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# A Green approach towards the synthesis of chiral alcohols using functionalized alginate immobilized *Saccharomyces cerevisiae* cells

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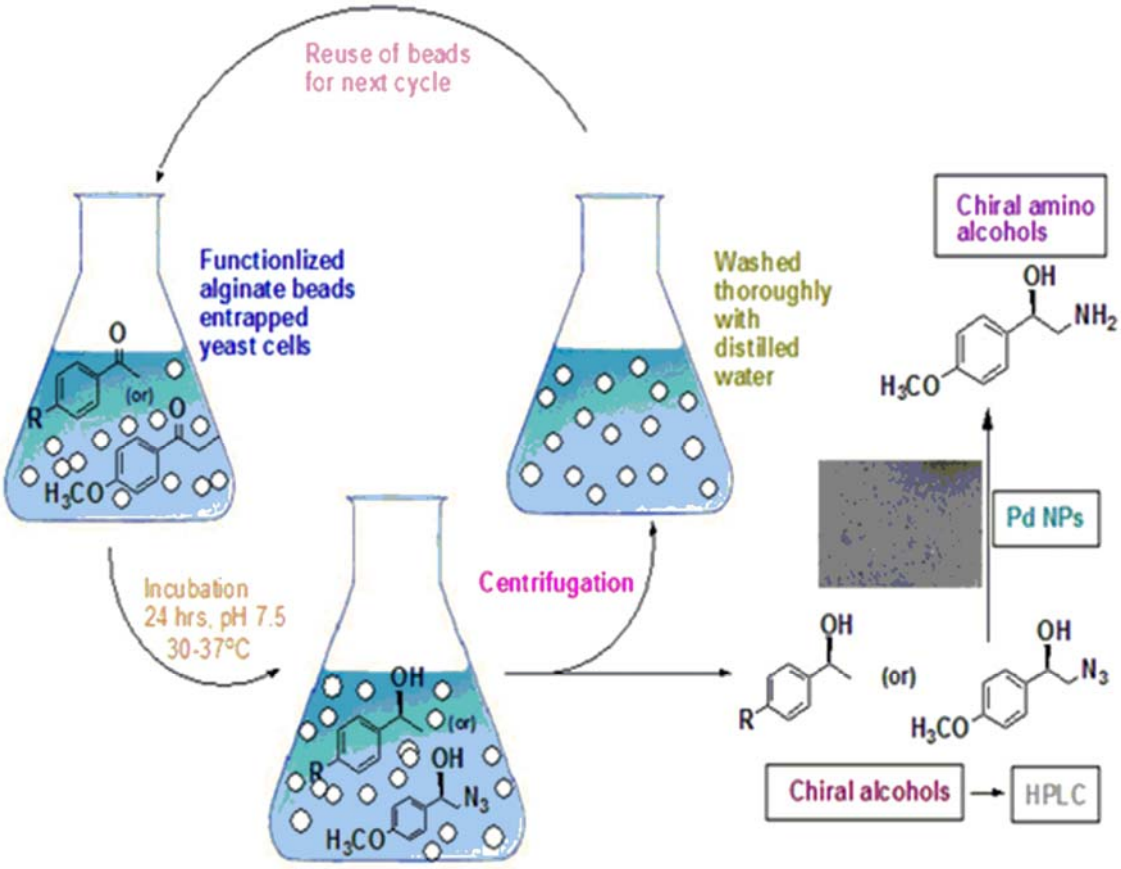
Graphical abstract



## HIGHLIGHTS

The present study highlights the following;

- Synthesis of chiral alcohols and chiral amino alcohols by developing simple chemo-enzymatic, inexpensive, green process
- Functionalized alginate beads entrapped yeast cells
- Reuse of immobilized yeast beads for continuous synthesis of chiral alcohols
- Hydrogenation of chiral azido alcohols into respective chiral amino alcohols by incorporating Palladium (Pd) nanoparticles (<5nm)
- The methodology increase the scope of the synthesis of pharmaceutically important chiral precursors by simple fashion to reach industrial demand.

