid bodies (Li *et al*., 2008), most times more than 20 % of their cell biomass under suitable cultivation conditions of limiting Nitrogen, phosphate (Muniraj *et al*., 2013), and the presence of excess carbon (Mane and Raut, 2016). Their relatively high rate of growth and the similarity of their triacylglycerols (TAGs) to the oils produced by plants (Pan *et al*., 2009; Kosa and Ragauskas, 2010) recommend them for biodiesel production. Furthermore, their ability to accumulate and produce oil is not influenced by external factors such as their origin, climatic changes or season (Thiru and Rangaswamy, 2011).

The potential of these microorganisms for application as lipid-producing sources has attracted a lot of interest because of the similarity between the lipids they accumulate, also called single-cell oils, to the oil obtained from plants such as Jatropha, which are used as substrates in biodiesel production (Amaretti *et al*., 2012). Microbial oils are economical because they can be derived from low cost sources such as corn stalk, rice straw, and even from forest residues and as such do not impact the ecological food chain (Mullner and Daum, 2004; Melickova *et al*., 2004; Li *et al*., 2008; Drucken, 2008). Oleaginous yeasts attract a lot of attention because of their high growth rates and their ability to use different carbon sources. They can also utilize low-cost fermentation media such as waste materials from agricultural and industrial products (Amaretti *et al*., 2012, Benjamas and Louhasakul, 2013).

The use of these oleaginous organisms isolated from palm oil mill effluents can maximize the value of biomass and minimize the wastes generated by recycling waste streams within the bio-refinery (Clark and Deswarte, 2008). The recycling of waste streams by such microbial lipid production processes, the search and determination of new lipid producing microorganisms, which are also able to convert complex second generation biomass, are challenges for the development of microbial oil production processes that will compete with conventional methods of oil production (Lee and Lavoie, 2013).

Lipid production formerly based on animal fats and plant oils competes with crops production for land leading to the expansion of bioenergy crop cultures, posing crops scarcity to some extent. It is therefore necessary to search for a sustainable alternative using oleaginous yeasts capable of utilizing low-cost substrates from renewable sources. Therefore, using wastes from palm oil mill effluents for the isolation of yeasts that are highquality single cell oil producers with dense intracellular lipid droplets within their cells offer great possibilities.

Materials and Methods

2.1 *Isolation of Yeast Strain*

Samples of Palm Oil Mill Effluents were aseptically collected from different local palm oil mill industries in Ede Road Ile-Ife (N 7° 28', 4° 34' E) and Akiriboto, Gbogan (N 7° 28', 4° 21' E) all in Osun State. The palm oil mill effluents were collected using clean sterile McCartney bottles and transported immediately to the laboratory for yeast isolation.

1 ml of palm oil mill effluent sample was added into 50 mL of glucose-enrichment medium containing (g/L) glucose (20), $(NH_4)_2SO_4$ (5), KH_2PO_4 (1), $MgSO_4 \cdot 7H_2O$ (0.5), yeast extract (0.5)in a 250-mL Erlenmeyer flask with 0.1mL of 95% streptomycin solution to inhibit bacterial growth. It was incubated in a 180 rpm rotary shaker at 37°C for 96 hours according to the method of Pan *et al*. (2009).One millilitre of the pre-cultured samples was serially-diluted ten (10) fold using sterile distilled water and 0.1mL of the serially-diluted samples were spread from 10-3, 10-5, 10-7 and 10-9 dilutions, with bent glass rod on Glucose Salt Medium (GSM) plates which contains (g/L): glucose (20), $(NH_4)_2SO_4$ (5), KH_2PO_4 (1), $MgSO_4 \cdot 7H_2O$ (0.5), $CaCl_2.2H_2O$

(0.1), yeast extract (0.5) and Agar-agar 2% in distilled water with 0.1 mL of 95% Streptomycin solution. The plates were incubated in Illuminated cooled Incubator (Gallenhamp IH- 16) at 37^{o}C for 2 days. Plates were observed for growth and isolated colonies were sub-cultured several times on the Glucose Salt Medium (GSM) until pure isolates of the culture were obtained and each isolate was maintained on yeast peptone glucose agar (YPGA) slant composed of (g/L) : Yeast extract (4), Peptone (4) and Glucose (4) in 200 mL distilled water at 4ºC.

