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Bioreduction of a Drug Intermediate in Presence of Hexane and Surfactants

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Enhancing the dispersion and dissolution of substrate particles in substrate/water suspension is a feasible way to improve enzyme substrate contact. The aim of the present study is to investigate the effects of organic solvents like hexane and surfactants like sodium lauryl sulphate (SLS) and cetyltrimethyl ammonium bromide (CTAB) on bioreduction of 3-[5-[(4-flurophenyl)-1,5, di-oxopentol]-yl]-4-(s)-phenyloxazolidin-2-one using *Sacchromyces cerevisiae* as biocatalyst. Effect of variations in the ratio of hexane to water and the concentration of an anionic and cationic surfactants, were studied to see their effect on the bioreduction of the above mentioned ketone. As the substrate is hydrophobic, the bioreduction was tried in a biphasic system using solvent like hexane. The overall yield of the alcohol decreased significantly when the reaction was carried out in presence of hexane as compared to aqueous medium. The yield of alcohol increased when the ratio of hexane to water was 2:1, but decreased significantly with further increase in hexane concentration. The use of surfactants has been reported extensively in microbial biotransformation reactions. Hence the effect of both anionic (sodium lauryl sulphate) and cationic (cetyltrimethyl ammonium bromide) surfactants on the above said bioreduction was considered for the study. The results showed that cetyltrimethyl ammonium bromide has insignificant effect in bringing about ketone reduction while sodium lauryl sulphate exhibited three fold increase in the yield.

Key Words: Biocatalysis, Sodium lauryl sulphate, Cetyltrimethyl ammonium bromide, Hexane, *Saccharomyces cerevisiae*.

INTRODUCTION

There has been an increasing awareness of the enormous potential of microorganisms and enzymes for the transformation of synthetic chemicals with high chemo-, regio- and enantioselectivity[1,2]. Chiral alcohols are very important precursors for a large number of pharmaceuticals and their production by asymmetric bioreduction of a prochiral carbonyl precursor is now well recognized in the field of biocatalysis[3-5].

The whole cell biotransformation is well established in conventional aqueous systems. However many substrates are too hydrophobic to react with enzymes in aqueous medium. This problem is overcome by using organic solvents in whole cell bioprocess[6,7]. One of the possible drawback of this technique is the deactivation of whole cells in organic solvents. Therefore, the selection of a suitable solvent with biocompatibility is the most important criteria. Hexane is a nonpolar solvent and therefore may not distort the water coat of the biocatalyst present in the biphasic system[8]. Hence it has been used as an alternative to aqueous medium.

One of the probable reasons for the low degree of bioconversion in aqueous medium is the hydrophobicity of the substrate molecule. This problem can be overcome by the use of surfactants like triton X-100, sodium lauryl sulphate, cetyltrimethyl ammonium bromide, Tween 20, Tween 80 and methyl-β-cyclodextrin[9,10]. Living systems accomplish high levels of selectivity and efficiency using compartmentalization through self assembling systems often composed by amphiphatic units similar to those of synthetic surfactants which form micellar systems above the critical micellar concentration. Direct micelles are capable to solubilize amounts of a polar compound in their core. Such systems can be regarded as chemical microreactors, where hydrophobic substances stored in the apolar center can diffuse to the hydrophilic aqueous medium or directly to the adjoining cell membranes.

3-[5-[(4-Flurophenyl)-1,5, di-oxopentol]-yl]-4-(s)-phenyl-oxazolidin-2-one is an intermediate in the synthesis of Ezetimibe, which is a cholesterol lowering agent. The intermediate is poorly soluble in water and this result in low productivity in aqueous medium. In this work we have studied the effect of hexane and surfactants on bioreduction of the above mentioned ketone intermediate.

EXPERIMENTAL

Microorganism: *Saccharomyces cerevisiae*: MTCC 174 was obtained from MTCC, Chandigarh. The organism was maintained on YEPD media containing yeast extract 3 g, peptone 10 g, dextrose 20 g, agar 20 g and distilled water 1000 mL.

Cultivation of *Saccharomyces cerevisiae*: The organism from the slant culture was subcultured into 300 mL YEPD medium containing yeast extract 0.3 g, peptone 1.0 g, dextrose 2.0 g and distilled water 100 mL, pH was adjusted to 7.0 and was sterilized at 121 ºC for 15 min. The culture was grown at 30 ºC, 160 rpm for 24 h. 10 % volume of the fermentation medium was used for inoculation of 2.5 L of fermentation medium. The inoculated medium was incubated at 30 ºC, 160 rpm for 48 h. After 48 h of growth, the cells were separated by filtration using buchner funnel and the biomass was washed with phosphate buffer twice.