**Abstract**

The stereochemistry of the drug molecule is gaining greater therapeutic importance and thus much attention was drawn in synthesis of chiral compounds by the pharmaceutical industry. In this study *Saccharomyces cerevisiae* cells immobilized on functionalized alginate beads, catalyze the bio-reduction of prochiral ketones **1a-12a** to their corresponding chiral alcohols **1b-12b** in higher yields of 60-99% and.excellent optical purity 75-97%. The synthesized chiral azido alcohols **10b-12b** were further subjected to hydrogenation using Palladium(Pd) nanoparticles (≤5nm), to obtain chiral amino alcohols **10c-12c** of therapeutic importance. Thus, a simple, green and inexpensive continuous chemo-enzymatic process has been developed in the synthesis of chiral alcohols/ amino alcohols to enhance the scope of the methodology towards industrial application.

**Key words:** *Saccharomyces cerevisia* cells, functionlized alginate immobilized beads, Palladium nanoparticles, chiral amino alcohols

## 1. Introduction

Chiral compounds are important synthons in the synthesis of numerous pharmaceuticals, agrochemicals, flavors and other value added fine chemicals [1-2]. Strategies to obtain molecules having a single stereogenic centre have gained importance in drug discovery

because one of the enantiomer having an effective therapeutic action, while the other isomer exhibits undesirable therapeutic effect or toxic effect [3-4]. A number of chemical and bio-catalytic methodologies are known in the synthesis of value added chiral compounds. Biocatalytic reactions have proven to be a promising green methodology in the synthesis of chiral compounds. Mostly, the conventional chemical synthesis use expensive chemical catalyst or transition metals and requires multiple steps to get the desired product, whereas using biocatalyst (whole cells or by isolated enzymes) the reactions are carried out in single step under mild reaction conditions [5-7]. The biocatalytic reactions show substrate specificity and the products obtained are in good yields with high enantio- selectivity/ purity.

Though, it is believed that biocatalytic processes are superior to chemical methodologies in the synthesis of chiral molecules, the use of the expensive co-factors (NADH, NADPH) has limited their scope for commercial applications. An alternative approach to improve the biocatalytic efficiency in the synthesis of chiral molecules is by immobilizing biocatalysts (cells/ enzymes); that enable the re-use of biocatalyst, resulting in enhancing their ability to catalyze a wide range of stereo selective reactions [8]. Several chiral alcohols/ amino alcohols are known potential precursors in synthesis of biologically and pharmaceutically active molecules. In

continuation to our interest in synthesis of novel chiral molecules of medicinal importance using biocatalysts [9-11], the present study is focused on extending our methodology for a continuous process of chiral alcohols/ amino alcohols which are known building blocks for various pharmaceuticals and agrochemical products.

There has been an increasing awareness in the use of biocatalysts in the biotransformation of pro-chiral compounds to their corresponding chiral products with high chemo-, regio- and enantio-selectivity [12]. Bio-reduction of pro-chiral ketones to their corresponding chiral alcohols transformation using whole microbial cell system are advantageous over the use of isolated pure enzymes dehydrogenases (ADH) as they do not require expensive co-enzyme regeneration system (NADH, NADPH) [13]. Immobilization of whole cells/enzymes demonstrate several advantages like easy separation of cells from the reaction mixture, repeated or continuous use of biocatalyst with enhanced stability and enantio-selective properties. Keeping in the view the pros and cons of yeast immobilization, we here in report the synthesis of chiral alcohols using *Saccharomyces cerevisiae* (Baker's yeast) immobilized over functionalized alginate beads to give optimum bead stability and no loss of enzyme activity with a potential for continuous process.

## 2. Experimental

### *2.1. Materials*

Baker's yeast from *Saccharomyces cerevisiae* (Type II) was purchased from Sigma-Aldrich (USA). Substituted acetophenones, 1-(2-hydroxyphenyl) propan-1-one, 1-(3-(trifluoromethyl) phenyl) propan-2-one, azido ketones, sodium alginate, succinic anhydride and Palladium chloride (II) bought from Sigma-Aldrich (USA). Calcium chloride purchased from Hi-Media Pvt. Ltd, Mumbai. All the analytical and HPLC grade chemicals and solvents used in the study were purchased from Merck, India.

