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Lipid Production Of Yarrowia Lipolytica Using Food Waste Anaerobic Digestate

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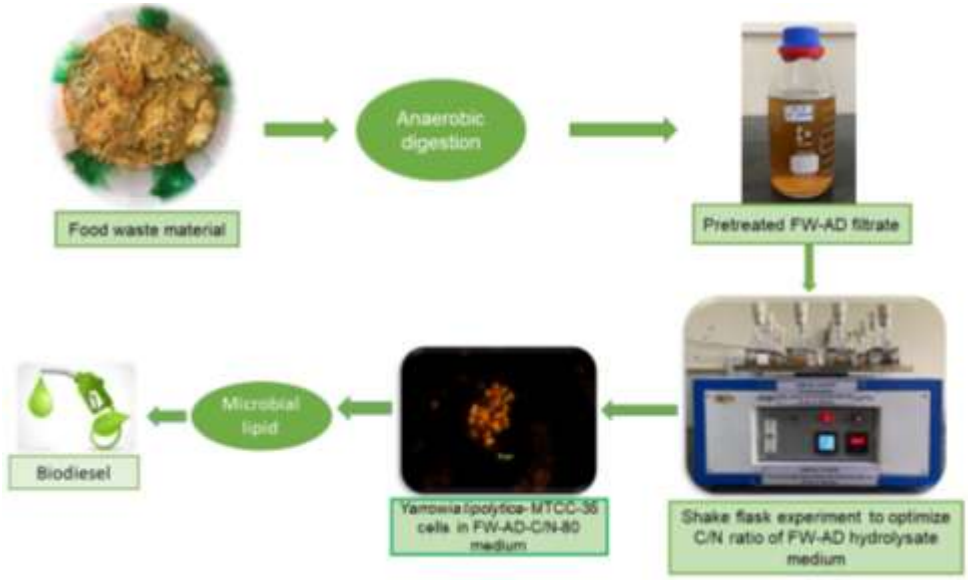
Abstract: *Microbial lipid production from waste derived material act as an alternative sustainable approach for fossil fuels. In this study, food waste anaerobic digestate (FWAD) was employed as culture medium for oleaginous yeast strain Yarrowia lipolytica MTCC 35. To optimize the culture conditions, the initial C/N ratios of the medium were adjusted to 20 using glycerol and after three days C/N ratios of the culture media were adjusted to 40 (C/N-40), 60 (C/N-60), 80 (C/N-80), 100 (C/N-100) and 120 (C/N-120). The following three treatments without C/N ratio adjustment served as controls to reveal the effectiveness of C/N adjustment: (C1) YPD medium, (C2) FWAD without pretreatment and (C3) FWAD with pretreatment C1-YL, C2-YL and C3-YL without any C/N ratio adjustment. Biomass production was the highest in YPD medium (6.73 g/L, dw basis) while the lipid content was the highest (12.53 %, w/w) in FWAD-hydrolysate medium with C/N-80. Results suggested that C/N ratio adjustment of FWAD-hydrolysate medium significantly increase the lipid production of Y. lipolytica and C/N ratio 80 of FWAD-hydrolysate medium was found optimum for high lipid production.*

Key Words: *FWAD-Hydrolysate, Yarrowia Lipolytica, C/N Ratio, Microbial Lipid Production.*

Graphical Abstract



1. INTRODUCTION

Over exploitation of fossil fuels due to population growth leads to greenhouse gas emission, climate change and other environmental problems. To alleviate these environmental consequences and minimize the fossil fuel consumption, development of alternative renewable fuels from renewable feedstock has received high priority over the past two decades. The concept of microbial lipid production using oleaginous microorganisms is becoming more widespread using a variety of renewable substrates including molasses, agro-industrial wastes, agro biomass, etc. In addition to that food waste has been

identified as a promising inexpensive substrate recently. It was reported that 1.05 billion tonnes of food waste were generated across the household, food service and retail sectors [1]. Considering this huge amount of food waste generation and the need to dispose this waste material it is recognized as an excellent alternative renewable feedstock for microbial lipid production. Microbial organisms which have the capacity to accumulate more than 20 % w/w of lipids on the basis of dry cell weight intercellularly are referred as oleaginous microorganisms [2]. The major oleaginous microbial species include microalgae, bacteria, fungi and yeasts [3]. Among these organisms, oleaginous yeasts are widely preferred for microbial lipid production owing to these organism's short life cycle, fast growth rate, less labor intensive and less cultivation area. Mostly studied oleaginous yeast species include *Candida*, *Lipomyces, Rhodotorula, Cryptococcus, Trichosporon, Rhodosporidium,* and *Yarrowia* [4]. In this study, a non-conventional yeast strain *Yarrowia lipolytica* was used for the lipid production from the food waste anaerobic digestate (FWAD). It has the ability to utilize a diverse array of low cost, renewable substrates including alkanes, fatty acids, organic acids and proteins [5]. It can tolerate severe environmental conditions like low temperature, acidic and alkaline pH and high saline condition [6]. It is reported that the wild type strain of *Y. lipolytica* can accumulate lipids up to 70% of their cell dry weight [7]. During growth phase, Oleaginous microorganisms should require nitrogen, one of the key nutrients for their cell proliferation and after the growth phase they started to accumulate lipids when the major nutrients like phosphorus and nitrogen gets exhausted from the culture medium and the carbon source is present in higher amount. The excess carbon content was converted into lipid reserves by unique biosynthetic pathways [8]. In this study nitrogen limitation condition was achieved by adjusting the carbon to nitrogen (C/N) ratio of the culture medium by supplementing glycerol as carbon source. A two-stage batch experiment was conducted to optimize the C/N ratio of the FWAD hydrolysate medium for improved biomass production followed by enhanced lipid production of *Y. lipolytica* MTCC 35

