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Effect of pre-treatment of Food waste on lipid production of the two oleaginous yeast strains *Cryptococcus curvatus* 2698 and *Yarrowia lipolytica 35*

Arjunan Umapathi [1], Kumarasamy Murugesan [2], Ammaiyappan Selvam[1*]

[1] Department of Plant Science, Manonmaniam Sundaranar University, Abhishekapatti, Tirunelveli, Tamil Nadu, India

[2] Department of Environmental Science, Periyar University, Salem, Tamil Nadu, India

[*]Corresponding author: selvam@msuniv.ac.in

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ABSTRACT

A batch experiment was conducted to study the effect of different pretreatment methods of food waste (FW) on the growth and lipid accumulation of two oleaginous yeasts *Cryptococcus curvatus* 2698 and *Yarrowia lipolytica* 35. Food waste was pretreated by acid (A), thermal (T) and acid-thermal (AT) hydrolysis methods. The resulting FW filtrate, without any nutrient supplementation, was used as culture medium for both the yeasts. Compared to other pretreatment methods, acid-thermal hydrolysis method solubilized more carbon content from the food waste material. This higher carbon content of FW-AT medium supported higher biomass production of the yeast strains *C. curvatus* (10.35 ± 0.51 g/L) and *Y. lipolytica* (9.80 ± 1.2 g/L). Compared to *Y. lipolytica*, higher lipid content was obtained from *C.curvatus* from the acid treated food waste medium (8.81 ± 1.17 %, g/g). It was observed that the culture medium from food waste with acid-thermal pretreatment is suited for biomass production and food waste with acid pretreatment medium enhanced the lipid production among the other treatments.

INTRODUCTION

According to UNEP food waste index report 2024 (UNEP, 2024), globally 1.05 billion tonnes of food was wasted in 2022. In India, 68.7 million tonnes of household food is wasted every year. Due to the increasing population, food waste production also gradually increasing. Improper waste management methods lead to serious threats to the environment. Food waste are rich in organic compounds such as carbohydrates, protein, lipid and fraction of inorganic compounds like sodium, magnesium, calcium, iron, phosphorus and nitrogen (Lin *et al.*, 2013, Dahiya *et al.*, 2015). Microbial lipid production from food waste using oleaginous microorganisms is considered as one of the alternative methods to reduce the amount of food waste; and the resulting lipids extracted from the yeast can be used as biofuels (Donzella *et al.*, 2022).

Microorganisms which are able to accumulate lipids more than 20 % of their dry biomass as lipid are known as oleaginous microorganisms (Patel *et al.*, 2020). It includes microalgae, yeasts, fungi, and even bacteria. It was reported that among 1,958 numbers of known yeast species only approximately 160 species of yeast are found oleaginous in nature (Abeln and Chuck, 2021, Boekhout *et al.*, 2022 and Poontawee *et al.*, 2023). Among the oleaginous yeasts *Cryptococcus curvatus* have the capacity to accumulate large amounts of lipid, up to 60% of the cell's dry weight (Hassan et al, 1996). It can utilize a very broad range of substrates such as corn cob hydrolysate (Chang et al 2015), vegetable waste hydrolysate (Chatterjee & Mohan .2018), corn stover (Gong *et al.*, 2014), sweet sorghum bagasse (Liang *et al.*, 2014), spent coffee grounds (Titiri *et al.*, 2023), crude-glycerol (Signori *et al.*, 2016), wheat straw hydrolysate (Yu *et al.*, 2014) and waste office paper (Gong *et al.*, 2024) as carbon source for their growth and lipid accumulation.

Pretreatment methods are required to solubilize the available nutrients in the food waste material from its complex structure into simple sugars that can be readily available for the microorganisms (Chua *et al.*, 2021). Wang *et al.*, 2024 found that acidification pretreatment improved the efficiency of hydrolysis and acidogenesis in acidified food waste. The aim of the present study was to pretreat the food waste material by using three different hydrolysis methods such as acid, thermal and combination of both acid and thermal to increase the solubility of FW material. The resulting FW hydrolysate from different pretreatments was used as the culture medium for the growth and lipid production of the two oleaginous yeast *Cryptococcus curvatus* 2698 and *Yarrowia lipolytica 35*.