### *2.2. Instruments*

$^{1}H$ and $^{13}C$ NMR spectra were recorded on Bruker Avance-400MHz spectrometer. IR spectra were recorded on Thermo Nicolet FT/IR-5700 spectrometer. Mass spectra were recorded using Waters mass spectrometer. Polari meter-Perkin Elmer model 341, HPLC Gilson solution 321, UK, SHIMADZU- GC-2010 were used to record the HPLC and GC spectra respectively.

### *2.3. Synthesis of chiral alcohols using functionalized alginate immobilized yeast cells*

The fermentation medium, in a total volume of 1L, consists of 20 g $L^{-1}$ glucose, 20 g $L^{-1}$ peptone and 10 g $L^{-1}$ yeast extract, to this yeast cells were inoculated, followed by incubation at 28-30 $^{\circ}C$ for 48 h. After optimal growth, the medium was centrifuged to isolate

fermented yeast cells, which were then washed with sterile distilled water. These fermented yeast cells were immobilized different alginate matrix i.e. a) Calcium alginate immobilized yeast beads and b) Succinyl modified alginate immobilized beads as described in our earlier studies [14]. The degree of succinylation was determined by the titration method as described by Wurzburg [15]. To the 4% sodium alginate/succinylated alginate solution, 2% (v/v) of fermented *S.cerevisiae* cells were added and the resultant cell suspension ($10^7$cfu/$cm^3$) was extruded through a needle with diameter-0.5 mm injector added as droplets into 2% calcium chloride solution under continuous stirring to get the calcium alginate/succinylated alginate-immobilized beads. Beads having a diameter in the range of 1.5 to 2.5 mm were selected for subsequent fermentation experiments. Cell viability was determined by plate counts. The stability of yeast entrapped in the succinyl alginate matrix (beads) was measured as described in [14]. The binding efficiency of yeast cells in the alginate/ functionalized alginate matrix has been confirmed by SEM studies (**Fig: 1**).

To the above immobilized yeast cells (10 gm in 250 mL of phosphate buffer, pH 7.5), the pro-chiral ketones **1a-9a** or azido ketones **10a-12a**, (100 mg/ mL) each were added and incubated at 30-37 $^{\circ}$C. The progress of the reactions at different time intervals

was monitored by TLC and HPLC. After optimal biotransformation of ketones to respective chiral alcohols the immobilized beads containing yeast cells were separated and reused up to 7 cycles without much loss enzyme activity. Thus collected reaction medium containing products was extracted with equal amounts of ethyl acetate and the products obtained were isolated and purified by column chromatography. The spectral data of all the chiral products synthesized has confirmed by literature [9, 10, 17-23]. Further the chiral azido alcohols **10b-12b** obtained were subjected to hydrogenation by using Pd nanoparticles to obtain chiral amino alcohols.

***2.4. Synthesis of chiral amino alcohols by using Palladium (Pd) nanoparticles***

The chloride salt of palladium used for preparation of palladium nanoparticles according to the reported method [16], In brief palladium nanoparticles were prepared by adding 50 mg of $PdCl_2$, 6 mL of 0.02M HCl and make up the volume to 250 mL with water to get 1.2 Mm $H_2PdCl_4$ stock solution. The prepared solution was sonicated for 3h and incubated at room temperature for 24 h before use. Add 10 mL of 1.2 mM $H_2PdCl_4$ to the 10 mL of de-ionized water maintaining pH 2.5, by adding 0.1M HCl. To this added 30 mg of PVP, the solution was heated upto 100 °C for 1 h by adding ethanol drop wise (10 mL) the solution was refluxed for

3 h, which resulted in a dark brown colloidal Pd solution. Thus obtained Pd nanoparticles were separated and incorporated into the hydrogenation reaction of chiral azido alcohols **10b-12b** to give chiral amino alcohols **10c-12c** as described in the literature [13].