2. MATERIALS AND METHODS

2.1. Yeast Strain

The oleaginous yeast strain *Yarrowia lipolytica* MTCC 35 was purchased from Microbial Type Culture collection and Gene Bank (MTCC), Chandigarh, India. The yeast strain was maintained on YPD agar medium (Yeast extract-10 g/L, Peptone- 20 g/L, Dextrose- 20 g/L) and subcultured at regular intervals.

2.2. Food Waste Anaerobic Digestion and Pretreatment

Anaerobic digestion (AD) of food waste (FW) was carried out in a 5-L glass vessel with a working volume 4-L and the reactor was continuously stirred using magnetic stirrer. Cow dung was used as pre-inoculum for the anaerobic digestion reaction. Every alternate day, 100 ml of FWAD was withdrawn from the reactor and 100 ml food waste slurry (1:3, FW: water) was fed to the reactor. The FWAD collected from the reactor was subjected to acid and thermal pretreatment by adjusting the pH of the effluent into 3 by using 4M HCl for 24 h followed by autoclaving the sample at 121℃ for 15 min. The pretreated effluent was filtered and used as culture medium for *Y. lipolytica*.

2.3. Culture of *Y. Lipolytica*-35 on FWAD-Hydrolysate Medium

Experiment was conducted in 250-ml conical flask with 100 ml culture medium. Each treatment was inoculated with 10% of 24 h old *Y. lipolytica* cells grown in YPD broth. Then the culture flasks were incubated on an orbital shaker at 120 rpm at room temperature. To assess the effectiveness of adjusting the C/N ratio, YPD control (C1), FWAD without pretreatment (C2) and FWAD-hydrolysate with pretreatment (C3) were set as control. In these treatments C/N ratio was not adjusted. For treatments with adjusted C/N ratio of FWAD-hydrolysate medium, the C/N ratio was initially set as 20 to promote the biomass production and after 3 days of cultivation the C/N ratio was adjusted using glycerol to 40 (C/N-40), 60 (C/N-60), 80 (C/N-80), 100 (C/N-100) and 120 (C/N-120) to promote lipid accumulation The experiment was conducted for 8 days. The physicochemical properties of the culture medium were monitored at regular intervals.

2.4. Analytical Methods

The growth of the yeast strain was monitored by spectrophotometric method. Briefly, 1 ml of culture broth was withdrawn from the culture medium and the cells were centrifuged at 6000 rpm for 30 min.

Then the cells were washed twice with distilled water and re-suspended in phosphate buffered saline (PBS, pH 7.4) and diluted to 100 times using distilled water. Then the absorbance was read at 600 nm using the spectrophotometer. pH of the culture medium was measured using Eutech pH meter. Total organic carbon content (TOC) was analyzed using Walkley-Black method. Total Kjeldahl nitrogen (TKN), available nitrogen, total phosphorus (TP) and available phosphorus were measured using the Standard methods [9]. After 8 days of cultivation, the yeast cells were harvested by centrifugation and washed twice with distilled water. The wet cells were dried at 105 ° C for 24 h. Then the biomass was calculated by gravimetric method. Lipids were extracted from the dried biomass using the method of Li et al [10] with some modifications. For lipid extraction the dried biomass was acid treated with 4 M HCl for 2 h at 70 ° C. After digestion, the mixtures were centrifuged at 6000 rpm for 15 min. The supernatant from the biomass was collected separately and extracted twice with methanol/chloroform (1:1), then washed with 0.1% NaCl solution. Then the chloroform layer containing lipids was collected in a pre -weighed glass vial. The samples were dried in hot air oven for 24 h and the total lipid content of the samples was determined gravimetrically.

2.5. Nile Red Staining

Yarrowia lipolytica cells were stained with Nile red using the method followed by Kimura et al. [11]. Briefly, 0.1 g of wet cells were suspended in PBS buffer (pH 7.4), stained with Nile red dye dissolved in acetone (1 mg/ml), and incubated in dark condition for 30 mins. After the incubation period the stained cells were washed twice with distilled water and observed under advanced light and fluorescence microscope (Nikon 8i Eclipse) equipped with an excitation filter of 470/40 nm.