2. Materials and methods

2.1. Preparation of food waste material

Simulated food waste material was prepared by mixing boiled rice (~37 %, wt./vol.), boiled dahl (~7.5 %, wt./vol.), fried chapati (~18.5 %, wt./vol.), vegetables such as potato and cabbage (~37 %, wt./vol.) The physicochemical properties like pH, electrical conductivity (EC), moisture content, total organic carbon (TOC), and total Kjeldahl nitrogen (TKN) of the collected food waste material were analyzed.

2.2. Pre-treatment of food waste material

The prepared food waste material was ground well with the addition of water in 1:3 ratio using a mechanical blender. Then the FW slurry was pre-treated by different hydrolysis methods (acid, thermal and acid-thermal) following the protocols reported by Karthikeyan et al. (2018). After the pre-treatment, the FW hydrolysate was filtered through a fine cheese cloth followed by centrifugation. The resulting supernatant from the FW filtrate was used as the medium for cultivation of oleaginous yeast strains.

2.3. Yeast strain

The two oleaginous yeast *Cryptococcus curvatus* MTCC-2698 and *Yarrowia lipolytica* MTCC-35 were procured from Microbial type culture collection center and gene bank (MTCC), Chandigarh, India. The cells of the yeast strain were revived from the freeze dried condition and maintained on YPD agar medium (dextrose 20 g/L, yeast extract 10 g/L, peptone 20 g/L and agar 15 g/L, pH- 6.5). For pre-inoculum preparation, a loop full of culture was inoculated into a 250-ml shake flask containing 100 ml of YPD broth and incubated in an orbital shaker at 120 rpm for 24 h at room temperature.

2.4. Cultivation of yeast strain on FW medium

Prior to inoculation, the pH value of each medium was set as 6.5 using 1 N NaOH. Food waste medium without any pre-treatment and YPD broth medium were set as controls. For inoculation, 0.1 ml of 24 h old culture was used to inoculate into a 250-ml conical flask containing 100 ml of pre-treated FW medium. Then the flasks were incubated in an orbital shaker at 120 rpm at room temperature. After 192 h of cultivation the biomass from each treatment was harvested by centrifugation and the lipid content was extracted from the dried biomass.

2.5. Analytical methods

For analysis of the yeast growth, 1 ml of culture broth from each culture medium was withdrawn under sterile condition and centrifuged. The pellet was washed twice with distilled water and suspended in same volume of PBS buffer (pH 7.4). Then the suspension was diluted to 100 ml with distilled water and the growth was monitored by reading the absorbance at 600 nm. The physicochemical properties like moisture content, pH, electrical conductivity, total Kjeldahl nitrogen (TKN) and available nitrogen were determined by using the standard methods (APHA, 2017). Total organic carbon (TOC) content was determined by using modified Walkley-Black method. Total carbohydrate of food waste material was determined by using Phenol-sulphuric acid method (Dubois *et al.,* 1956). After harvesting the yeast cells, the cells were washed twice with distilled water to remove the debris and the gravimetric method was employed to calculate the final biomass of the yeast strains. From the dried biomass the lipid content was derived using the method reported by Li et al (2020) with some minor modifications. Initially, the dried biomass of the both oleaginous yeast strains collected from different treatments were digested with 4 M HCl for 2 h at 70˚C. After that the acid treated biomass was centrifuged at 6000 rpm for 15 min; supernatant was extracted twice with methanol/chloroform (1:1) mixture. Then the chloroform layer containing lipids was transferred into a pre-weighed glass vial and washed with 0.1% NaCl solution. The solvent from lipid samples was evaporated in a hot air oven; and the total lipid content was determined gravimetrically.

3. Results and discussion

3.1. Properties of food waste material

The initial physicochemical properties like pH, EC, moisture content, TOC, TKN, TP, total carbohydrate and C/N ratio of the simulated food waste material were analyzed and are presented in Table 1. In accordance with the food waste material composition and the region where it was generated the physico-chemical properties varied (Slopiecka *et al.,* 2022).