2.2 *Primary screening of yeast for lipid production*

Lipid production of *Debaryamyces* isolates were evaluated qualitatively by making alight smear of the test yeast on a clean glass slide, heat-fixed and stained using Sudan Black B staining technique of Thakur *et al.* (2012) and observed under the microscope on oil immersion lens for the presence of blue or grayish coloured fat globules within the cells.

2.3 *Secondary screening of yeast for lipid production*

Oleaginous yeasts colonies were inoculated into sterile medium containing (g/L): glucose (15); $(NH_4)_2SO_4$ (5); KH_2PO_4 (1); $MgSO_4.7H_2O$ (0.5); and yeast extract (0.5), incubated at 37ºC at 180 rpm for 48 hours (Pan *et al*., 2009; Kraisintu *et al*., 2010). Five (5) mL from the 48 hours old inoculum was transferred to 45 mL of nitrogen-limited medium composed of (g/L): glucose (40); $(NH_4)_2SO_4$ (2); KH_2PO_4 (7); NaH_2PO_4 (2); $MgSO_4.7H_2O$ (1.5).The yeast extract was supplemented with a 1 mL of 100-fold diluted trace element solution (Wu *et al*.,2010) containing trace element solution (g/L): $CaCl_2.2H_2O$ (0.75), $FeCl_3$ (0.005), $ZnCl_2$ (0.005), pH adjusted to 7.0. The medium was sterilized by autoclaving at 121 °C for 15 mins in 250 mL Erlenmeyer flask, (Wu *et al*.,2011). The culture was incubated on a rotary shaker at 180 rpm and 37ºC for 5 days for complete enrichment and fermentation.

2.4 *Determination of yeast biomass*

Portions of 15 mL cultures were harvested from the nitrogen-limited fermentation medium by centrifugation using table size centrifuge (Helmreasinn 80-2)at 4000 rpm for 10 mins. Harvested biomass was washed twice with 5 mL of distilled water, filtered with Whatman No. 1 filter paper and then dried in an oven (ArtellHearson-Hot) at 40°C to constant mass. The biomass was determined gravimetrically according to Shi *et al*. (2011) using the formula

Biomass = $W_2 - W_1$

Where:W_2 = weight of Whatman No. 1 filter paper with dry biomass in g
W_1 = weight of empty Whatman No. 1 filter paper in g

2.5 *Lipid extraction*

Lipid extraction was carried out based on Bligh and Dyer (1958) method with few modifications by Pan *et al*. (2009). A sample of 15 mL from the nitrogen-limited fermentation medium was centrifuged at 4000 rpm for 15 minutes to obtain the residue. The yeast cells residue was washed with 5 mL of distilled water centrifuged and the supernatant decanted. This process was repeated twice. A 5 mL of 4M HCl was added to the washed cells and the mixture was kept at room temperature for 30 min before it was kept in refrigerator at -5^{o}C for 10 minutes and subsequently in boiling water for 10 min. The freezing and thawing process was repeated thrice in order to break up the yeast cells. Ten (10) millilitres of chloroform/methanol mixture (1:1) was added to the acid hydrolyzed mass, stirred vigorously with vortex (Helmreasinn 60-10) for 10 mins. The whole mixture was centrifuged at 4000 rpm for 3-5 mins to separate the aqueous upper phase and organic lower phases. The lower phase containing lipid was recovered with Pasteur pipette; allowed to evaporate by air exposure overnight and the dried lipid was weighed.

