Utilization of *Yarrowia Lipolytica Strain CBS 6303* in Palm Oil Mill Effluent Degradation

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Abstract :—**Palm oil is one of the two most important vegetable oils. Its extraction and the purification processes generate different kinds of waste generally known as palm oil mill effluent (POME). The environmental impact of POME cannot be over emphasized. Release to POME in nature without adequate treatment is extremely harmful for environment. Hence the need for treatment measures to reduce these impacts before discharge. *Yarrowia lipolytica* CBS 6303 strain was used to degrade POME at a concentration of 10^7 cells/ml. Biodegradation of fat effluents by *Y. Lipolytica* during palm oil production resulted in COD and BOD_5reductions of about 16% and 27%, respectively. Addition of purified lipase produced by *Y. Lipolytica* strain LGx6481 yielded even higher reduction rates of 26% for COD and 3 7 % for BOD_5.**

Keywords-component: *Yarrowia lipolytica*, lipase, biodegradation, POME.

I. INTRODUCTION

Palm oil and soybean oil are the two most important vegetable oils in the world [1]. Crude palm oil contains esters composed of glycerol and several types of fatty acids, which are commonly referred to as triglycerides. They cover a large part of the world's need for edible oils and fats. Palm oil is composed of approximately 50% saturated fats (primarily palmitic acid) and 40% unsaturated fats (principally linolenic and oleic acid); this represents a unique composition compared with other major fats [2].The extraction and purification processes of palm oil generate different kinds of waste [3]. Palm oil producing countries face serious problems of disposing their waste water, which is a mixture of oil and water commonly called Palm Oil Mill Effluent (POME). These highly polluting waters heavily loaded with organic compounds particulary affect the quality of the waters in which they are discharged (receiving environments).The high load of chemical oxygen demand (COD) and especially in biochemical oxygen demand after 5 days (BOD_5) effluent, prevents the self-purification of surface water when it discharged [4].The organic and inorganic compounds lead not only to a coloration of the waters but also to high oxygen consumption causing eutrophication [5].The release of POME into the environment therefore generates various impacts on water resources, soils and the cultures [6]. Considering these environmental impacts, it appears necessary to explore the techniques of effective and cheaper treatments to purify POME before discharge into receiving waters [7]. However the enhancement of aerobic microbiological treatment using yeast strains of microorganisms non-pathogenic bacteria or degrading able of fat and assimilate, in synergy with the physico-chemical treatments would Facilitate Their degradation [8] .The yeast *Y. lipolytica* has the capacity to grow in POME and to produce less polluting biomass [9]. *Y. lipolytica* has a very high lipolytic capacity. Reference [10] showed that there exist at least two systems for the degradation of fatty acids, on specific for C_{12} or C_{14} fatty acids and the other specific for C_{16} orC_{18} saturated or unsaturated. The development of technical processes for waste water treatment requires experiments with pilot plants. The work presented here established that POME, particularly the fat fraction responsible for numerous pollution, can be degraded by using either *Y. lipolytica* strain CBS6303 or the lipase produced by the mutant strain LGx6481.

II. MATERIALS AND METHODS

A. Biological material

Cells of *Y. lipolytica* strain CBS 6303wereused to study POME degradation. POME degradation was also carried out with strain CBS6303 and added extracellular lipase overproduced by the *Y. lipolytica* strain LGx6481. The lipase was spray dried according to [11].

B. Biomass production

Y. lipolytica cells were grown on 868 medium (yeast, pepton, dextrose, agar) at 30°C with shaking. Cultures were inoculated with 10% preculture and incubated for 48h. Cells were centrifuged at 1500 rpm for 15 min and the obtained biomass was washed with physiological water (three times) and stored at 4°C.

C. Greasy waste splitting

Cells were homogenized and 1 kg was lysed by heating to 70°C during 15 min. Three distinct phases appeared after cell decantation. The upper phase contains fat waste.

D. Determination of physico-chemical parameters

Samples were taken during a 3 months period (from July to September) with two samples per month in shady tanks. Physico-chemical parameters such as pH, suspended solids (TSS), chemical oxygen demand (COD) and biochemical oxygen demand (BOD_5) were followed during 5 days by using the method described by[9]12.