Table 1. Properties of Food waste

Parameter	Value
pH	6.03 ± 0.01
Electrical conductivity (mS/cm)	4.59 ± 0.87
Moisture content (%)	62.76 ± 3.2
Total Organic Carbon (%, dry wt. basis)	50.31 ± 4.41
Total Kjeldahl Nitrogen (%, dry wt. basis)	1.20 ± 0.12
Total Phosphorus (%)	0.41± 0.03
C/N ratio	42.61 ± 5.77
Total carbohydrate (%dry wt. basis)	75.72 ± 9.14

The values are mean ± standard deviation (n=3)

3.2. Growth of *Cryptococcus curvatus* 2698 and *Yarrowia lipolytica* 35

The growth pattern of two oleaginous yeast strains *C. curvatus* and *Y. lipolytica* in different treatments were monitored at regular intervals for 192 h. *Cryptococcus curvatus* showed the maximum growth after 2 days of incubation in all the treatments after that gradual increase in growth curve was observed. Compared to control media pretreated FW media showed higher growth rate. After 96 h, *C. curvatus* cells reached their stationary phase in two control medium (FW-C and YPD-C). Approximately similar growth pattern was also observed for the yeast strain *Y. lipolytica* in FW pretreated culture medium. After 4 days in two control media (FW-C and YPD-C), it showed slow decline in growth (Figure 1). It was observed that FW medium with acid-thermal pretreatment was more suitable for promoting the growth of the yeast strains than the control media.

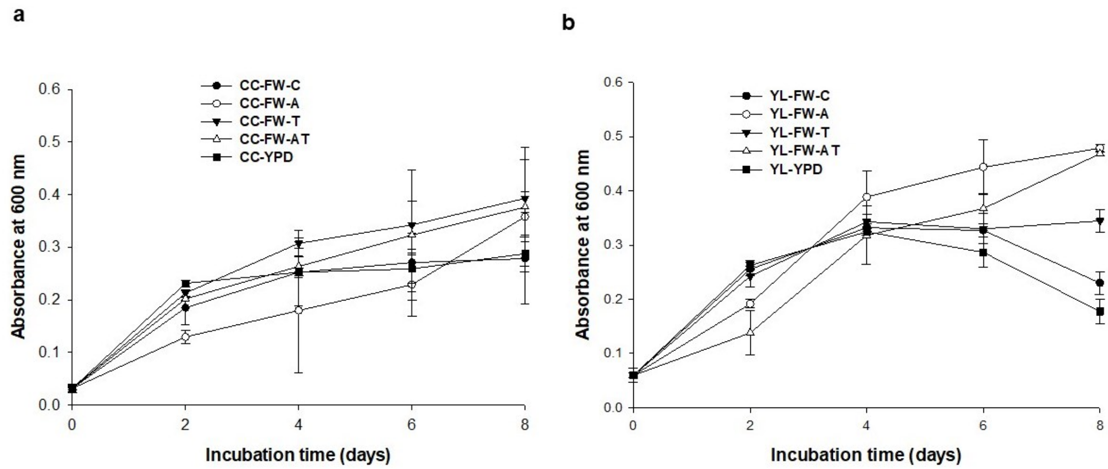

Figure 1. Growth of a) *Cryptococcus curvatus* 2698 and b) *Yarrowia lipolytica* 35 on different FW media and control media. FW-C- Food waste [control], FW-A - food waste medium with acid pretreatment, FW-T - food waste medium with thermal pretreatment, FW-AT - food waste medium with acid and thermal pretreatment and YPD-C - yeast peptone dextrose broth medium [control].

3.3. Changes in pH of the different culture medium

The pH of the culture medium is a critical factor affecting the growth and lipid accumulation of oleaginous yeast strain; and it differs based on the type of culture medium and yeast strain. Due to the formation of some organic acids on day 2, there was a marginal decrease in pH of all the culture media was observed. After 2 days of growth, the pH of all the media were gradually increased (Figure 2). The vast uptake of ammonium from the culture medium leads to rapid decrease in pH for the first few days (Zheng *et al.*, 2013). Liu *et al.*, (2017) reported that the yeast strain *C. curvatus* -ATCC20509 produced high lipid content under alkaline pH conditions (pH 8.0 to 10.0) than the neutral pH 7.0. Gong *et al.*, (2015) found that at the end of culture the pH of the culture medium was increased to above 9.0 when acetic acid was used as culture medium for the oleaginous yeast *C. curvatus*. In contrast to the observed results Chang et al. (2015) reported that after 144 h cultivation of *Cryptococcus* sp. on corn cob hydrolysate medium the pH of the culture medium was decreased from 6.0 to 3.2. Chatterjee and Mohan (2018) also reported decrease in pH values from 6.5 to 3.1 when acid pre-treated vegetable waste hydrolysate was used as culture medium.