3. RESULTS AND DISCUSSION

3.1. Properties of FWAD-Hydrolysate

Selected physicochemical properties of pretreated FWAD are listed in the Table.1. Due to the acid – thermal pretreatment, the FWAD hydrolysate showed acidic pH and high electrical conductivity. Total carbon content of the FWAD is low and within 1%. Chatterjee and Mohan [12] reported that compared to alkali pretreatment of vegetable waste acid pretreatment catalyzes the breakdown of complex sugars into monosaccharides more efficiently.

Table 1. Physicochemical properties of FWAD-hydrolysate

Parameter	Value
pH	3.08 ± 0.015
Electrical conductivity (mS/cm)	27.43 ± 1.064
Total organic carbon (%)	0.510 ± 0.009
Total Kjeldahl nitrogen (mg/L)	0.534 ± 0.053
Available nitrogen (mg/L)	0.355 ± 0.020
Total phosphorus (mg/L)	4.50 ± 0.63
Available phosphorus (mg/L)	0.794 ± 0.025
C/N ratio	5.73 ± 0.06

3.2. Growth Pattern of *Y. Lipolytica*-35 in Different Culture Medium

The growth of the yeast strain was determined by spectrophotometric method at two-day interval. As shown in Fig.1, *Y. lipolytica* exhibited longer lag period in all the culture medium and after 2 days only it started to grow slowly. This may results from the initial drop in culture medium pH (Fig.2). Compared to other culture medium, the cells in C1 treatment (YPD medium) showed rapid growth rate due to the presence of all the essential nutrients available but in other treatments, except glycerol addition for C/N ratio adjustment, there is no key nutrients were supplemented. After 6 days, the cells in different culture medium reached their stationary growth phase except in C1 and C/N-80.

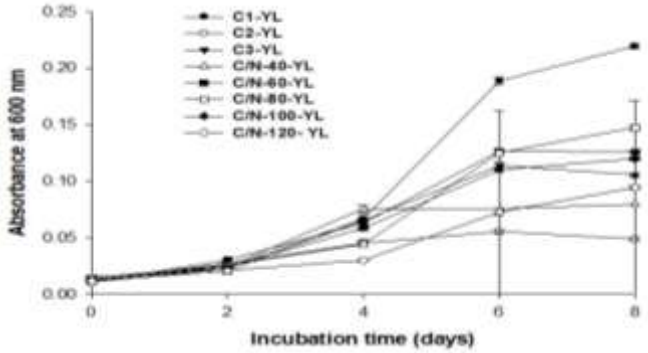


Figure 1. Growth pattern of *Yarrowia lipolytica* MTCC 35 in different culture medium. C1- YPD control; C2- FW AD without pretreatment; and C3- FWAD-hydrolysate with pretreatment. For C/N treatments, the initial C/N was set to 20, and changed to 40 (C/-40), 60 (C/N-60), 80 (C/N-80), 100 (C/N-100), and 120 (C/N-120) after three days.

3.2. Impact of pH of the Culture Medium on Biomass Yield and Lipid Accumulation

Among the other factors pH of the culture medium is considered as a significant factor affecting the proliferation of cells and accumulation of lipids by the yeast strain *Y. lipolytica*. Based on the nature of organism and carbon sources used for cultivation, the optimum pH value varies. The changes in pH of the different medium during the experiment are depicted in Fig.2. Initial pH values for all the treatments were set as 6.5. The pH of the culture medium was measured at two-day interval. It was observed that in all treatments except control medium C2, pH was decreased on day 2. Similar result was observed by Gao et al. [13] using acetic acid as culture medium for *Y. lipolytica.* Zheng et al. [14] reported that rapid decrease in pH on the first few days results from massive uptake of ammonium from the culture medium and further increase in pH on the following days due to the consumption of acetate ions and production of OH^-groups.

In this study after 2 days, in all C/N ratio altered medium (C/N-40 to C/N-120) pH declined for the entire experimental time. In contrast, in the three control treatments (C1, C2, and C3), pH increased from acidic to alkaline condition. Gao et al., [13] suggested that initial pH of 8 was found optimal for *Y. lipolytica* and significantly improve biomass and lipid production. Zhang et al. [15] found that *Y. lipolytica* W29 showed enhanced citric acid production at neutral pH while improved lipid production at more acidic pH values (pH-2) and also reported that when the pH of the culture medium changed from pH 5 to 6, the cells were ceased to grow in that medium. Dobrowolski et al. [16] achieved high lipid production from engineered *Y. lipolytica* strain at pH 3 using crude glycerol medium. Yang et al. [17] utilized food waste derived VFA supplemented with nutrients for biomass and single cell protein production; and reported high biomass production (9.2 ± 0.3 g/L) at pH 4.5 and high SCP production from pH 6.5. They also reported that culture medium with pH 4.5 metabolized the organics of the substrate into biomass while at the pH 6.5 the organics were metabolized into SCP. Xu et al. [18] found that volatile fatty acid medium derived from food waste hydrolysate with pH 5 is most suitable for lipid production of *Y. lipolytica* but when synthetic VFA was used as culture medium the pH values raised from initial 7.0 to 8.43.

