

Effect of carbon and nitrogen sources on the production of lipase by *Candida cylindracea* 2031 in Batch Fermentation

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Abstract

Production of extracellular lipase by *Candida cylindracea* DSMZ 2031 was studied in a seven litres batch bioreactor, using palm oil (PO), palmitic acid (PA), lauric acid (LA), olive oil (OO) and cooking oil (CO) as carbon source. The effect of carbon and nitrogen sources was studied by measuring the lipase activity. The maximum lipase activity was found to be 12.7 kLU on palm oil as carbon source and urea as nitrogen source. This was achieved at a temperature of 30° C, pH of 6.0, agitation speed of 500 rpm and aeration of 1vvm.

Keywords: Lipase production; Candida cylindracea; palm oil; stirred bioreactor

1.0 Introduction

Interest in lipase enzymes has been greatly developed in the past few years due to their potential application in fat splitting as well as in synthesis of glycerides, the large number of reaction, not necessarily esterification reaction that they can catalyse. The advantages of the enzymatic hydrolysis over the chemical process are less energy requirements and higher quality of the obtained product. [1, 2].

The main uses of industrial lipases are as additives to washing detergents and in food industry, such as cheese ripening, preparation of cocoa butter substitutes and flavour production. Lipases have also been used for tanning, sewage treatment, in cosmetic industries and for transesterification of triglycerides.

Lipase activity has been found in different mold, yeast, and bacteria. Although numerous papers have been published on selection of lipase producers, there is less available information on the fermentation process [3, 4]. This information is important in order to identify the optimal operation condition for enzyme production.

The highest activity of lipase obtainable depended on the type cell of used for its production. In fungi, although lipidic substrates and fatty acid generally act as inducers, lipase is produced constitutively. The yeast *Candida cylindracea* is an important lipase producer. Among microbial cells the most commonly used for lipase production is the yeast, *Candida cylindracea*. [5, 6, 7]. *Candida cylindracea* produces extracellular lipases in the presence of lipid material, especially when a fat and a steroid are present simultaneously [8]. In spite of its wide use, there are not many information in the open literature about the factors and condition that control its biosynthesis and secretion of lipase production on palm oil by *Candida cylindracea* available. However, a common characteristic in lipase production is the

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use of a lipid such as triglycerides or fatty acid, solely or jointly with glucose as carbon source, as inducer of the production. Nevertheless, the role of inducer is not clearly understood [9]. Valero et al. [10, 11] showed that lipase production was sensitive to glucose repression. This paper discusses of the effect of carbon and nitrogen sources on the production lipase by *Candida cylindracea*.

2.0 Materials and methods

2.1 Materials

Materials used in this research were microorganisms, chemicals for preservation of the microbe in slant, culture medium and for analytical procedures.

2.1.1 Microorganism

Candida cylindracea DSM 2031 obtained from German Collection of Microorganisms and Cell Cultures, Mascheroder Weg 1 b, Braunschweig, Germany, was maintained on universal yeast-agar (UY) medium at 4° C as described by Benjamin and Pandey [12].

2.1.2 Chemicals

All chemicals and medium components used for the experiments were of analytical grade. Peptone, yeast extract, malt extract, KH_2PO_4 , $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$, Urea, $\text{FeCl}_3 \cdot 6\text{H}_2\text{O}$ were purchased from Sigma Chemical Company, USA. The trace elements are inositol, thiamine hydrochloride and biotin were obtained from Fluka Chemie AG, Switzerland. The carbon substrate used were glucose purchased from Ajax Chemicals, Auburn NSW, Australia, and palm oil (PO) obtained from Palm Oleo Sdn Bhd, Kuala Lumpur.

2.2 Methods

2.2.1 Preparation of universal yeast (UY) agar

Candida cylindracea (DSM 2031) was grown and maintained using the Universal Yeast (UY) - Agar medium at 4° C. The medium consists of (g.L⁻¹): glucose 10, peptone 5, yeast extract 3, malt extract 3 and agar 15 (pH 7.0) in distilled water.