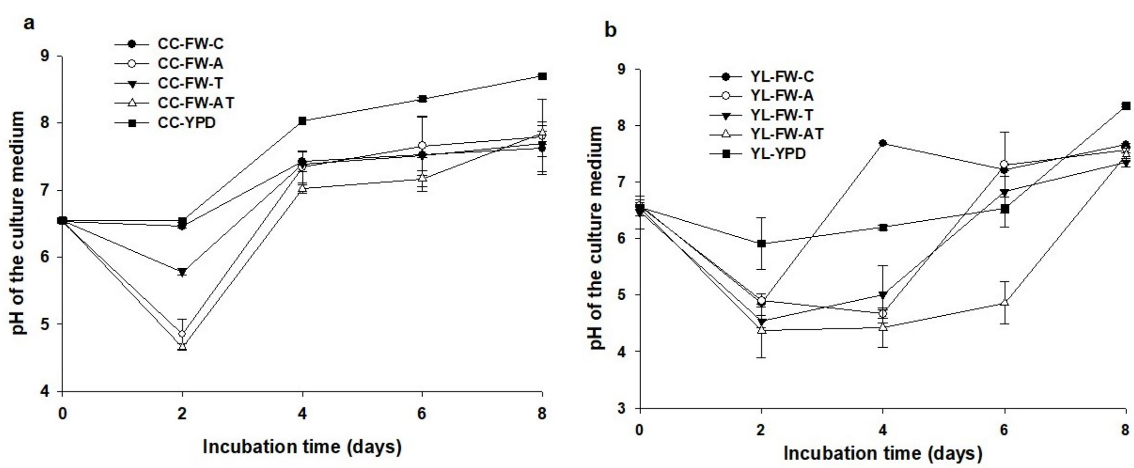

Figure 2. Changes in pH of the culture medium during cultivation of a) *Cryptococcus curvatus* 2698 and b) *Yarrowia lipolytica* 35 on different FW media and control media. FW-C- Food waste [control], FW-A - food waste medium with acid pretreatment, FW-T - food waste medium with thermal pretreatment, FW-AT - food waste medium with acid and thermal pretreatment and YPD-C - yeast peptone dextrose broth medium [control].

3.4. Changes in TOC, TKN and available nitrogen of the culture media

Carbon and nitrogen contents of the culture medium are the major factors for determining the growth and lipid accumulation of the oleaginous yeast strain. Compared to acid pretreatment, combined acid-thermal (FW-AT) and thermal (FW-T) pretreatments solubilized more carbon content from the food waste medium as shown in Figure 3a-b. The acid hydrolysis method is more efficient in the conversion of complex sugars into monosaccharides than the alkali pretreatment as reported previously (Chatterjee and Mohan. 2018).

Due to the gradual increase in the growth rate of the both yeast strains *C. curvatus* and *Y. lipolytica* in the culture media, the carbon and nitrogen contents decreased from day 0. FW medium contained low amount of nitrogen content than the YPD control medium. Among the FW culture media, acid-thermal treated FW medium contained higher TKN content than other media on day 0 (Figure 4 a-b). Chua *et al.*, (2020) reported that hydrothermal pretreatment of FW above 120ºC resulted in double the amount of carbohydrate yield but the protein content was slightly decreased. Similarly, in this study FW-T medium consists of comparatively low amount of TKN (24.41± 2.29 mg/100 ml). Compared with other FW media, rapid decrease in carbon and TKN contents from day 0 to 4 was observed in from FW-A medium. It implies that the nutrients from the acid treated FW medium were readily available for the yeast strain. Changes in available nitrogen content of the different culture medium of the yeast strains *C. curvatus* and *Y. lipolytica* are presented in Figure 4c-d.