E. Amounts of free fatty acids

Base neutralization was used for free fatty acids determination. 50ml of neutralized technical ethanol were added to 10 g of sample before titration with 0.1N NaOH and phenolphthalein as pH indicator. The percentage of free fatty acids was estimated by the following formula:

$$\text{Free fatty acids (\%)} = \text{volume base} \times \text{N NaOH} \times 136 \times 10^{-1} / (\text{sample weight})$$

136: molar mass of palmitic acid; N: Normality of NaOH

F. Biodegradation test

10 g of decanting fat were used for biodegradation test and fat was inoculated at 10% volume. This medium was maintained at 30°C with stirring. In order to analyse the ability of *Y. lipolytica* to degrade fat, cell concentration was determined such as 10^7 cells/ml. In the same way, degradation of fat was analysed with cell and enzyme (lipase) produced by *Y. lipolytica* LGx6481. Cells were used at 10^7 cells/ml and lipase at 10,000 U/ml. The biodegradation assay was performed during 48 h.

G. Gas Chromatography (GC)

Gas chromatography was used to determine proportions of fatty acids in the decanted fat and after biodegradation.

H. Statistical analyses

The software R was used for statistical analyses. The homogeneity of variances was tested by ANOVA. Means from the different treatments were compared by the method of Newman and Keuls (least significant difference at $\alpha = 0.05$).

III. RESULTS

A. Effluent and decanted fat Physico-chemical parameters

The physico-chemical characteristics were identified and the results were used to determine the average values of COD, BOD_5, and TSS. We show in Table 1 different average of COD concentrations, BOD_5, TSS, pH and COD/BOD_5 biodegradability of the effluent and decanted fat. The COD of the effluent ranges from 10 0 , 20 to 35 145 mg O2 / l with an average value of 23 495 ± 12 700 mg O2 / l, while that of the decanted fat ranges from 4520 to19 010 mg O2 / l with an average value of 411.67 ± 9 8310 mg O2 / l. The BOD_5 effluent numbers range from 5 000 to 9000 mg O2 / l with an average value of 7000 ± 2000 mg O2 / l, while those of decanted fat range from 800 to 1600 with an average value of 1133.33 ± 416 mg O2 / l.
The TSS effluent ranges from 1 400 to 8 900 with a mean value of 5190 ± 3750 mg / l. The COD / BOD_5 effluent ranges from 1.11 to 7.03 with a mean value of 3.92 ± 2.97. However The COD/BOD_5decanting fat has evolved from 2.94 to 19.01 with a mean of 9.02 ± 8.60.Regarding the pH, values range from 4.2 to 4.7 with a mean value of 4.50 ± 0.27 for the effluent, while that of the decanted fat ranges from 4.52 to 4.84 with a mean value of 4.73 ± 0.18. Fat as the effluent are acidic.

TABLE 1: PHYSICO-CHEMICAL PARAMETERS OF EFFLUENT AND FAT DECANTED

Parameters	**Values effluent**	**Values fat decanted**	**Mean values effluent**	**Mean values fat decanted**
pH	*4.2-4.7*	*4.52-4.84*	*4.5 ± 0.27*[a]	*4.73 ± 0.18*[a]
COD (mgO_2/l)	*10020-35145*	*4520-19010*	*23495 ± 12700*[a]	*411.67 ± 98310*[b]
BOD_5 (mgO_2/l)	*5000-9000*	*800-1600*	*7000 ± 2000*[a]	*1133.33 ± 416*[b]
TSS (mg/l)	*1400-8900*	-	*5190 ± 3750*	-
COD/BOD_5	*1.11-7.03*	*2.94-19.01*	*3.92 ± 2.97*[a]	*9.02 ± 8.60*[b]