***2.5. HPLC and GC Analysis:***

The progress of product formation was monitored by TLC and HPLC analysis. Waters C18 column (250× 4.6 mm) using mobile phase Actonitrile:Water (80:20) at 254 nm and the enantiomeric excess (ee) was determined by HPLC using Diacel-chiracel OD-H (50 x 4.6mm x 3mm) and OJ-H using mobile phase n-Hexane: isopropanol (95:5) and PDA Multi detector at 265 nm, with 1ml/min flow rate.

GC analysis: For determining the enantiomeric excess of products **6b and 8b**, (SHIMADZU, GC-2010) equipped with a flame ionization detector and a HP Chiral 10% cyclodextrin chromatography column (30 m×0.25 mm×0.25 μm). $N_2$ was used as carrier gas and a sample volume of 0.1μl was injected by using a split ratio of 20∶1. The column temperature was held at 120 °C for 1 min and was increased to 220 °C at a rate of 2 °C·min$^{-1}$, and then kept constant for 10 min. The temperature of the injector and the detector were 240 °C and 250 °C, respectively [26].

**3. Results and discussion**

In this study the optimum reaction conditions in the synthesis of chiral alcohols **1a-12a** by fermented yeast cells immobilized on derivatized alginate beads found to be pH 7.5 and incubation temperature was 30-37 °C (**Fig: S1 and S2**). The results show that maximum conversion of pro-chiral ketones **1a-12a** to respective chiral alcohols **1b-12b** was observed after 24 h by fermented yeast cells immobilized on to the alginate and functionalized alginate matrices (**Scheme 1**, **Scheme 2, Scheme 3**). When compared to other immobilized matrices the succinyl alginate beads immobilized yeast cells has shown promising reproducible yields of 60-99% and high enantiomeric excess of 93-97% ee (**Table: 1**). The *ortho* substituted compound 1-(2-hydroxyphenyl) propan-1-one **9b** has shown lower yields (50%) and enantiomeric excess (>20%) when compared to *para* substituted acetophenones **1b-6b**, azido ketones **10b-11b** and *meta* substituted compounds **8b** [1]. The progress of bio-reduction of keto compounds to respective chiral alcohols by functional alginate immobilized yeast cells was summarized (**Table: S1** and **S2**). The absolute configuration of chiral alcohols **1b-9b** and **12b** was found to be '**S**' (Prelog's rule) [9], [17-19], whereas **10b, 11b** has shown '**R**' configuration (anti-*Prelog*'s rule) [10]. The functionalized alginate beads shown maximum bead stability without functionalized alginate bead degeneration and thus improving the enzyme activity and enantio-

selectivity (ee) even after repeated use of beads in a continuous process up to 7 cycles, (**Fig: 2**). Where as in calcium alginate immobilized yeast cells, diminution of enzymatic activity which is associated with loss of cell viability or decline in intracellular co-factor regeneration (NADH, NADPH) cycle resulting in loss of stability of alginate beads, resulting in decrease in enzyme activity after 3rd cycle. The improved enzyme activity in functionalized alginate immobilized beads containing yeast cells, may be due to high amount of water/ medium around the cavities of beads, which helps in maintaining cell viability and enzyme activity (ADH), and also takes part in the internal cofactor (NADH, NADPH) regeneration system via, glucose present in the medium [20]. Thus the results establishes that the succinic anhydride derivatized alginate beads provide better integrity and intact support to the immobilized yeast cells without altering the extracellular (ADH) enzyme activity, in comparison to conventional calcium alginate beads. Several immobilized methods have been reported for immobilization of baker's yeast cells on different matrices, resulted in low yields of 70-80% and enantiomeric purity 50-90% when compared to present study [21-22]. The main drawbacks of all these methods were decreased in the enzyme activity and stability of immobilized beads after few cycles due to dissolution of the matrix support. Therefore, all these known methods have

limited scope in scale up and continuous production of chiral intermediates. Chiral compounds **1b-5b**, **7b** are the important intermediates in development of various drugs and agrochemicals [9]. Compound **6b** used as chiral intermediate in the synthesis of anti viral CCR5 antagonists [17]. The compound **8b** has its importance in the synthesis of drug (S) - (-) - fenfluramine [18], **10b-12b** are the intermediates for synthesis of drugs (R) - (-) - tembamide, β-adrenergic blocker, (S) - (+) - fluoxetine respectively [23-25].