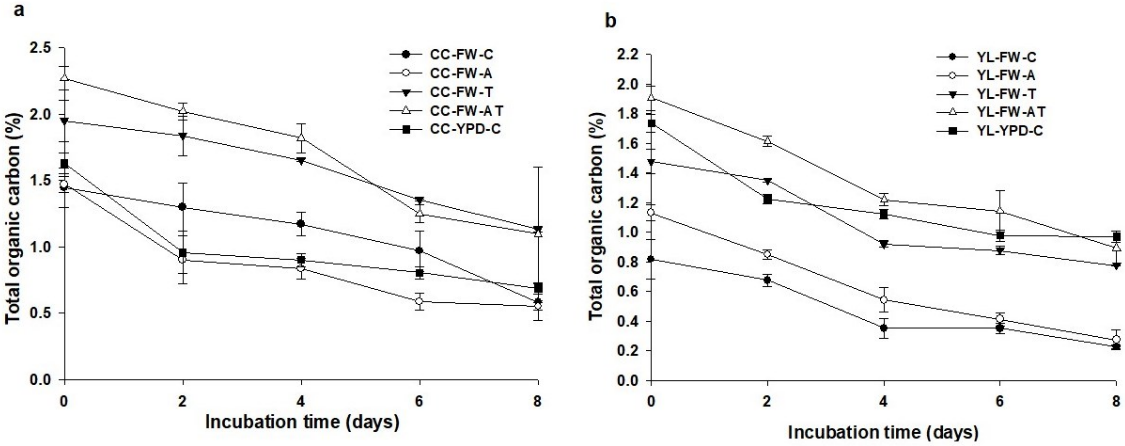

Figure 3. Changes in the total organic carbon content in the culture media during the cultivation of a) *Cryptococcus curvatus* 2698 and b) *Yarrowia lipolytica* 35. FW-C - Food waste [control], FW-A - food waste medium with acid pretreatment, FW-T - food waste medium with thermal pretreatment, FW-AT - food waste medium with acid and thermal pretreatment and YPD-C - yeast peptone dextrose broth medium [control].