2.6 *Identification of the Test Yeast Isolate*

Pure isolate of the test yeast was identified according to Lodder (1970) and Barnett *et al*. (2000). Sporulation test was carried out according to Barnett *et al*. (2000). The following media were used: Yeast extract 1g, malt extract 0.5g, glucose 2g, peptone 1g and 3% agar in 100 mL distilled water; Acetate agar, which was prepared by dissolving 9.8g potassium acetate, 1.0g glucose, 1.2g sodium chloride, 0.7g magnesium sulphate, 2.5 g yeast extract and 3 g agar in a litre of distilled water and 3 g Potato Dextrose Agar (PDA)

10 mL each of the above media were dispensed into McCartney bottles as slopes and autoclaved at 121ºC for 15 minutes. The test yeast isolate was then inoculated in each slope and incubated at 28ºC for four weeks. Thin smear from each of the medium were prepared and stained with 5% malachite green, then examined under the microscope for ascospore formation.

2.7 *Test for growth at 37ºC and 40ºC*

For the test for growth at 37ºC and 40ºC, yeast malt agar (YPGA) media (3g yeast extract, 3g malt extract, 10g glucose, 5g peptone and 20g agar in 1000 mL distilled water) was inoculated with fresh culture of yeast and incubated aerobically for 3 – 4 days at 37ºC and 40ºC respectively. The growth on the slope was then examined and recorded (Barnett *et al*., 2000).

2.8 *Test for growth on high D-glucose concentration*

For the test of growth on high D-Glucose concentration (56g of D-glucose, 1g of yeast extract, 3% agar homogenized in 100 mL of distilled water). 10 mL each of the medium were distributed into test tubes and autoclaved for 15 minutes at 121ºC then sloped. The test yeast isolate was inoculated in the test tubes by streaking and incubated at 25ºC for 4 weeks. The growth in slope was then examined and recorded (Barnett *et al*., 2000).

2.9 *Assimilation of carbohydrates*

The method of Lodder, (1970) with modifications was adopted for carbohydrate assimilation. Small test tubes were filled each with 10 mL of sterile medium (0.5% peptone water and 4% test sugars: glucose, mannose, sucrose, maltose, lactose, manitol, galactose, melibiose and raffinose) with inverted Durham tubes. The tubes were inoculated with the 48 hour old isolated yeast culture and incubated at 25ºC for four to five days. Fermentation was detected by the production of gas bubbles in the test tubes.

2.10 *Effect of incubation time on biomass and lipid production*

The optimum incubation time for lipid production was determined using the method of Kraisintu*et al*., (2010) by varying incubation time of the fermentation medium from 24, 48, 72, 96, 120 and 144 hrs. Five (5) mL of the inoculum was inoculated into a 45 mL sterile nitrogen-limited fermentation medium in 100 mL Erlenmeyer flask and incubated with shaking at 180 rpm and 37^{o}C. The cultures were analyzed after 24 hours by withdrawing 15 mL of the fermented medium into a centrifugal tube for centrifugation at 4000 rpm for 10 min to determine both the biomass and lipid content as described in Sections 3.2.4.1 and 3.2.4.2 respectively.

2.11 *Effect of different inoculum volume on biomass/ lipid production*

Inoculating medium of various volumes and various volumes of inoculum in the following ratio from 50:0, 48:2, 46:4, 45:5, 44:6, 42:8, and 40:10 mL were varied and incubated with shaking at 180 rpm for 5 days at 37^{o}C. Fifteen (15) mL each of the fermented medium was withdrawn and centrifuged for both the biomass and lipid content as described in Sections 3.2.4.1 and 3.2.4.2 respectively (Kraisintu *et al*., 2010).

2.12 *Effect of pH on biomass/ lipid production*

Optimum pH for the biomass and lipid production was determined by varying the pH of minimal

medium (45mL) from pH 3 to pH 11 at interval of 0.5. This was then inoculated with 5 mL of 48 hour old test yeast inoculum and incubated in rotary shaker at 180 rpm for 5 day at 37^{o}C. After incubation, fifteen (15) mL of the fermented medium was centrifuge at 4000 rpm for 10 min to determine both the biomass and lipid content as described in Sections 3.2.4.1 and 3.2.4.2 respectively (Kraisintu *et al*., 2010).