2.2.2 Inoculum Preparation

Four 500 mL conical flask containing 125 mL growth medium (in g.L⁻¹) contained KH_2PO_4 6, $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$ 1, urea 4, and micronutrients (in mg.L⁻¹): $\text{FeCl}_3 \cdot 6\text{H}_2\text{O}$ 10, inositol 0.4, thiamine hydrochloride 0.2 and biotin 0.8 with glucose 10 g.L⁻¹ as carbon sources were sterilized in an autoclave at 121° C for 20 minutes. For preparing the inoculum, a loop full of cells from a freshly grown culture (agar slant) of *Candida cylindracea* was transferred to the flask. The flask was incubated at 30° ± 1° C on a rotary shaker at 200 rpm for 36 hrs. The medium used for preparing the inoculum is the same as the production medium but with only glucose as the carbon source. Palm oil (PO) was not used in the inoculum preparation medium.

2.2.3 *Batch fermentation*

50 gram of sterile palm oil (PO) in a storage bottle was transferred aseptically using a peristaltic pump to the sterilised medium in the fermentor. The inoculum used 10% of the working volume. This was transferred aseptically by bunsen burning around the septum port. The inoculum medium was of similar composition and concentration as the media for production.

Samples of 50 mL were withdrawn aseptically at regular time interval for analysis. The fermentation was carried out at a temperature of 30.0° C, pH of 6.0, aeration of 1.0 vvm and stirrer speed of 500 rpm. The pH of the fermentation was maintained constant by addition of sterile 3N NaOH and 3N H₂SO₄ solution. Exhaust gases were removed through the exit gas filter stream at the top of the fermentor. The exhaust gas line was connected to an off line oxygen / carbon dioxide monitoring unit to measure its composition [13].

2.3 *Analytical methods*

2.3.1 *Dry weight biomass*

At regular intervals, a 10 cm³ sample was collected from the bioreactor and filtered through a Whatman filter paper (glass microfibre filters (gf/c), pore diameter 0.45 µm), washed with 10 cm³ of ethanol (70 %), followed by 10 cm³ of n-Hexane to remove traces of crude palm oil, and finally with 40 cm³ distilled water. The filter paper was dried at 85° C for 10 hours to constant weight [13]. Sample was measured on either direct or diluted at 640 nm absorbance against water as blank in a spectrophotometer. A calibration curve of absorbance against dry weight was prepared.

2.3.2 *Estimation of glucose*

Glucose was estimated by an enzymatic method using hexokinase and glucose-6-phosphate dehydrogenase [14]. The absorbance of NADPH was measured at 340 nm.

2.3.3 *Estimation of lipase activity*

Lipase activity was determined using tributyrin as the substrate [15]. The activity was expressed as unit per mL. One unit of lipase activity was defined as the amount necessary to hydrolyze 1 µmol of ester bond per minute under the assay conditions.

3.0 **Results and Discussions**

3.1 *Effect of different carbon sources*

Carbon is the main component of cells and some natural fats or oils have been used as carbon sources and inducers for lipase fermentation. The effect of carbon source is shown in Table 1. Plant oils containing palmitic acid, lauric acid such as palm oil and olive oil are suitable for biosynthesis of lipase from *Candida cylindracea* DSM 2031.

To assess the effect of different substrates as carbon sources for lipase production, experiments were carried out using palm oil (PO), olive oil (OO), glucose, palmitic acid

(PA), lauric acid (LA) and palm cooking oil (PCO) as carbon sources. PO, OO and glucose, PA, LA and CO were incorporated in the medium to promote lipase induction.

The fermentation profiles in these six substrates are shown in Figures 1 to 4. The final cell concentration and lipase activity obtained, lag period, the estimated maximum specific growth rate, average enzyme productivity, fatty acid produced, the yield of product – substrate ($Y_{P/S}$), the yield of biomass – substrate ($Y_{X/S}$) and fatty acid produced are shown in Table 1. It can be seen from the Figures that the yeast grew in all the substrates but it exhibited a longer lag phase in olive oil (16 h) compared to the lag in crude palm oil (10.5 h) and in glucose (6.0 h).