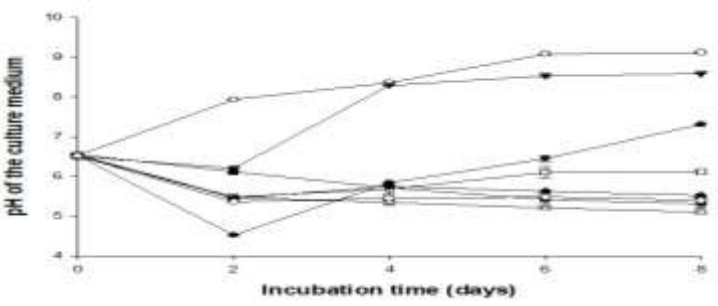


*Figure 2.*Changes in pH value of the different culture medium. C1- YPD control; C2- FW AD without pretreatment; and C3- FWAD-hydrolysate with pretreatment. For C/N treatments, the initial C/N was

set to 20, and changed to 40 (C/-40), 60 (C/N-60), 80 (C/N-80), 100 (C/N-100), and 120 (C/N-120) after three days.

3.4. Consumption of Nutrients from the Different Culture Medium by *Y. Lipolytica*.

Compared with three control medium, carbon consumption rate was slower in all other treatments which may be due to the addition of glycerol for initial adjustment of C/N ratio to 20. It was noted that in all the C/N ratio adjusted treatments (C/N-40 to C/N-120) after 8 days of cultivation significantly high quantity of carbon was left underutilized by the yeast strain (Fig. 3). Similar result was observed by Bellou et al. [19] using glycerol as carbon source for the lipid production of *Y. lipolytica*. Except in C1 and C2 treatments, in all the other treatments rapid consumption of available nitrogen was observed which eventually lead to drop in pH values (Fig. 2 and Fig. 4b).

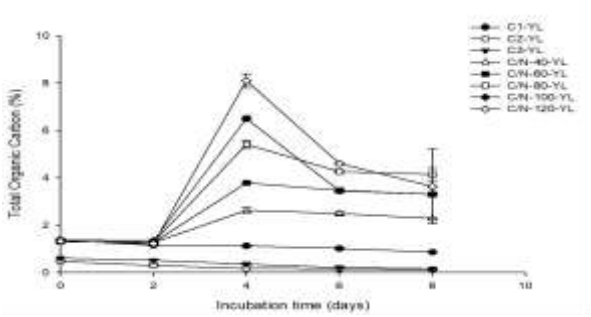


Figure 3. Changes in total organic carbon content (%) of the different culture medium. C1- YPD control; C2- FWAD without pretreatment; and C3- FWAD-hydrolysate with pretreatment. For C/N treatments, the initial C/N was set to 20, and changed to 40 (C/-40), 60 (C/N-60), 80 (C/N-80), 100 (C/N-100), and 120 (C/N-120) after three days.

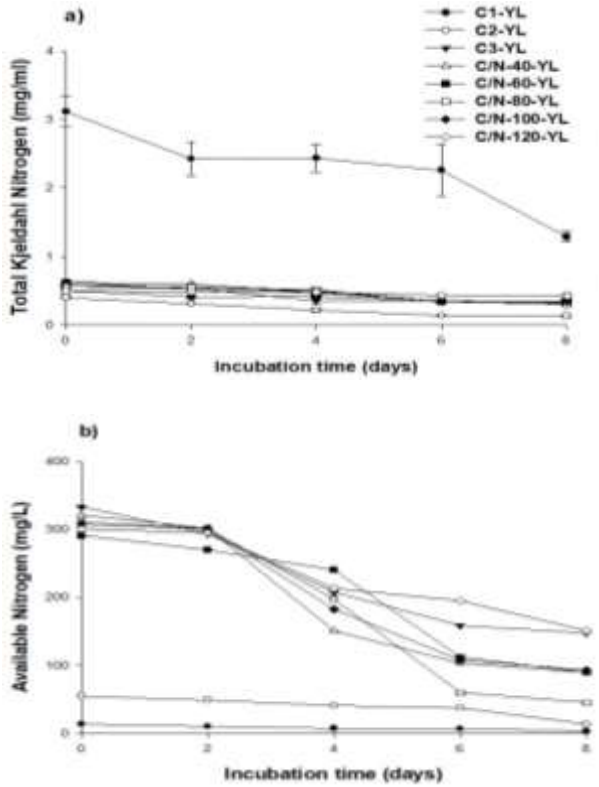


Figure 4. (a) Changes in total Kjeldahl nitrogen (mg/ml) and (b) available nitrogen (mg/L) of the culture time during the cultivation period. C1- YPD control; C2- FWAD without pretreatment; *and* C3- FWAD-

hydrolysate with pretreatment. For C/N treatments, the initial C/N was set to 20, and changed to 40 (C/-40), 60 (C/N-60), 80 (C/N-80), 100 (C/N-100), and 120 (C/N-120) after three days.

