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Biocatalytic transformation of cyclohexanone by *Fusarium* sp.

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Abstract

The ε-caprolactone was obtained from cyclohexanone in high selectivity (99+%) by using whole-cell of fungus *Fusarium oxysporum* f. sp. *ciceri* NCIM 1282. The new oxidative activity of the fungus has been studied and potentiality of the biocatalyst towards high concentration of substrate has been investigated. Quite interestingly, present biocatalyst exhibits redox activity, first converting cyclohexanone to cyclohexanol followed by the formation of caprolactone.

Keywords: Biotransformation; Ketone; *Fusarium oxysporum*; Lactone; Microorganism

1. Introduction

Biotransformations that involve the introduction of an oxygen atom into the substrate to give oxidized product are potentially of great value in synthetic organic chemistry. Enzymes can directly incorporate the molecular oxygen into an organic molecule. The oxidation of ketones to esters and lactones by the Baeyer–Villiger reaction [1–6] is a reliable and highly useful transformation in synthetic chemistry. The most common reagents used for this reaction are peroxycarboxylic acids (e.g., peracetic acid, *m*-chloroperbenzoic acid). These are commercially available on a large scale, but their industrial use is either costly or requires significant safety considerations. Moreover, these peracids yield the corresponding carboxylic acids, raising separation and recycling concerns. Recently, a catalytic method using titanium silicate molecular sieve (TS-1)—H_2O_2 system has also been reported for catalyzing cyclohexanone conversion into caprolactone [7]. The oxidation of cyclohexanone to ε-caprolactone is of particular industrial interest since the product is extensively used in the synthesis of polycaprolactone (PCL) which is a well-known biodegradable polyester that can be prepared by the ring-opening insertion polymerization of caprolactone.

The biotransformation of cyclohexanone to ε-caprolactone has been reported using enzymes, isolated from microorganism *Acinetobacter* [8,9] and *Nocardia globerula* CL1 [10] in the presence of co-factor NADPH. Alphand et al. [11,12] have compared the whole culture of *Acinetobacter* TD63 and MO2 enzyme isolated from *Pseudomonas putida* NCIMB 10007 in the Baeyer–Villiger oxidation of α-substituted cyclohexanone into optically active corresponding caprolactone. We have found that the fungus *Fusarium oxysporum* f. sp. *ciceri* NCIM 1282 is capable of biotransforming cyclohexanone to caprolactone with remarkable activity and selectivity. The fungus *F. oxysporum* exhibits redox activity (reduction followed by oxidation) and such dual (redox) activity has also been observed in the biotransformation

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of ketones by fractured cells of *Acinetobacter* [13] and whole-cells of *Beauveria bassiana* [14].

Here, we report the biotransformation of cyclohexanone to ε-caprolactone selectively via cyclohexanol formation by using whole-cells of *F. oxysporum*. Although it is reported to catalyze many different types of oxidative reactions such as epoxidation of styrene [15], hydroxylation of steroids [16] and sulfoxidation of alkyl aryl sulfide [17], to the best of our knowledge, this type of oxidation (Baeyer–Villiger type) reaction has not been reported with this biocatalyst. One major advantage of our method is that the whole biomass (cell) can be used as biocatalyst. Further, there is absolutely no need for any co-factor like NADPH and isolation of enzyme from the biomass.

2. Experimental

2.1. Microorganism, maintenance and growth

Fungus *F. oxysporum* f. sp. *ciceri* NCIM 1282 was obtained from the National Collection of Industrial Microorganism (NCIM), National Chemical Laboratory, Pune, India. *F. oxysporum* was routinely maintained by subculturing on potato-dextrose agar (PDA) slants at 25 °C. The fungus was grown in 500 ml Erlenmeyer flasks each containing 100 ml autoclaved MGYP medium, composed of malt extract (0.3%), glucose (1.0%), yeast extract (0.3%), and peptone (0.5%) at 25–28 °C under shaking condition (200 rpm) along with a drop of the substrate (ca. 20 mg) for 96 h.

2.2. Biotransformation

After 96 h of fermentation, mycelia were separated from the culture broth by centrifugation (5000 rpm) at 10 °C for 20 min and settled mycelia was washed with sterile distilled water twice, each time centrifuging and removing the supernatant to remove any traces of the medium. Harvested mycelial mass (30 g wet wt., unless stated otherwise) was then resuspended in 100 ml sterile distilled water in 500 ml Erlenmeyer flasks at pH = 5.5–6.0 and the suspension was used immediately for biotransformations. The substrate (100 mg) which was predissolved in ethanol (0.5 g) in sterile tube was added to the cell suspension. The whole mixture was put into a shaker at 28 °C (200 rpm). Ethanol was used for solubilising the substrate in water. As a control under the same reaction conditions, the mycelial mass with ethanol (0.5 g) was suspended in water without substrate. The ethanol was found not to be utilized by the microorganism.