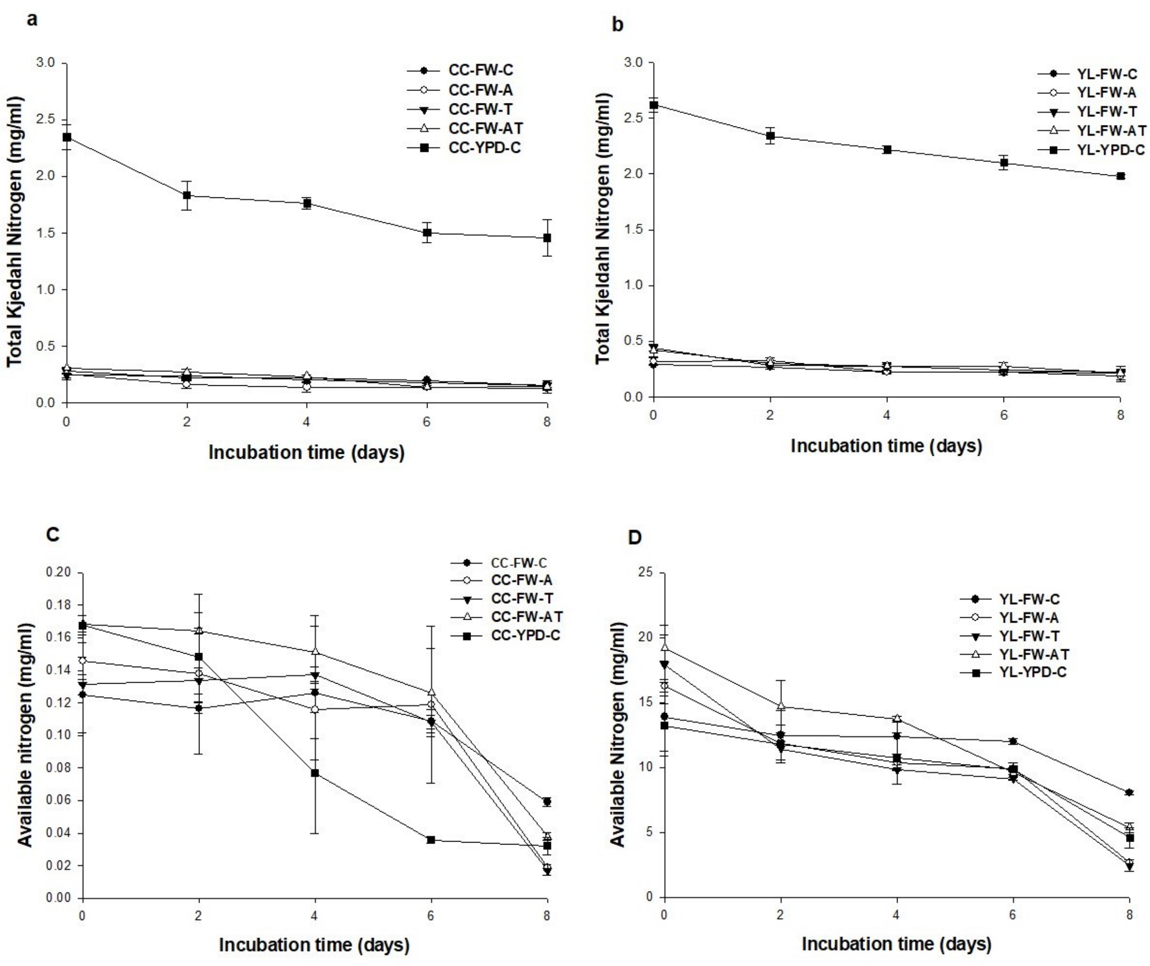

Figure 4. Changes in total Kjeldahl nitrogen and available nitrogen concentrations in the culture media during the cultivation of *Cryptococcus curvatus* 2698 (a and b) and *Yarrowia lipolytica* 35 (c and d). FW-C - Food waste [control], FW-A - food waste medium with acid pretreatment, FW-T - food waste medium with thermal pretreatment, FW-AT - food waste medium with acid and thermal pretreatment and YPD-C - yeast peptone dextrose broth medium [control].

3.5. Final Biomass and lipid content of *yeast strains* from food waste medium

Biomass from each culture medium was harvested by centrifugation after 192 h of cultivation. From the dried biomass, lipids were extracted. As presented in Figure 5, *C. curvatus* produced higher quantity of biomass (10.35 ± 0.51g/L) from FW-AT culture medium followed by FW-T and FW-A culture media. Compared with YPD-control medium, *C. curvatus* produced two-fold higher biomass in FW-AT medium. Similarly, *Y. lipolytica* 35 produced 9.80 ± 1.25 g/L of biomass from FW-AT medium followed by FW-A (5.82 ± 0.27 g/L) and FW-T (5.53± 0.47 g/L) media.