This study was further extended in hydrogenation of chiral azido alcohols **10b-12b** to obtain respective chiral amino alcohols **10c-12c** in good yields (85-92%), by implementing the Pd nanoparticles ≤ 5nm, prepared by ethanol reduction and the particle size along with distribution was confirmed by TEM (**Fig: 3 and 4**), The absolute configuration of the synthesized chiral amino alcohols **10c, 11c** found to be (**R**) configuration and **12c** was (**S**) , these values were compared with literature values [23-25].

The chiral amino alcohols synthesized (**Scheme 3**), are the known intermediates/ precursors of the anti-hyperglycemic drug R - (-) - tembamide, β-adrenergic blocker, anti-depressant drug (S) - (+) – fluoxetine, respectively.

Thus the study highlights the use of yeast cell bound functionalized alginate immobilized beads, which are stable and can be reused for

more number of cycles without degeneration of beads and leakage of yeast cells. The developed methodology has shown greater advantage in synthesis of chiral compounds over conventional methods in demonstrating the recyclability, easiness and the use of yeast cell bound functionalized alginate immobilized beads without degeneration of beads and leakage of yeast cells, followed by the use of palladium nanoparticles in selective hydrogenation to obtain chiral amino alcohols. Thus the process enlightens the scope in the synthesis of chiral alcohols/ amino alcohols of pharmaceutical importance.

## 4. Conclusions

In summary, a continuous chemo-enzymatic synthesis of chiral alcohols followed by amino alcohols was achieved successfully by using functionalized alginate immobilized beads containing yeast cells with increased efficiency and high enantio selectivity by improving cell viability and co-enzyme regeneration system. This green process developed also overcome the drawbacks like waste management and product loss and has the advantage in continuous use of yeast cells immobilized in alginate beads. The yeast cells show broad substrate specificity and it has been observed that electronic effects were not influencing the stereo chemical features and enantio-selectivity during the process of bio-

reduction the keto compounds. Thus, the method developed provides a green approach to ameliorate the efficiency of biocatalyst in the synthesis of optically pure chiral alcohols and provides tremendous opportunity in developing simple, easy, inexpensive and scalable route in synthesis of chiral compounds of biological interest.

**Conflict of interest**

The authors do not declare any conflict of interest, whatsoever, either financially or otherwise.

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**List of Figures and Tables:**

**Figures and Tables**

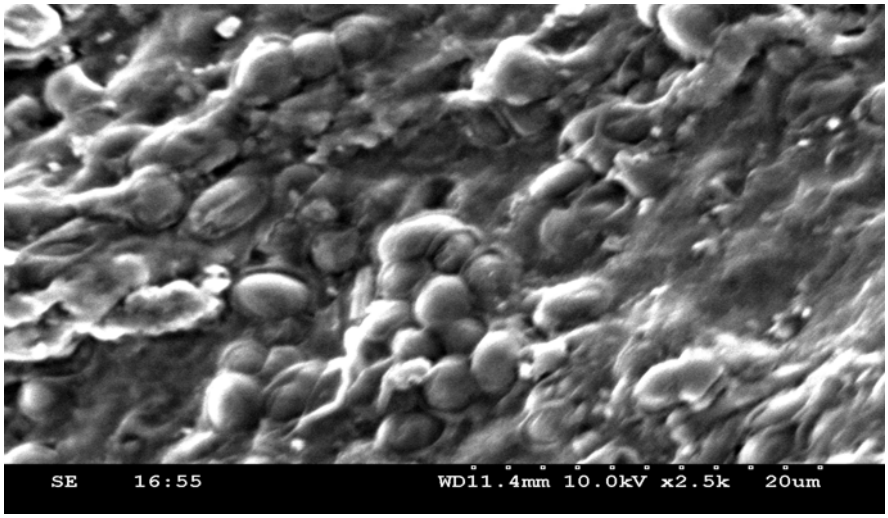



**Fig: 1.** Scanning Electronic Microscopic (SEM) image of functionalized alginate immobilized alginate beads entrapped yeast cells