2.13 *Effect of temperature on biomass/ lipid production*

The optimum temperature for biomass and lipid production was determined by varying incubation temperatures of the culture from 25, 35, 45, 55 and 65^{o}C. Forty five milliliter (45 mL) of sterile minimal medium was inoculated with 5 mL of the enriched inoculum and incubated with shaking at 180 rpm for 5 days respectively. For each temperature, 15 mL each of the fermented medium was centrifuged at 4000 rpm for 10 min for both the biomass and lipid content as described in sSections 3.2.4.1 and 3.2.4.2 respectively (Kraisintu*et al*., 2010).

2.14 *Effect of different carbon sources on biomass/ lipid production*

The effect of various carbon sources were studied on the biomass and lipid production by using different sugars such as fructose, glucose, galactose, Lactose, sucrose, starch and carboxyl methyl cellulose (CMC) at 1% concentration as sole carbon sources in the production medium. Conical flask (100 mL) containing 45 mL of the minimal medium of appropriate carbon source was inoculated with 5 mL of 48 hour-old yeast inoculum. The flasks were incubated with shaking at 180 rpm for 5 days at 37^{o}C. Fifteen (15) mL each of the fermented medium was centrifuged at 4000 rpm for 10 min for both the biomass and lipid content as described in Sections 3.2.4.1 and 3.2.4.2 respectively (Kraisintu*et al*., 2010).

2.15 *Effect of different nitrogen sources on biomass/ lipid production*

Different nitrogen sources were investigated for their effect on biomass and lipid content by replacing the $(NH_4)_2SO_4$ in the minimal medium with NH_4Cl, $NaNO_3$, KNO_3, Urea, Casein and Yeast extract. The nitrogen sources were added at 1% concentration for each. One hundred (100) mL conical flasks containing 45 mL of the sterile media of appropriate nitrogen source were inoculated with 5 mL of 48 hour old enriched yeast inoculum and the flasks were incubated with shaking at 180 rpm for 5 days at 37^{o}C. From the fermented medium, 15 mL was pipetted from each and centrifuged at 4000 rpm for 10 mins for both the biomass and lipid content as described in Sections 3.2.4.1 and 3.2.4.2 respectively (Kraisintu *et al*., 2010).

2.16 *Bulk Production of Biomass/ Lipid from the Yeast Isolate*

One thousand milliliter of the production medium was prepared at the optimum conditions obtained from the optimization stage. One hundred milliliter (100 mL) of 48 hour-old enriched yeast inoculum was used to inoculate the minimal production medium which was incubated in rotary shaker at 180 rpm at 35^{o}C for 120 hrs. The fermented culture was centrifuged at 4000 rpm for 10 mins and the residue was analyzed for both the biomass and lipid content as described in Sections 3.2.4.1 and 3.2.4.2 respectively.

2.17 *Determination of Iodine Value of the Yeast Lipid Produced*

The Enshaeieh *et al*. (2014) method was used in determining the iodine value of the oil sample obtained from bulk production. A total of 0.25 g of the oil sample was weighed and dissolved in a 200 mL flat Erlenmeyer flask and 10 mL of chloroform was added to the flask. The flask was gently swirled to dissolve the oil and the mixture was tightly closed in a brown bottle and kept in the dark in a fume-hood for 30 minutes. A freshly prepared 10 mL of Hanus reagent (which was prepared by dissolving 3 grams of iodine in 200 mL of warm glacial acetic acid, cooled to room temperature with the addition of 0.63 mL of potassium solution). The mixture (potassium

iodide solution) was added followed with shaking and with the addition of 100 mL of distilled water. The resulting solution was yellowish in colour and 1 mL of 1% concentration of starch was added to assist in dictating the end point during titration with 0.1 M of sodium thiosulphate solution. The same content but void of the test lipid was set as blank control and all titration was done in triplicate.

$$\text{Iodine value} = \frac{(B - S) \times N \times 0.127 g/meg \times 100}{W}$$

Where:
W= weight of oil sample
N= normality of $Na_2S_2O_3$ used to titrate
B=volume of $Na_2S_2O_3$ solution for blank
S= volume of $Na_2S_2O_3$ solution for sample