Comparing the maximum specific growth of the cells in the six substrates, the yeast grew more rapidly in glucose (0.137 h^{-1}) than in other sources (about 0.10 to 0.65 h^{-1}). Also the final activity of lipase obtained was higher in PO (12.70 kLU.L^{-1} at 34.5 h) compared to in OO (2.26 kLU.L^{-1} at 36 h) and glucose (0.22 kLU.L^{-1} at 36 h) and also in LA, PA and PCO (about $1.52 - 1.62 \text{ kLU.L}^{-1}$ at 36 h). However, the final biomass concentration obtained was higher in glucose (7.54 g.L^{-1}) and increased with an increase in glucose concentration compared to other substrates. The enzyme productivity in PO was $0.368 \text{ kLU.L}^{-1}.\text{h}^{-1}$ and is higher compared to in other oils.

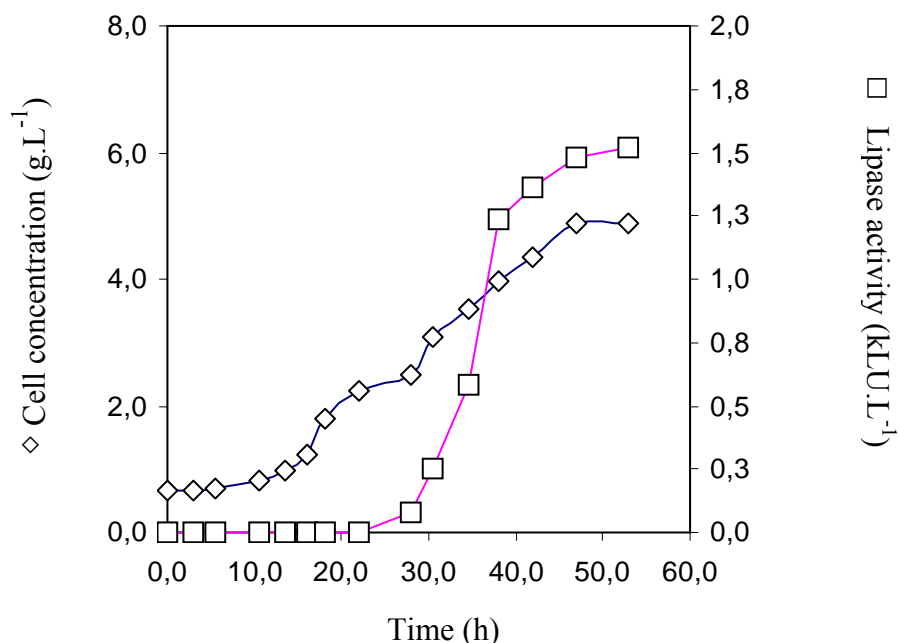
The maximum cell concentration in PO was observed to be lower than in palm cooking oil and glucose, but lipase activity was higher in the former. With OO, oleic acid (composition: 18:2) maybe produced and then consumed by the cells. However with PO, palmitic acid (composition: 16:0) would be the acid produced and then consumed by the cells.

In the absence of glucose in the OO and PO and other oil media, the cells were induced to produce lipase to break down the lipid to derive energy and carbon for growth. Initially the growth in PO was exponential (10 to 18 h). After 18 h, the growth is linear as the availability of lipid due to its low solubility in the aqueous medium may be limiting the growth.