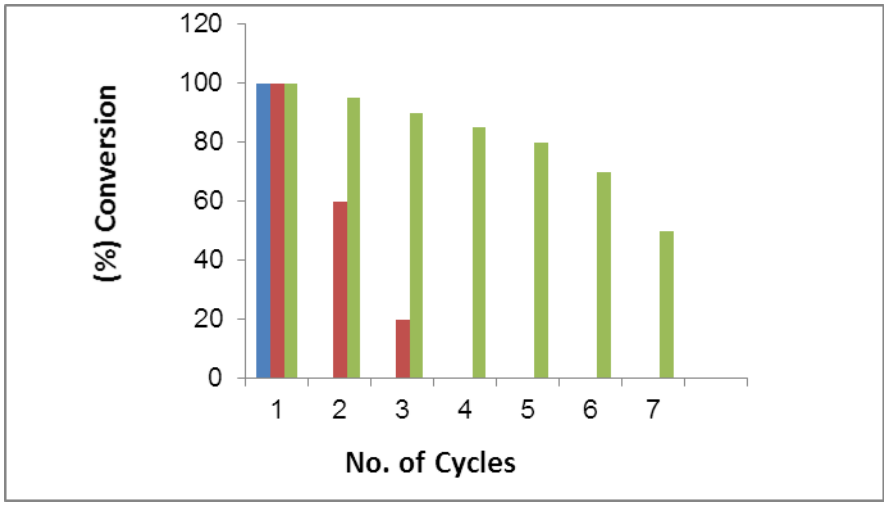



**Fig: 2.** Represents number of reuse cycles of functionalized alginate immobilized yeast beads

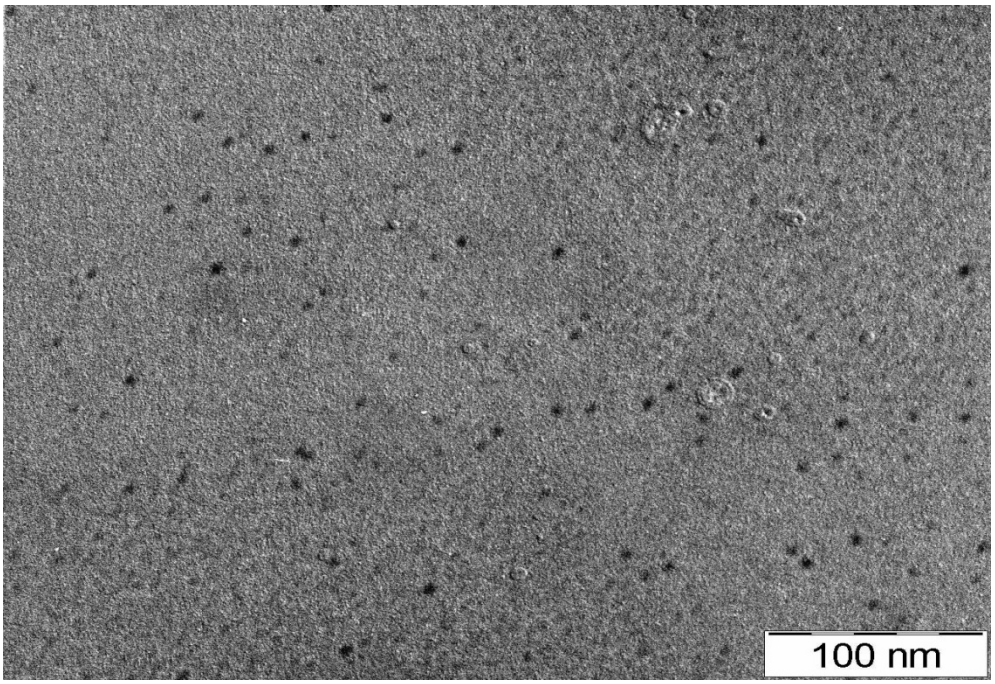



**Fig: 3.** Transmission electron microscopy (TEM) image to represents Palladium (Pd) nanoparticles

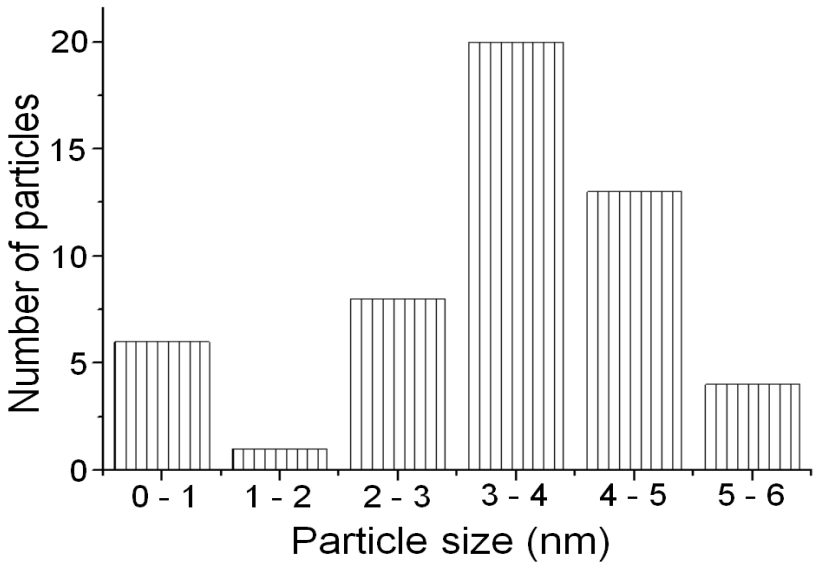