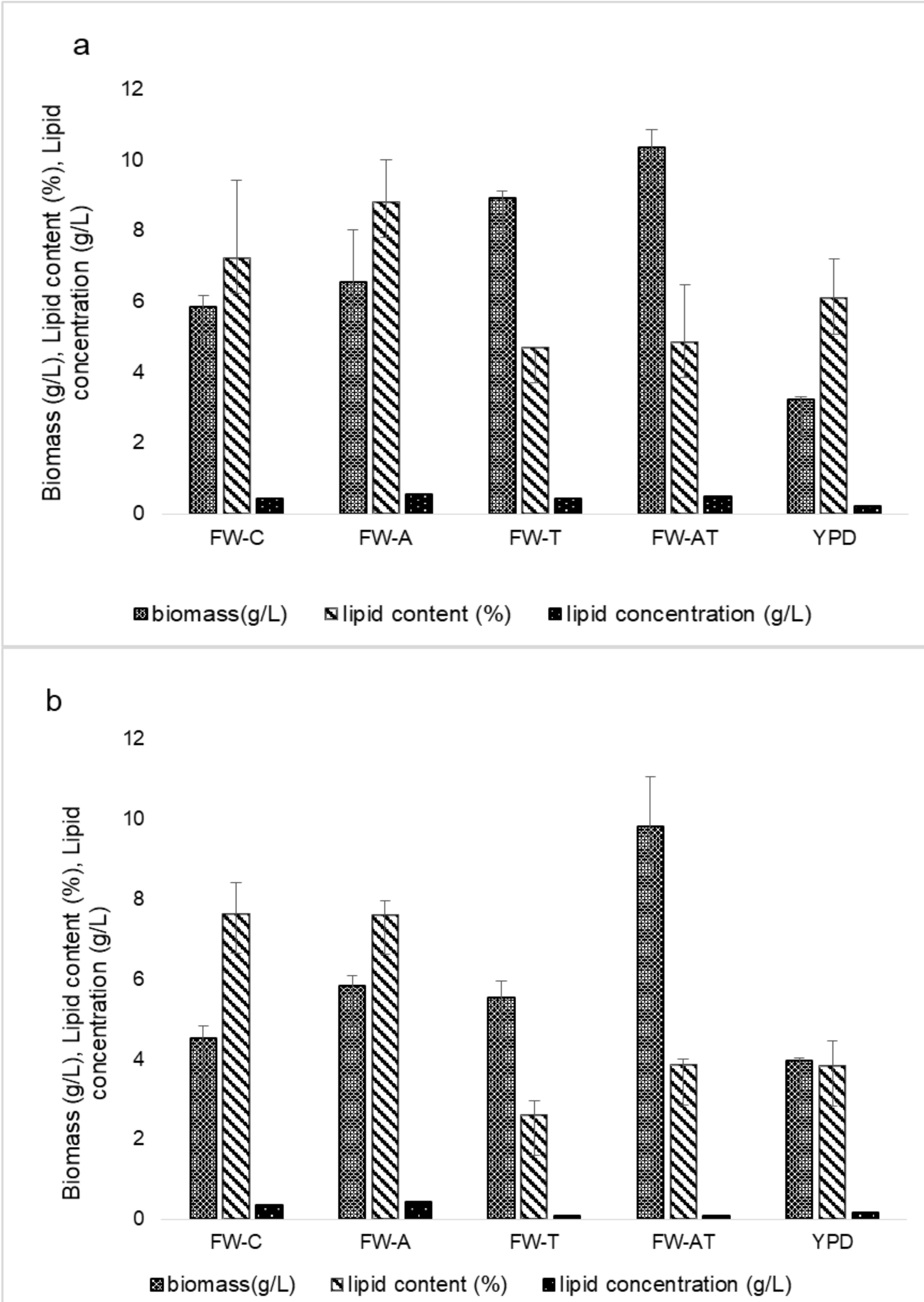

Figure 5. Biomass (g/L), lipid content (%, w/w) and lipid concentration (g/L) of (a) *Cryptococcus curvatus* 2698 and (b) *Yarrowia lipolytica* 35 cultivated in different media. FW-C - Food waste [control], FW-A - food waste medium with acid pretreatment, FW-T - food waste medium with thermal pretreatment, FW-AT - food waste medium with acid and thermal pretreatment and YPD-C - yeast peptone dextrose broth medium [control].

Considering the lipid content on w/w dry basis, *C. curvatus* produced 8.81± 1.17 % from FW-A medium followed by FW-C (7.21± 2.20 %) and YPD-C medium (6.86 ± 1.11%). In case of *Y. lipolytica*, lipid content was the highest in FW-C medium (7.63 ± 0.78 %) followed by FW-A (7.60 ± 0.35%) while the lowest lipid content was obtained from FW-T (2.58±0.38%) medium. Although FW-AT and FW-T medium supported higher biomass production of both the yeast strains, lipid production was lower. Chi et al. (2011) found that when food waste-hydrogen production effluent was used as culture medium for *C. curvatus* ATCC 20509 it only supported the growth and inhibitors present in the effluent inhibited the lipid production efficiency. Chang et al. (2015) reported that when the glucose concentration reached above 6% with low C/N ratio, lipid production of the yeast strain *Cryptococcus* sp.SM5S05 was decreased. Similar results was also observed by Park et al. (2017) using volatile fatty acids from rice hydrolysate as carbon source at 5 g/L concentration and the yeast *C. curvatus* produced 8.98 ± 0.15 % of lipid content. Chi et al. (2011) reported that from municipal waste water medium without any additional nutrients, *C. curvatus* strain produced 11.1±1.0% of lipid content. Johnravindar et al. (2018) reported that from FW leachate medium *C. curvatus* produced 20% lipid content and *Y.*

lipolytica produced 48% of lipid content. They also found that *C. curvatus* biomass yield was 4-7 times higher in FW leachate medium than the glucose medium. Under N limitation condition, *Y. lipolytica* from glycerol medium produced lipid cotent ranging from 4.7± 0.2 % to 10.9± 0.3% at various glycerol concentration (Bellou *et al.*, 2016). From purified glycerol *Y. lipolytica* SKY7 accumulated 7.3% of lipid content (Kumar *et al.*, 2020). Similarly, *Y. lipolytica* MTCC-9519 produced 7.9% of lipid content from grass hydrolysate based culture medium (Kommoji *et al.*, 2021)

CONCLUSION

Biomass and lipid production of two oleaginous yeast strains *Cryptococcus curvatus* 2698 and *Yarrowia lipolytica* 35 in food waste hydrolysate filtrate medium without any nutrient supplement was compared with synthetic medium. Combined acid-thermal pretreatment method solubilized more carbon content than acid and thermal pretreatment methods. Comparatively high lipid content was produced by *C.curvatus* (8.81 ± 1.17 %, g/g) from the acid treated FW medium. However both the yeast strains produced higher biomass from the acid-thermal treated food waste medium.

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