2.3. Isolation, identification and characterization of products

The biotransformations were routinely monitored by periodic sampling of aliquots (2 ml) which were extracted with dichloromethane and analyzed by GC. After completion of the biotransformation, the mycelia were removed by centrifugation (5000 rpm) at 10 °C for 20 min and the supernatant was extracted with dichloromethane (3 × volume of the aqueous phase). Extracted solvent was dried over anhydrous Na_2SO_4 and concentrated under pressure. Crude reaction mixture was analyzed by GC Shimadzu 17A using Supelco BETA-DEX 110 (30MX0.25) capillary column (column temperature 100 °C, 5 min, 4 °C/min, 220 °C, 10 min). The products were also confirmed by GC–MS.

3. Results and discussion

The effect of reaction time on the conversion of cyclohexanone and product distribution using whole-cells of *F. oxysporum* as biocatalyst is depicted in Fig. 1. Large amounts of reduction product, the corresponding alcohol (cyclohexanol) was observed after 24 h. It was found that ca. up to 50% of the conversion of cyclohexanone, the only product was cyclohexanol (with 100% selectivity, when compared with an authentic sample). Later on with further increase in ketone conversion, ε-caprolactone, a Baeyer–Villiger oxidation product of cyclohexanone, started forming at the expense of cyclohexanol and cyclohexanone. At complete conversion, the caprolactone was obtained with 99+% selectivity as assessed by GC analysis.

The ability of washed-cell suspensions of *F. oxysporum* to biotransform cyclohexanone was studied under various conditions to maximize the conversion.

The mass balance of the reaction was about 75%. Since the mycelia is in living condition after completion of the reaction, the remaining 25% material

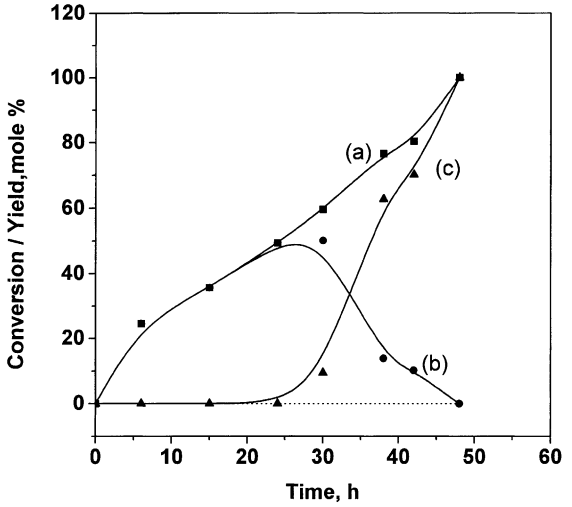

Fig. 1. Progress curve in cyclohexanone biotransformation. (a) Conversion of cyclohexanone, (b) yield of cyclohexanol, (c) yield of caprolactone. Reaction conditions: substrate concentration, 1 mM; catalyst concentration, 30 g wet wt./100 ml reaction medium; temperature, 28 °C; shaker speed, 200 rpm.

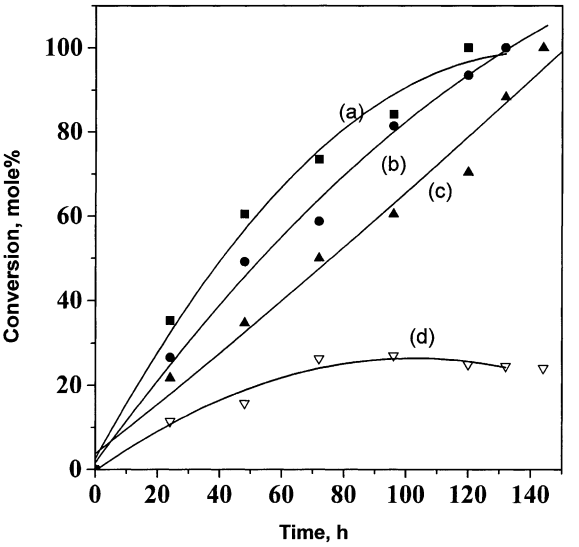

Fig. 2. Effect of substrate concentration in cyclohexanone transformation. (a) 4 mM, (b) 6 mM, (c) 8 mM, (d) 10 mM. Reaction conditions: catalyst concentration, 30 g wet wt./100 ml reaction medium; temperature, 28 °C; shaker speed, 200 rpm.

was probably being utilized by the mycelia as energy-source for growing along with some handling loss.