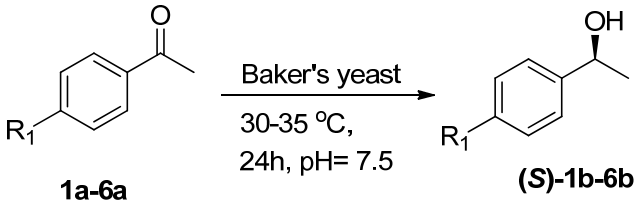
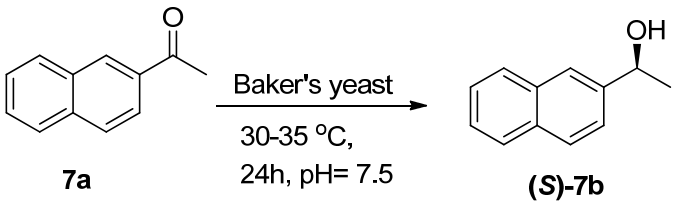


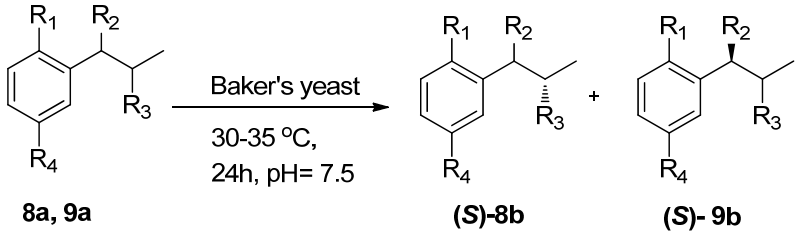

**Fig: 4.** Particle size distribution of Palladium (Pd) nanoparticles (≤5nm)




($R_1$= **1a**- Me; **2a**-$NO_2$; **3a**- Cl; **4a**- Br; **5a**-$OCH_3$; **6a**- $OCF_3$)