Mathiazhagan et al. [20] found that glycerol uptake was increased with rise in glycerol concentration and also reported that the glycerol consumption rate was high at low C/N ratio of 25 while it started decreasing with further raise in C/N ratio to 150. It was reported that glycerol is one of the most preferred carbon sources for *Y. lipolytica* and the glycerol can repress the uptake of other substrates even glucose [21-22]. Another important nutrient phosphorus consumption from the culture medium was shown in the Fig. 5a and 5b.

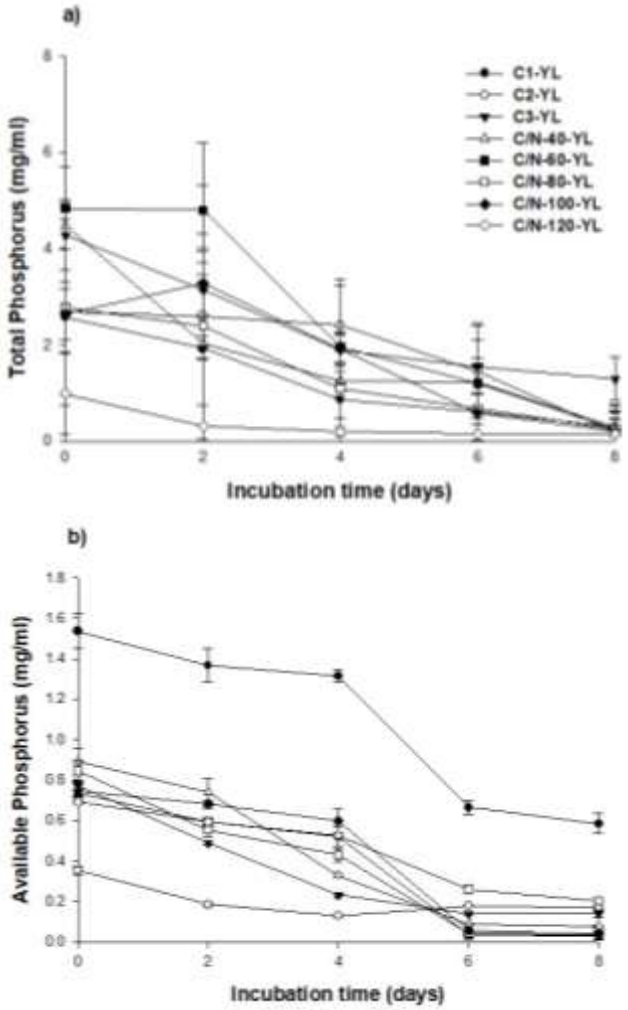


Figure 5. (a) Changes in total phosphorus (mg/ml) and (b) available phosphorus (mg/ml) from the different culture time during the cultivation period. C1- YPD control; C2- FW AD without pretreatment; *and* C3- FWAD-hydrolysate with pretreatment. For C/N treatments, the initial C/N was set to 20, and changed to 40 (C/-40), 60 (C/N-60), 80 (C/N-80), 100 (C/N-100), and 120 (C/N-120) after three days.

3.5. Effect of C/N Ratio on the Biomass and Lipid Production of *Y. Lipolytica*

To induce lipid accumulation, nitrogen limitation and high C/N ratio are the commonly practiced strategies for the oleaginous microorganisms [19]. Fig. 6 shows fluorescence microscopic observation of Nile red dye stained cells of *Y. lipolytica*-35 cultivated in C3 medium and C/N-80 medium. It was observed that the cells are oval in shape with numerous lipid bodies inside the cells appeared in golden yellow colour. Compared to all the treatments a biomass of 6.73 g/L was obtained from C1 medium followed

by 4.62 g/L from the treatment C/N-80 (Fig. 7). Similar quantity of biomass (6.3±0.2 g/L) was obtained from *Y. lipolytica* ACA-DC 50109 with glycerol as carbon source and yeast extract as nitrogen source at C/N ratio 103 [19]. Slightly lower amount of biomass (3.15 g/L) was harvested from the treatment C/N-120. From C/N-40 to 100 treatments almost similar amount of biomass ranging from 4.27 g/L to 4.62 g/L was obtained. Least amount of biomass (1.04 g/L) was obtained from C2 treatment. It was reported that from food waste leachate medium *Y. lipolytica* produced 48% lipid content in total biomass [23]. It was found that lower C/N ratio of the culture medium enhanced the biomass production of the yeast strain *Y. lipolytica*. [36].Gao et al. [13] reported that *Y. lipolytica* yielded 14.65 g/L of biomass and 21.86% of lipid content from volatile fatty acids medium derived from FW fermentation.