Bioreduction in aqueous medium: 5 g of wet biomass was taken in 20 mL of phosphate buffer pH 7.6 and 2.5 g of glucose was added. 4 mg of the substrate dissolved in dimethyl sulphoxide was added to the above mixture and incubated at 30 ºC, 160 rpm for 48 h. The reaction mixture was filtered and the filtrate was extracted with 20 mL methylene dichloride thrice and washed with brine twice and dried over anhydrous sodium sulphate.

Bioreduction in presence of hexane: 10 mg of substrate was dissolved in DMSO and added to 20 mL reaction mixture having 5 g of biomass, 2.5 g of glucose and different concentrations of hexane and pH 7.6 buffer The volume of hexane was varied *i.e.*, 4, 8, 12, 16 and 20 mL. The mixture was incubated at 30 ºC, 160 rpm for 48 h. After incubation, the hexane layer was separated and dried over anhydrous sodium sulphate. The aqueous layer of the biphasic culture was extracted with methylene dichloride thrice and washed with brine twice, dried over anhydrous sodium sulphate and combined with hexane portion.

Bioreduction in presence of surfactants: 4 mg of substrate dissolved in DMSO was mixed with 12 mg of sodium lauryl sulphate and stirred for 10 min. The solvent was then removed under reduced pressure and the solid obtained was added to 20 mL of pH 7.6 buffer with 5 g of wet biomass and 2.5 g of glucose. The reaction mixture was incubated at 30 ºC, 160 rpm for 48 h.

The reaction mixture was filtered and the filtrate was extracted with 20 mL methylene dichloride thrice and washed with brine twice and dried over anhydrous sodium sulphate. The experiment was repeated in a similar manner with another surfactant, cetyltrimethyl ammounim bromide (cetyltrimethyl ammonium bromide).

Since there was significant increase in the yield of alcohol with sodium lauryl sulphate treated cells, the same experiment was carried out using different concentrations of sodium lauryl sulphate. 4 mg of substrate dissolved in DMSO was mixed with 4, 12 and 20 mg of sodium lauryl sulphate and the experiment was carried out as mentioned above.

Bioreduction in presence of sodium lauryl sulphate and hexane: The above experiment was repeated with 40 % hexane in pH 7.6 buffer.

RESULTS AND DISCUSSION

The overall yield of alcohol was maximum when the reaction was carried out in aqueous medium *i.e.*, 7.03 mg/L (Table-1). In biphasic system the yield decreased condiderably with increase in hexane concentration probably due to the toxic effect of hexane on the enzyme system.

TABLE-1
EFFECT OF HEXANE CONCENTRATION ON BIOREDUCTION

Hexane (%)	Concentration of alcohol (mg/L)
20	2.27
40	3.28
60	1.17
80	–
100	–
00	7.03

There was significant increase in the concentration of the product in presence of sodium lauryl sulphate which is an anionic surfactant (Table-2). But the presence of cetyltrimethyl ammonium bromide showed detrimental effect on the enzyme and on the yield of the product. The result could probably be due to the reason that sodium lauryl sulphate entrapped the substrate and whole cell together for the reaction to occur at a faster rate where as cetyltrimethyl ammonium bromide did not have the same effect.

TABLE-2
EFFECT OF ANIONIC AND CATIONIC SURFACTANT ON BIOREDUCTION

Surfactant	Concentration of alcohol (mg/L)
Sodium lauryl sulphate	11.078
Cetyltrimethyl ammonium bromide	–

Low concentration of sodium lauryl sulphate resulted in good yield as compared to higher concentration (Table-3). This may be probably due to the toxic effect of the surfactant on the enzyme.

TABLE-3
EFFECT OF SODIUM LAURYL SULPHATE CONCENTRATION ON BIOREDUCTION

Substrate: SLS* ratio	Concentration of alcohol (mg/L)
1:1	18.67
1:3	11.07
1:5	12.50

* Sodium lauryl sulphate.

There was marked decrease in the yield when the reaction was carried out in presence of sodium lauryl sulphate and hexane (Table-4). This again proved that hexane has toxic effect on the enzyme.

TABLE-4
EFFECT OF SODIUM LAURYL SULPHATE AND HEXANE ON BIOREDUCTION

Substrate: SLS* ratio	Concentration of alcohol (mg/L)
1:1	3.28
1:3	1.45
1:5	–
pH 7.6 buffer	6.25

* Sodium lauryl sulphate.

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