Table 1 Effect of different carbon sources

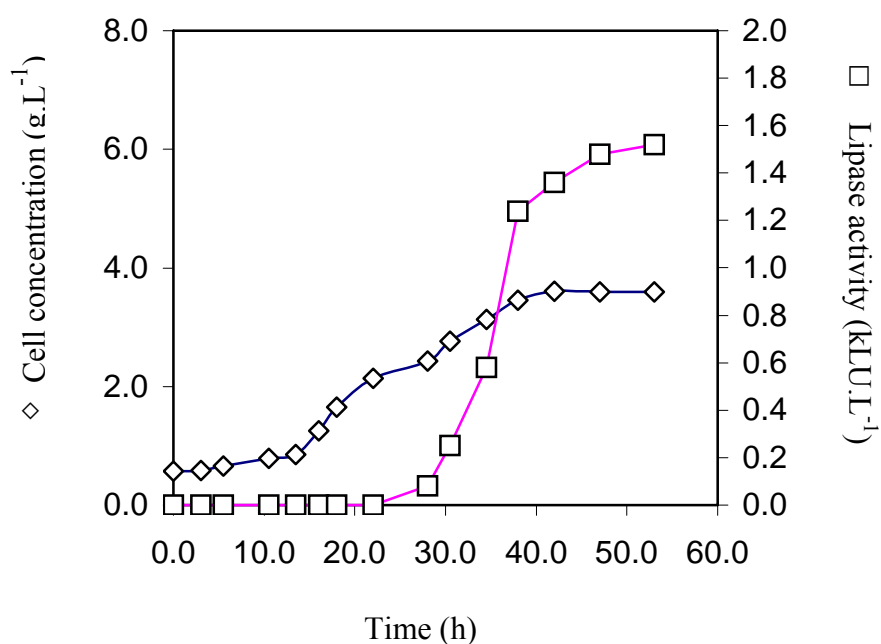
Parameter	Range of variable (o C)	Lag period (h)	μ_{max} (h^{-1})	Max. Cell Conc. (g L^{-1})	Max. lipase activity (kLU L^{-1})	Productivity ($\text{kLU L}^{-1} \text{h}^{-1}$)	$Y_{X/S}$ of lipase production	$Y_{P/S}$ of lipase production
Different carbon sources	Glucose	4.5	0.137	7.54	0.22	0.006	0.71	0.06
	Palm oil	10.5	0.101	5.8	12.7	0.368	2.36	5.75
	Palmitic acid	10.5	0.053	4.89	1.52	0.038	5.3	1.9
	Lauric acid	13	0.645	3.6	1.55	0.043	7.575	3.8
	Olive oil	16	0.082	5.17	2.26	0.074	2.86	1.413
	Palm Cooking oil	12	0.81	6.9	1.68	0.047	5.24	1.4

Operation condition: pH of 6.0, Agitation speed of 500 rpm, temperature 30°C and aeration 1 vvm, concentration of carbon sources of 10 g.L^{-1} .



(pH=6.0, T= 30° C, Aeration = 1 vvm, Agitation = 500 rpm

Figure 1 Profile of lipase production in 10 g L⁻¹ palmitic acid



(pH=6.0, T= 30° C, Aeration = 1 vvm, Agitation = 500 rpm)