Using purified glycerol as culture medium, *Y. lipolytica* SKY produced 19.5 g/L biomass and 7.3% lipid content [24]. Kommoji et al. [25] reported that from grass hydrolysate culture medium with C/N ratio 50, *Y. lipolytica* MTCC 9519 produced 17.5 g/L biomass and 7.9 % of lipid content.

According to Gao et al. [26], complex composition of FW fermentate contain some unknown inhibitors which may adversely affect the lipogenesis of the yeast strain. Oleaginous microorganisms generally prefer Carbon to Nitrogen (C/N) ratios varies from 40 to 80 for their appropriate lipid accumulation and they are unable to accumulate high content of lipid with C/N ratio lower than 20 [27]. In this study, among all the treatments high lipid content was obtained from C/N-80 medium (12.53 % w/w) followed by C3 medium (8.29% w/w) and it also observed that in the FW AT medium with C/N ratio above 80 the lipid content started decreasing. It was reported that when the C/N ratio of the culture medium above 200 the lipid concentration was decreased for the yeast strain *Cryptococcus curvatus* in synthetic medium with hemicellulose prehydrolysate liquor [29].

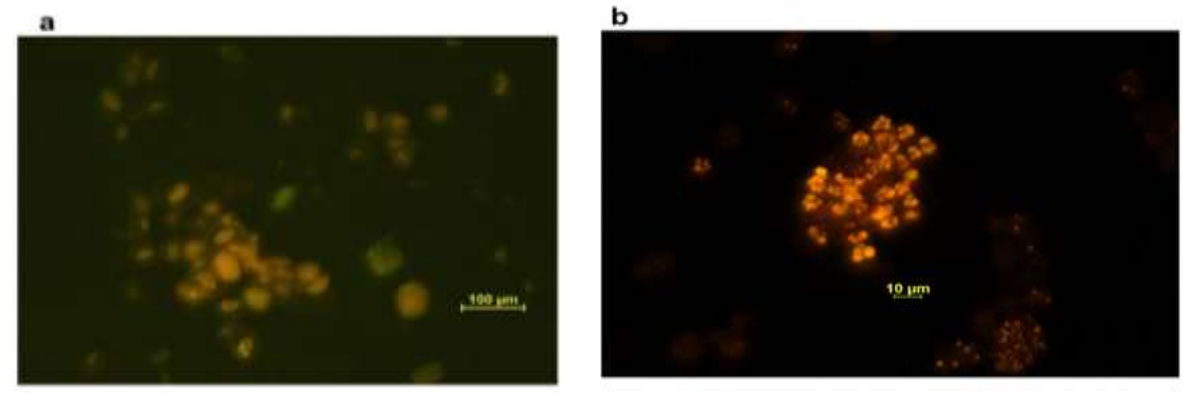


Figure 6. Microscopic observation of *Yarrowia lipolytica* MTCC 35 cells stained with Nile red cultivated in (a) C3 medium, FWAD-hydrolysate with pretreatment and (b) C/N-80 medium - the initial C/N was set to 20, and after three days changed to 80.

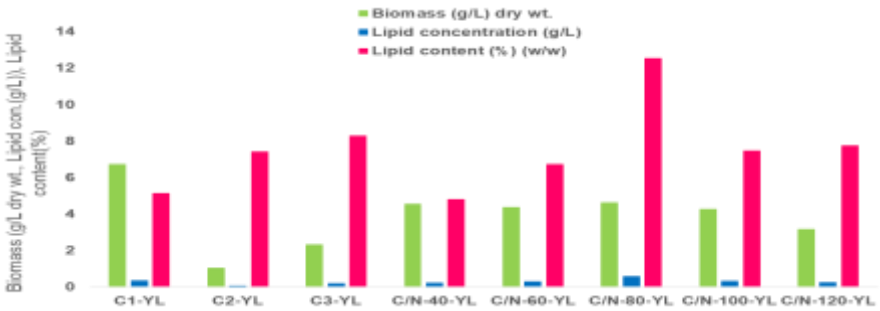


Figure 7. Biomass and lipid content of *Yarrowia lipolytica* MTCC-35 from different culture medium. C1- YPD control; C2- FW AD without pretreatment; *and* C3- FW AD hydrolysate with pretreatment. For

C/N treatments, the initial C/N was set to 20, and changed to 40 (C/-40), 60 (C/N-60), 80 (C/N-80), 100 (C/N-100), and 120 (C/N-120) after three days.

Bellou et al. [20] reported that using glucose as carbon source and yeast extract as nitrogen source the yeast strain *Y. lipolytica* produced 12.2 g/L of biomass and 7.8 % w/w lipid content. They also found that using glycerol or glucose as carbon source, nitrogen limitation condition was not suitable for enhancing lipogenesis in *Y. lipolytica*.
To achieve high C/N ratio, high amount of carbon sources are needed to add in to the system [13]. Mathiazhakan et al. [21] found that at C/N ratio of 100 and glycerol concentration of 100 g/L, *Y. lipolytica* SKY 7 showed improved biomass and lipid production. Vajpeyi and Chandran [28] reported that using VFA derived from food waste the yeast strain *Cryptococcus albidus* produced 14.9% lipid content. Similarly From oat bran hydrolysate medium wild-type strain *Y. lipolytica* A101 produced 11% w/w of lipid content [33]. Gao et al. [27] found that VFA derived from restaurant FW as culture medium for *Y. lipolytica*, a final lipid content of 18.23% was attained. From the current study it was found that the lipid production was enhanced in considerable amount when C/N ratio of the medium was adjusted to 80 when compared with three control mediums C1, C2 and C3.