2.18 *Determination of Saponification Value of the Yeast Lipid Produced*

A total of 1.0 g of the oil was mixed with 25 mL of potassium hydroxide ethanol solution in the ratio of 3:1 for saponification with 0.5 mL of phenolphthalein solution as an indicator. A total volume of 0.5 molar Hydrochloric acid standard solution was used to titrate the remaining potassium hydroxide. The same content but void of the test lipid was set as blank control at the same time. Titrations were carried out in triplicate. The amount of potassium hydroxide consumed in titration marked the saponification value of the microbial lipid produced (Enshaeieh*et al*., 2014).

$$S\ V = \frac{56.1 \times (B - S) \times N}{W}$$

Where:

SV = saponification value
B = blank's titre value in mL
S = oil sample's titre value in mL
N = normality of hydrochloric acid in M
W = weight of the oil sample in g

2.19 *Determination of Fatty Acid Profile of the Yeast Lipid Produced*

The composition of fatty acids in the extracted lipids was determined by gas chromatography method of Mane and Raut (2016), using the gas chromatography machine (GC-14B, Shimadzu Corporation, Japan). The GC was equipped with a flame ionization detector and a capillary column (Packed Flame wax by Restek, USA). The column temperature was programmed to increase from 80°C to 360°C at 10°C/min and kept at 360°C for 10 minsfor thermal stability before use. The temperature of both the injector and the detector was maintained at 370°C. A twenty microgram of sample was dissolved in 1 mL of ethyl acetate and 5 µL of sample was taken and injected into the GC machine. Nitrogen gas was used as the carrier gas at a flow rate of 0.80 mL/min. Fatty acids were identified by comparison of their retention times with those of standard ones, quantified based on their respective peak areas according to Wei *et al.*(2015).

Optimization of Production Conditions

Effect of incubation time on biomass/ lipid production

The effect of incubation time on lipid and biomass production was varied ranging from 1– 6 days. The optimum production of lipid and biomass was observed at day 5 with productivity of 0.15 g/15mL of lipid and 0.23 g/15mL of biomass as shown in Figure 4.3**.**

Effect of different inoculum volume on biomass/ lipid production

The effect of various inoculum volumes on biomass and lipid production was varied ranging from 2 – 10 mL of the standardized inoculum. The optimum production of biomass and lipid was observed at the 10 mL of the standardized inoculum inoculated into 40 mL of the nitrogen limited fermentation medium (NLM) with productivity of 0.79 g/15 mL of biomass and 0.34 g/15 mL of lipid.

Results and Discussion

The optimum pH for the production of microbial lipid from yeast was at pH 6.0 with 0.79 g/15mL of biomass, 0.34 g/15mL of lipid and 43% of single cell oil percentage productivity. This was in accordance with Diana *et al*. (2015) who worked on production of microbial lipids by *Yarrowia lipolytica* and slightly different from the work of Syed *et al*. (2006) who reported maximum lipid production at pH 6.5. This showed that near neutral pH value was more favourable to biomass and lipid production by *Debaryamyces hansenii*. The lowest SCO productivity of 25% was observed at pH 10.0 showing that as low amounts of organic acids were produced in medium, restriction in microbial growth was observed.

The optimum temperature for the maximum production of intracellular lipid from yeast was 35^{o}C with single cell oil productivity of 41%. Temperature controls maximum growth and production of metabolites by microorganisms and usually varies from one organism to another (Koutinas*et al*., 2014).Yeasts have the ability to grow from 25 – 40^{o}C according to Husain *et al*. (2009) who obtained highest accumulation percentage production at 28^{o}C, followed by 24^{o}C and minimum production at 22^{o}C.