Figure 2 Profile of lipase production in 10 g L⁻¹ lauric acid

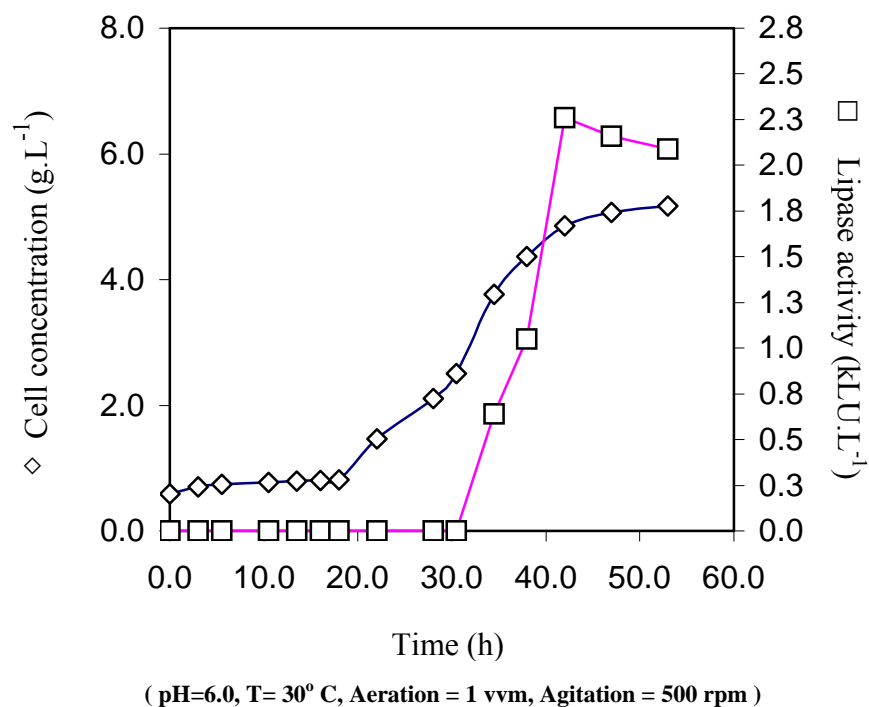


Figure 3. Profile of lipase production in 10 g L⁻¹ olive oil

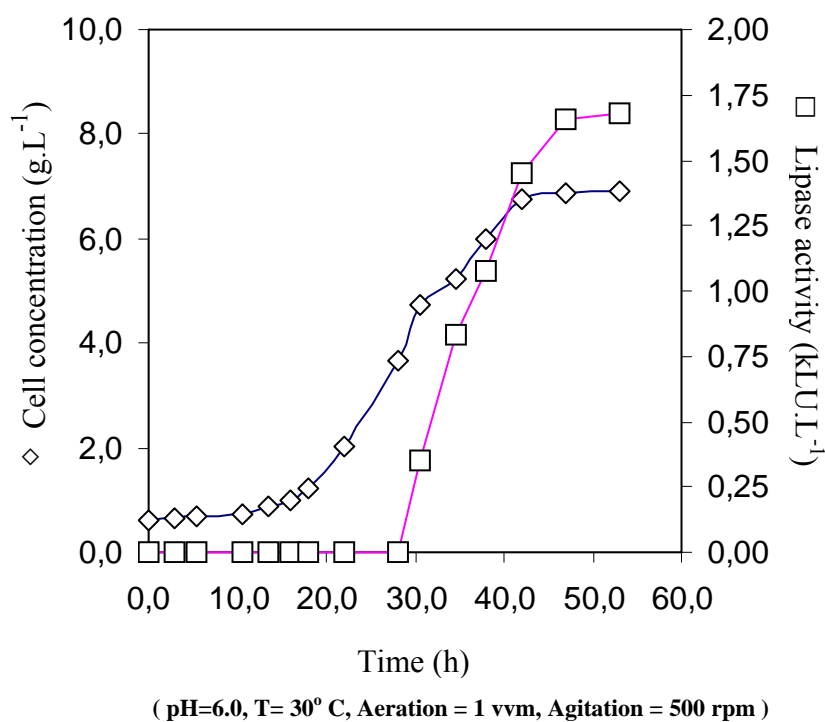
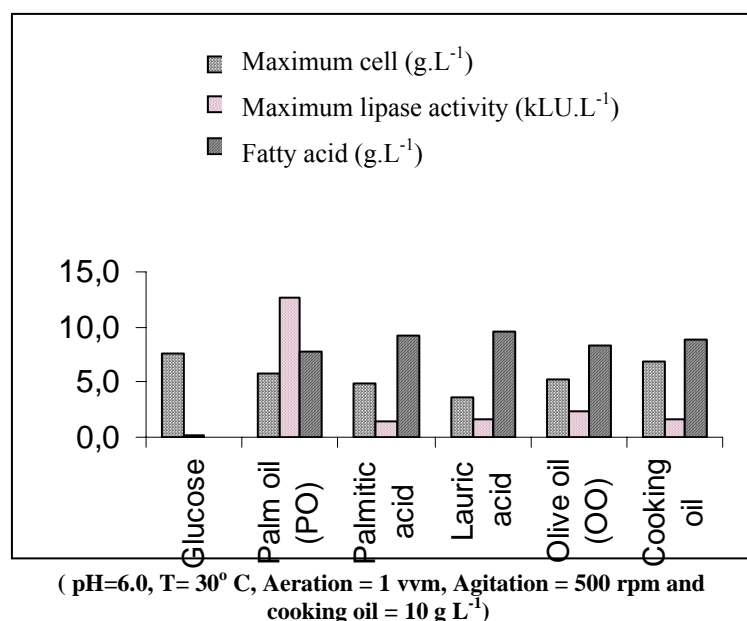