4. CONCLUSIONS

Pretreated food waste anaerobic digestate (FWAD) can be used as an alternative culture medium from a renewable source for the microbial lipid production of the yeast strain *Y. lipolytica*. Lipid and biomass production using FWAD-hydrolysate medium with C/N ratio ranging from 40 to 120 was compared with the three control medium (C1, C2 and C3) without C/N ratio adjustment. The yeast strain produced 6.73 g/L (dry wt. basis) of biomass from C1 control medium. A lipid content of 12.53 % was produced from FWAD-hydrolysate medium with C/N-80 while FWAD-hydrolysate medium with C/N ratio above 80, the lipid content started decreasing from 12.53 % to 7.47%. Although, it was found that C/N ratio 80 of FWAD-hydrolysate medium was suitable for the high lipid production of *Y. lipolytica*-35.

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Ethical Approvals: This study does not involve experiments on animals or human subjects.

Conflits of Interest: The authors declare that there are no conflicts of interest.

REFERENCES

[1] UNEP Food waste index report 2021.https:// www. unep. org/ resou rces/ report/ unep- food- waste- index- report- 2024.
[2] Patel, A., Karageorgou, D., Rova, E., Katapodis, P., Rova, U., Christakopoulos, P and Matsakas, L. (2020).An Overview of Potential Oleaginous Microorganisms and Their Role in Biodiesel and Omega-3 Fatty Acid-Based Industries. Microorganisms, 8(3):434.
[3] Vicente, G., Bautista, L., Rodríguez, R., Gutiérrez, F., Sadaba, I., Ruiz-Vazquez, R. M., Torres, S and Garre, V. (2009). Biodiesel production from biomass of an oleaginous fungus. Biochemical Engineering Journal. 48(1):22-27.
[4] Papanikolaou, S., Rontou, M., Belka, A., Athenaki, M., Chr, G., Mallouchos, A., Kalantzi, O., Koutinas, A.A., Kookos, I.K., Zeng, A.P and Aggelis, G, (2017). Conversion of biodiesel-derived glycerol into biotechnological products of industrial significance by yeast and fungal strains. Engineering in Life Sciences, 17: 262–281.
[5] Hu, M., Ge, J., Jiang, Y., Sun, X., Guo, D. and Gu, Y. (2024) Advances and perspectives in genetic expression and operation for the oleaginous yeast Yarrowia lipolytica. Synthetic and System Biotechnology; 9 (4):618-626.