The carbon source most suitable for intracellular lipid accumulation in yeast in this study was glucose with single cell oil productivity of 50% followed by sucrose with productivity of 37% while the lowest yield was observed in starch with 23% productivity. This has been reported by Hami and Bita (2012) and Diana *et al*. (2015) in the case of the fungus of *Mortierellais abellina.* This is different from the report of Shi *et al*. (2011) who reported D-xylose as the best carbon source for lipid and biomass production by fungus from soil. Basically, glucose is required for the growth and biosynthesis of oleaginous yeast (most especially when the nitrogen gets exhausted in the fermentation medium) as its presence is essential for both protein and nucleic acid biosynthesis. Excess carbon (glucose) present was converted into fatty acid and then to triacylglycerol by the suppression of the iso-citric dehydrogenase (ICDH), causing the blockage of the tricarboxylic acid (TCA) cycle. This leads to extra carbon being transformed to triacyglyceride (TAG) by series of enzymes such as the citric acid lytic enzyme, the malic acid enzyme and the fatty acid enzyme, hence completing fat accumulation (Amaretti *et al.*, 2012). If a limited nutrient like nitrogen still remains present in the culture condition, lipids produced will be metabolized (Beopoulous *et al.*, 2012). Therefore it is essential that the cells are promptly harvested and processed at the end of the lipid accumulation phase because the organism will begin to utilize the lipids, as the role of the accumulated material is to act as a reserve storage of carbon, energy, and possibly even water (Shi *et al*., 2011). The result from this study showed that total lipid increased when glucose was used as the sole carbon source in the medium. Apparently, better accumulation of biomass and lipids were achieved during the stationary growth phase during nitrogen exhaustion while the cells ceased to grow, instead continued to take up the surplus glucose from the medium for lipid biosynthesis.

The most suitable nitrogen source for optimum production of yeast biomass and lipid was yeast extract with 0.67g/15mL of biomass and 0.28 g/15 mL of lipid amounting to productivity of 41.8%. This was in accordance with Shi *et al*. (2011) and Syed *et al*. (2006) who worked on oleaginous yeast *Yarrowia lipolytica* as a source of microbial oil respectively and reported yeast extract as the best nitrogen source for intracellular lipid and biomass accumulation. Urea was the second best with 0.62 g/15 mL of biomass and 0.24 g/15 mL of lipid and with productivity yield of 38.7%. This was because Yeast extract contains abundant of vitamins, minerals and amino acids which are necessary for cell growth and lipid biosynthesis as reported by Enshaeieh *et al*. (2014).

Iodine value (IV) is a measure of the average degree of unsaturation of a lipid. The greater the iodine value, the greater the C=C double bonds in the lipid. The iodine value (IV) obtained from the

yeast lipid was 10.16I_2/100g. The iodine value obtained from the study was significantly low which depicts the presence of mostly saturated fatty acids in the oil while in the work of Shi*et al*. (2012), the iodine value obtained were high which indicated unsaturated fatty acids were mostly present in the lipid obtained from the microbial lipid isolated from Tibetan Plateau in China.

Saponification describes the molecular weight of the oil, which is the process of breaking down of neutral fat into glycerol and fatty acids by treatment with alkali. The saponification value (SV) obtained from the yeast oil was 2.8mgKOH/g. This low value indicates that the yeast oil is made up of high molecular weight fatty acids (or chain length) which makes it less likely to be used in soap making while in the report of Ajala and Adeleke (2014), the saponification value obtained from African star apple (*Chrysophyllum alibidum)* was significantly high (58.3mgKOH/g) which depicts that the lipids has high molecular weight and can be used in soap making.

Fatty acid profile result confirmed that yeast is a good source of succinic acid (Mane and Raut 2016; Nghiem *et al.,* 2017). In all cases, palmitic (C16:0) and oleic(C18:1), acids were the major components of the yeast lipid realized. Moreover, citric acid was found in low concentration less than 3% while fatty acids with more than four double bonds were absent. This is in accordance with the work of Wei *et al*., 2015; Mane and Raut 2016, who found out that the major fatty acids present in oleaginous yeast were (C14:0), palmitic acid (C16:0), stearic acid (C18:0), oleic acid (C18:1). The identified fatty acids can be used in cosmetic, health, food and pharmaceutical industries (Diana *et al*., 2015). These fatty acids found in oleaginous yeast lipid were similar to those found in various lipids from animal and vegetable origin.