Figure 4 Profile of lipase production in 10 g L⁻¹ palm cooking oil

Shimada et al [16] found out that lipase activity increased with the chain length of the fatty acid. It was suggested that long-chain fatty acids participated in the expression of lipase genes and induction is controlled at the level of transcription.



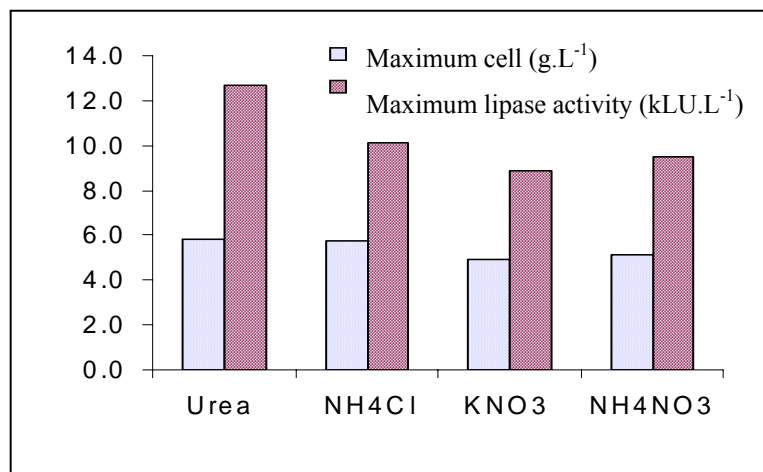
3.2 Effect of nitrogen sources on lipase production

The effect of nitrogen sources in the media on the lipase production was studied. The concentration of each of the nitrogen sources was 1% w/v. The results are given in Table 2. Figure 6 shows that a higher lipase activity was produced with urea compared to other sources. It was reported that secretion of lipase was maximum on addition of ammonium phosphate, by organism *Rhodotorula glutinis* [16].

Table 2. Effect of nitrogen sources

Parameter	Range of variable (o C)	Lag period (h)	□ max (h ⁻¹)	Max. Cell Conc. (g.L ⁻¹)	Max. lipase activity (kLU.L ⁻¹)	Productivity (kLU. L ⁻¹ .h ⁻¹)	Y _{X/S} of lipase production	Y _{P/S} of lipase production
Different nitrogen sources	Urea	10.5	0.101	5.8	12.7	0.368	2.36	5.75
	NH ₄ Cl	10.5	0.084	5.73	10.11	0.215	1.931	1.023
	KNO ₃	6	0.107	4.92	8.88	0.222	1.313	0.87
	NH ₄ NO ₃	5.5	0.622	5.12	9.5	0.211	1.57	3.393

Operation condition: pH of 6.0, Agitation speed of 500 rpm and temperature 30° C and aeration 1 vvm.



(pH=6.0, T= 30° C, Aeration = 1 vvm, Agitation = 500 rpm and palm oil = 10 g L⁻¹)

Figure 6 Effect of nitrogen sources on lipase production

4.0 Conclusion

Increasing palm oil concentration improved the synthesis of lipase but did not enhance growth of *Candida cylindracea*. Experiments carried out using palm oil (PO), olive oil (OO), glucose, palmitic acid (PA), lauric acid (LA) and palm cooking oil (PCO) as carbon sources, showed that the highest lipase activity was recorded with PO.

Different sources of nitrogen were tested in order to determine their influence on the synthesis of lipase. Results showed that maximum lipase activity was recorded when the media utilized urea.

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