[6] Zinjarde, S.S. (2014). Food-related applications of Yarrowia lipolytica. Food Chemistry, 152:1–10.
[7] Beopoulos, A and Nicaud, J.-M., (2012). Yeast: A new oil producer? Oléagineux, Corps gras, Lipides. 19: 22–28.
[8] Rossi, M., Amaretti, A., Raimondi, S and Leonardi, A. (2011). Getting Lipids for Biodiesel Production from Oleaginous Fungi. InTech. doi: 10.5772/25864
[9] APHA, 2017. Standard methods for the examination of Water and Wastewater, 23rd ed. American Public Health Association, American Water Works Association, Water Environmental Federation, Washington, DC, New York, USA.
[10] Li, Q., Kamal, R., Chu, Y., Wang, Q., Yu, X., Huang, Q. (2020). Automated pressurized liquid extraction of microbial lipids from oleaginous yeasts. Applied Biochemistry and Biotechnology, 192(3):283–295.
[11] Kimura, K., Yamaoka, M. and Kamisaka, Y. (2004). Rapid estimation of lipids in oleaginous fungi and yeasts using Nile red fluorescence, Journal of Microbiological Methods, 56, (3): 331-338.
[12] Chatterjee, S and Mohan, S.V. (2018).Microbial lipid production by Cryptococcus curvatus from vegetable waste hydrolysate. Bioresource Technology, 254:284-289.
[13] Bao, W, Li, Z, Wang, X, Gao, R, Zhou, X, Cheng, S, Men, Y, and Zheng, L (2021).Approaches to improve the lipid synthesis of oleaginous yeast Yarrowia lipolytica: A review, Renewable and Sustainable Energy Reviews, 149: 111386.
[14] Gao, R., Li, Z., Zhou, X., Bao, W., Cheng, S. and Zheng, L. (2020). Enhanced lipid production by Yarrowia lipolytica cultured with synthetic and waste-derived high-content volatile fatty acids under alkaline conditions. Biotechnology for Biofuels; 13, Article number: 3.
[15] Zheng, Y.B., Li, T.T., Yu, X.C., Bates, P.D., Dong, T and Chen S. (2013). High-density fed-batch culture of a thermotolerant microalga Chlorella sorokiniana for biofuel production. Applied Energy, 108:281–7.
[16] Zhang, S., Jagtap, S., Deewan, Anand Rao, C. (2018). pH selectively regulates citric acid and lipid production in Yarrowia lipolytica W29 during nitrogen-limited growth on glucose. Journal of Biotechnology, 290:10-15.
[17] Dobrowolski, A and Mironczuk, A.M. (2020).The influence of transketolase on lipid biosynthesis in the yeast Yarrowia lipolytica. Microbial Cell Factories; 19(1):138.
[18] Yang, R., Chen, Z., Hu, P., Zhang, S and Luo, G. (2022). Two-stage fermentation enhanced single-cell protein production by Yarrowia lipolytica from food waste. Bioresource Technology; 61: 127677.
[19] Xu, Y., Wang, X., Li, Z., Cheng, S and Jiang, J. (2022). Potential of food waste hydrolysate as an alternative carbon source for microbial oil synthesis. Bioresource Technology; 344 (Part B):126312.
[20] Bellou S, Triantaphyllidou IE, Mizerakis P, Aggelis G. (2016) High lipid accumulation in Yarrowia lipolytica cultivated under double limitation of nitrogen and magnesium. Journal of Biotechnology; 234:116–126.
[21] Mathiazhakan, K., Ayed, D and Tyagi, R.D. (2016) .Kinetics of lipid production at lab scale fermenters by a new isolate of Yarrowia lipolytica SKY7. Bioresource Technology. ; vol 221: 234-240.
[22] Mori, K., Iwama, R., Kobayashi, S., Horiuchi, H., Fukuda, R., Ohta, A., (2013). Transcriptional repression by glycerol of genes involved in the assimilation of N alkanes and fatty acids in yeast Yarrowia lipolytica. FEMS Yeast Research; 13(2): 233–240.
[23] Sabra, W., Bommareddy, R.R., Maheshwari, G., Papanikolaou, S and Zeng, A.P., (2017). Substrates and oxygen dependent citric acid production by Yarrowia lipolytica: in sights through transcriptome and fluxome analyses. Microbial Cell Factories; 16:1–14.
[24] Johnravindar, D., Karthikeyan, O.P., Selvam, A., Murugesan, K. and Wong, J.W.C. (2018). Lipid accumulation potential of oleaginous yeasts: A comparative evaluation using food waste leachate as a substrate. Bioresource Technology, 248: 221-228.
[25] Kumar, L.R., Yellapu, S.K., Tyagi, R.D and Drogui, P., (2020). Purified crude glycerol by acid treatment allows to improve lipid productivity by Yarrowia lipolytica SKY7. Process Biochemistry; 96:165–173.
[26] Kommoji, S., Gopinath, M., Satya Sagar, P., Yuvaraj, D., Iyyappan, J., Jaya Varsha, A, and Sunil,V. (2021). Lipid bio production from delignified native grass (Cyperus distans) hydrolysate by Yarrowia lipolytica. Bioresource Technology; 324:124659.
[27] Gao, R., Li, Z., Zhou, X., Cheng, S and Zheng, L. (2017).Oleaginous yeast Yarrowia lipolytica culture with synthetic and food waste-derived volatile fatty acids for lipid production. Biotechnology for Biofuels; 10:247-262.
[28] Seo, Y.H., Lee, I.G and Han, J.I. (2013). Cultivation and lipid production of yeast Cryptococcus curvatus using pretreated waste active sludge supernatant. Bioresource Technology; 135:304-308.
[29] Vajpeyi, S and Chandran, K., (2015). Microbial conversion of synthetic and food waste- derived volatile fatty acids to lipids. Bioresource Technology; 188, 49–55.
[30] Samavi, M., Uprety, B.K. and Rakshit, S. (2019) Bioconversion of Poplar Wood Hemicellulose Prehydrolysate to Microbial Oil Using Cryptococcus curvatus. Applied Biochemistry and Biotechnology, vol-189(2):626-637.
[31] Ichihara, K. I., and Fukubayashi, Y. (2010). Preparation of fatty acid methyl esters for gas-liquid chromatography. Journal of Lipid Research, 51(3): 635–640.
[32] Drzymała, K., Mironczuk, A.M., Pietrzak, W and Dobrowolski, A. Rye and oat agricultural wastes as substrate candidates for biomass production of the non- conventional yeast Yarrowia lipolytica, Sustainability, 12: 1–12.
[33] Robles-Iglesias, R., Nicaud, J. M., Veiga, M. C and Kennes, C. (2023). Integrated fermentative process for lipid and β-carotene production from acetogenic syngas fermentation using an engineered oleaginous Yarrowia lipolytica yeast. Bioresource technology, 389, 129815.