Table 1: Summary of the Best Optimization Conditions for Biomass and Lipid Production

Optimization conditions	Results
Effect of carbon sources	Glucose
Effect of nitrogen source	Yeast extract
Effect of temperature (^oC)	37
Effect of inoculum size (mL)	10.0
Effect of incubation time (h)	120
Effect of pH	6.0
Saponification value (mg KOH/g)	2.8
Iodine value (gI_2/100g)	10.16
Most common Fatty acids	Palmitic, Oleic and Succinic acids

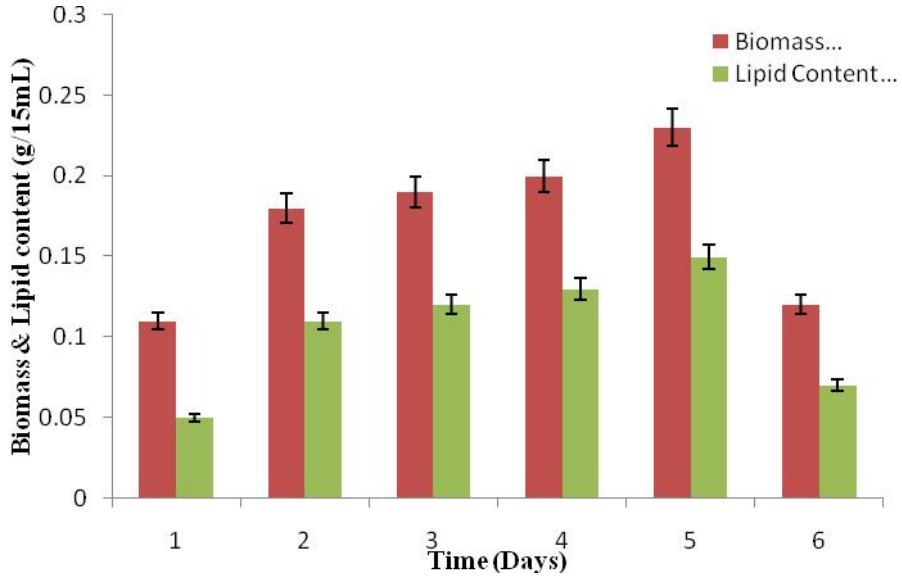

Figure 1: Effect of Incubation Time on Biomass and Lipid Production

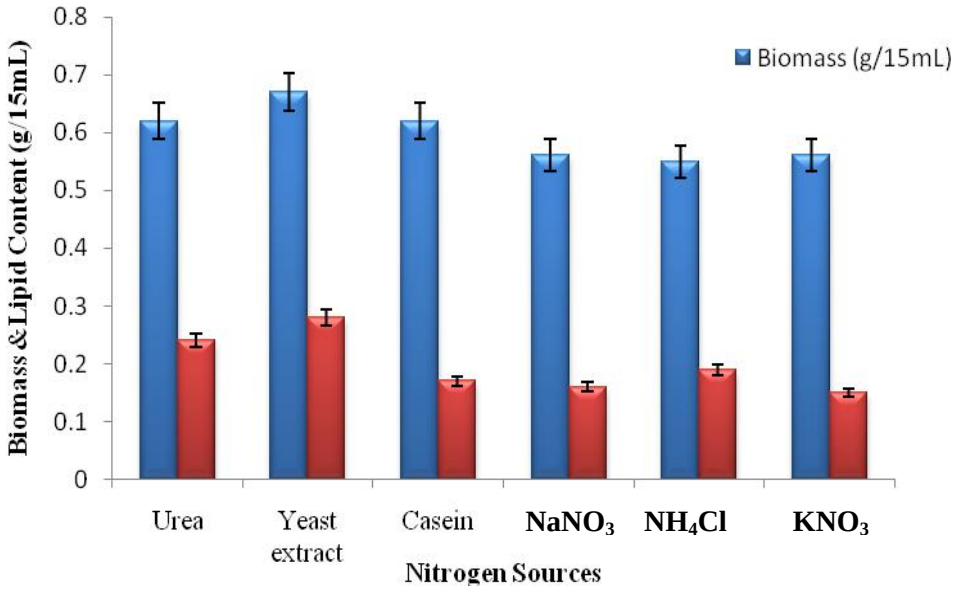

Figure 2: Effect of Different Nitrogen Sources on Biomass and Lipid Production

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