3.1. Effect of substrate concentration

The potentiality of the biocatalyst was investigated by varying the cyclohexanone concentration from 100 to 800 mg/100 ml of the reaction medium. Up to ca. 300 mg/100 ml of cell suspension the conversion was almost same in 48 h. With increasing substrate concentration (300–800 mg/100 ml of reaction medium), the conversion became steadily slower. The effect of substrate concentration on cyclohexanone transformation with the biocatalyst is shown in Fig. 2. This decrease in activity at higher concentration is probably due to a toxic effect of higher levels of cyclohexanone.

3.2. Effect of biomass concentration

The effect of biomass concentration on the conversion of cyclohexanone was studied starting from 10 to 30% wet wt. of the biomass. The rate of transformation of cyclohexanone depends on the amount of biomass of the grown culture. If the transformation is carried out in the presence of 15% wet wt. biomass (15 g wet biomass in 100 ml reaction medium), 99+% lactone can be obtained in 72 h, whereas in the presence of 30% biomass complete conversion of cyclohexanone into caprolactone was obtained in 48 h. The effect of biocatalyst concentration on cyclohexanone transformation and selectivity of products are shown in Table 1.

It was observed that initially the system tried to maintain an equilibrium between cyclohexanone and cyclohexanol (ca. 50% conversion) and then lactone formation started. The pathway of lactone formation through significant amount of cyclohexanol formation indicates that at least two types of enzymes were present in the microorganism. For maintaining the equilibrium between ketone and alcohol, an oxidoreductase enzyme is needed and for lactone formation, lactonization enzyme must be present. Another advantage of our biocatalyst is the absence of hydrolysis of lactone up to 48 h.

In order to corroborate this observation, we have carried out a control experiment where cyclohexanol was used as a substrate. The results are shown in Fig. 3. It is clear from these results that up to ca. 25%

Table 1
Effect of biocatalyst concentration on the biotransformation of cyclohexanone[a]

Biocatalyst concentration (% wet wt.)	Time (h)	Conversion (mol%)	Selectivity of products (%)	
			Cyclohexanol	Caprolactone
10	24	19.8	100	0.00
	48	31.5	100	0.00
	72	39.0	100	0.00
	96	86.8	67.0	33.0
15	24	27.4	100	0.00
	48	45.6	100	0.00
	60	76.5	18.2	81.8
	72	100	0.00	100
20	24	35.5	100	0.00
	36	48.6	100	0.00
	48	69.4	68.6	31.3
	60	100	0.00	100
30	15	35.5	100	0.00
	30	59.6	84.1	15.9
	42	80.4	12.7	87.3
	48	100	0.00	100

[a] Reaction conditions: substrate concentration, 1 mM; temperature, 28 °C; shaking speed, 200 rpm.

cyclohexanol conversion, no caprolactone was formed and the sole product was cyclohexanone. However, with increasing reaction time and conversion, the caprolactone started forming at the expense of ketone.

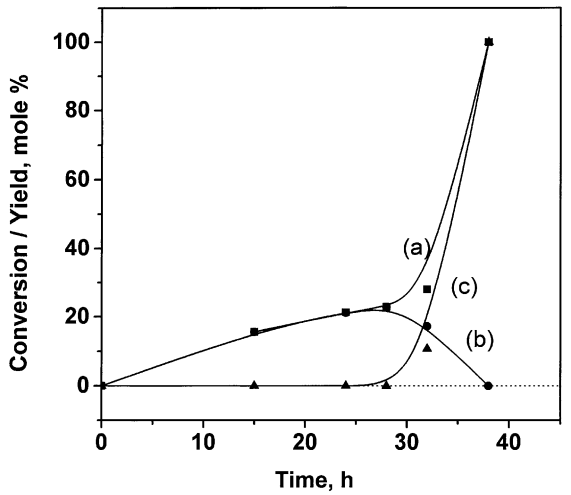

Fig. 3. Progress curve in cyclohexanol biotransformation. (a) Conversion of cyclohexanol, (b) selectivity of cyclohexanone, (c) selectivity of caprolactone. Reaction conditions: substrate concentration, 1 mM; catalyst concentration, 30 g wet wt./100 ml reaction medium; temperature, 28 °C; shaker speed, 200 rpm.

The formation of caprolactone became faster after the cyclohexanol conversion reached ca. 50%.

From the above results, it is postulated that an oxidoreductase pathway is operating. However, the exact mechanism is not clear at the moment.

4. Conclusions

In summary, a whole-cell mediated biocatalytic method for oxidation of cyclic ketones to lactones using fungus *F. oxysporum* has been demonstrated where it is found that the fungus *F. oxysporum* is an efficient biocatalyst for the synthesis of lactones from cyclic ketones with high selectivity (>99%). This biocatalyst can act on maximum 8 mM cyclohexanone per 100 ml reaction medium. At higher concentrations, like 10 mM/100 ml, the reaction becomes very slow and only ca. 25% maximum conversion is obtained.

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