a : no significant difference; b : significant difference

B. Biodegradation test

In order to analyse the ability of *Y. lipolytica* to degrade fat, different physic-chemical parameters previously described were used. In Fig.1, we reported the evolution of the COD, pH and BOD_5 fat decanted about biodegradation with *Y. lipolytica* and with extracellular lipase of *Y. lipolytica.*
In the presence of *Y. lipolytica* cells, we observed elevated COD values ranging from3410to18 440 mg O2 / l with an average of 8 514.67 ± 8600 mg O2 / l, whereas with lipase isolated from *Y. lipolytica*, values varied between 3038 and 16750 with an average of 7623.33 ± 7900 mg O_2/l.
The statistical test showed that there was no significant difference of the COD values ($p>0.05$) with lipase or without lipase.BOD_5 numbers extended from 900 to 4100 mg O2 / l with an average of 2 066.67 ± 1770 mg O2 / l in the presence of *Y. lipolytica* cells and reached between 800 and3100 with an average value of 1666.67 ± 1250 mg O_2/l when extracellular lipase was used.
In the presence of the strain only, the pH was between 4.7 and 4.91 with an average of 4.82 ± 0.11. However, when the lipase was added, pH values extended from 4.89 to 6.47 with a mean value of5.74 ± 0.78.

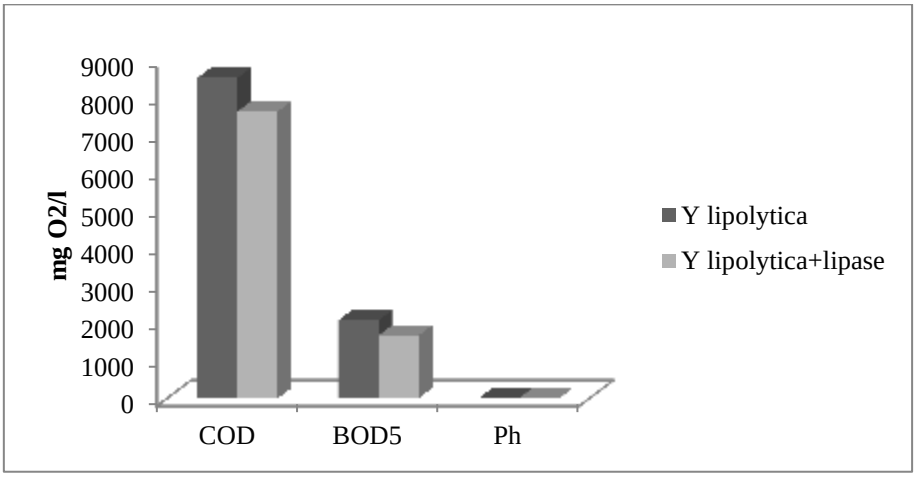

Figure 1: Evolution of parameters after biodegradation

C. Amounts of free fatty acids

We subsequently determined the amount of fatty acids produced with *Y. lipolytica* and with *Y. lipolytica* and lipase. We obtained a significant amount of free fatty acids in the presence of *Y. lipolytica* and lipase after degradation tests (Table 2). Addition of lipase to the biodegration medium produced a high level of free fatty acids (26.34 ± 0.34), whereas with *Y. lipolytica* only much lower amounts were obtained (7.28 ± 0.27).

TABLE 2: Amounts of free fatty acids in decanted fat.

Sample	**Amounts of free fatty acids (%)**
Fat decanted	*14.52 ± 0.18* ^{a}
Fat decanted + Y. lipolytica	*17.28 ± 0.27*^{a}
Fat decanted + Y. lipolytica+ lipase	*26.34 ± 0.34*^{b}

a: no significant difference b: significant difference

D. Gas chromatography (GC)

In order to determine the fatty acid composition in decanted fat and after biodegration with *Y. lipolytica* and *Y. lipolytica* added to lipase, gas chromatography was used. Gas chromatography experiments revealed 41,95 ± 0.36% palmitic acid, 9.95 ± 0.25% linoleic acid, 20.1 ± 0.27 and 16.78 ± 0.35 % stearic acid in decanted fat (Table 3).
We observed a significant decrease of the various fatty acids especially of polyunsaturated such as linoleic acid with *Y. lipolytica* cells.

With lipase addition, we noted a significant decrease also of saturated acid such as palmitic acid and stearic acid and polyunsaturated acids were absent.

TABLE 3: AMOUNTS OF FATTY ACIDS IN DECANTED FAT AFTER BIODEGRADATION.

Fatty acids in fat decanted	**Value (%)**	**Value after Biodegradation with *Y. lipolytica* (%)**	**Value after Biodegradation with *Y. lipolytica* + lipase (%)**
Palmitic acid	*41.95 ± 0.36*	*39 ± 0.20*	*22 ± 0.37*
Linoleic acid	*9.95 ± 0.25*	*5 ± 0.15*	-
Stearic acid	*16.78 ± 0.35*	*15 ± 0.34*	*8.28 ± 0.39*
Oleic acid	*20.1 ± 0.27*	*12.9 ± 0.25*	-

IV. DISCUSSION

POME is subject to many studies. In Ivory Coast, these harmful compounds are often released into the environment with an adverse impact on the environment. We have characterized the EPHP in order to determine the effluent pollution parameters. The concentrations of COD, BOD_5 and TSS effluent are significantly lower than those described by [13]. According to these authors, POME contains in average 25 g/l of BOD_5, 55.25 g/l of COD and 19.61 g/l of TSS. The samples were collected in the rainy season when the supply of raw materials is low, which probably explains the lower pollution parameters. Physico-chemical parameters are significantly higher than the Ivorian discharge standards set by [14]. According to the standards on the discharge of industrial effluents, the limit values are 300 mg / l for COD, 100 mg / l for BOD5, 50 mg / l for TSS and the pH should be in the range of 6.5 to 8.5. In our study, the pH of the effluent and the decanted fat was in therangeof4 to5. This would be conducive to growth of *Y. lipolytica* that supports pH values ranging from 3 to 8 [15].The biodegradation test with the fat of a decanted olive oil effluent with the same strain, the rate of reduction of the fatty acids was important during the addition of the lipase. This synergy allows the development of a yeast starter/lipase.

This work showed that the strain used is able to grow in these waters to lower COD and BOD_5 and produce biomass as was reported by [16]. However, this biological treatment offers purifying returns similar to a chemical treatment such as ozonation [17]. This work confirms that the treatment efficiency of an aerobic system compared to other system does not exceed 50% [18]. POME fact of his office organic matter composition must be diluted several times before undergoing an aerobic biological treatment [19]. In addition, when the preculture was carried out with the sterilized effluent, the decrease of the COD was found to be70% stronger than that reported by [20].The use of sterilized effluent shows that the microorganisms have an antagonistic action on the used strain. The amount of free fatty acids in the decanted fat .already increased with the addition of the strain, but was even more pronounced when the lipase was added.

The use of gas chromatography allowed to distinguish between saturated and unsaturated fatty acids. The obtained result is similar to those reported by [21]. A reduction of polyunsaturated fatty acids and saturated fatty acids was observed when *Y. lipolytica* was used to degraded fat. When lipase was added, total disappearance of unsaturated fatty acids (oleic and linoleic acid) and a significant decrease of saturated fatty acids (stearic and palmitic acids) was observed. A similar result was obtained by [9] in the biodegradation of olive oil effluents. The strain assimilates first unsaturated fatty acids before tackling the saturated fatty acids. Reference [22] showed that *Y. lipolytica* has affinity for the same fatty acids.

V.CONCLUSION

In this work, we were able to analyse the biodegradation of fat using strain and *Y. lipolytica* and *Y. lipolytica* complemented with lipase. We observed that *Y. lipolytica* cells at a concentration of 10^7 cells / ml lowered the relevant physico-chemical parameters and decreased the amount of fatty acids in the effluent. Even more strongly reduced parameters were observed when lipase was added to the medium and polyunsaturated fatty acids were entirely eliminated. We noticed that addition of lipase to the medium used to accelerate the elimination of fat by promoting hydrolysis of triglycerides to fatty acids and glycerol, thus facilitating the assimilation of fatty acids by the yeast. The results provided unsaturated fatty acids (oleic and linoleic) removing by *Y. lipolytica* that both saturated fatty acids (palmitic and stearic).These results pave the way for the development of a starter in the treatment of oily effluents.

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