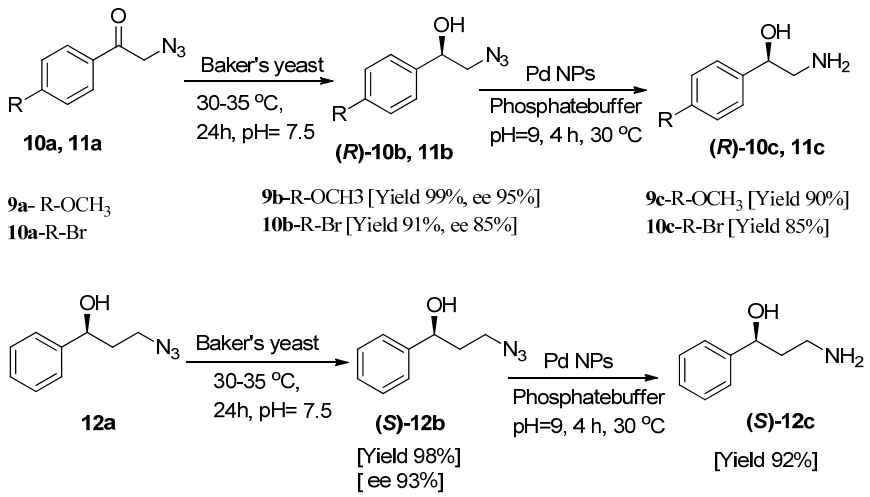

**Scheme 1:** Asymmetric reduction of *para*- substituted acetophenones **1a-6a** and 2-acetyl naphthone **7a** by functionalized alginate immobilized baker's yeast




**7a**-$R_1$, $R_2$-H; $R_3$-(=O); $R_4$- $CF_3$
**8a**- $R_1$-OH; $R_2$- (=O); $R_3$, $R_4$- H

**7b**-$R_1$, $R_2$-H; $R_3$-OH; $R_4$- $CF_3$
**8b**- $R_1$-OH; $R_2$- OH; $R_3$, $R_4$- H

**Scheme 2:** Asymmetric reduction of substituted propiophenones **8a, 9a** by functionalized alginate immobilized baker's yeast




**Scheme 3:** Chiral Reduction Azido ketones **10a-12a** by functionalized alginate immobilized Baker's yeast followed by hydrogenation with Pd nanoparticles.

**Table: 1.** Bio-reduction of pro-chiral ketones **1a-12a** with

*S .cerevisiae* cells

Table 1: Bio-reduction of pro-chiral ketones **1a-12a** by

by fermented *S. cerevisiae* cells

| Entry | Substrate | Product | Fermented yeast cells | | Alginate immobilized yeast cells | | Succinylated alginate Yeast cells | | Configuration |
|---|---|---|---|---|---|---|---|---|---|
| | | | Yield [%][a] | ee [%][b] | Yield [%][a] | ee [%][b] | Yield [%][a] | ee [%][b] | |
| 1. | **1a** | **1b** | 77 | 43 | 78 | 55 | 88 | 97 | S |
| 2. | **2a** | **2b** | 70 | 64 | 67 | 40 | 91 | 93 | S |
| 3. | **3a** | **3b** | 56 | 52 | 77 | 53 | 89 | 95 | S |
| 4. | **4a** | **4b** | 62 | 55 | 78 | 62 | 99 | 94 | S |
| 5. | **5a** | **5b** | 56 | 60 | 77 | 70 | 94 | 89 | S |
| 6. | **6a** | **6b** | 40 | 34 | 65 | 59 | 92 | 95 | S |
| 7. | **7a** | **7b** | 66 | 45 | 71 | 50 | 96 | 97 | S |
| 8. | **8a** | **8b** | 52 | 25 | 76 | 60 | 80 | 96 | S |
| 9. | **9a** | **9b** | 20 | - | 40 | <5 | 55 | 20 | S |
| 10. | **10a** | **10b** | 55 | 50 | 70 | 84 | 99 | 95 | R |
| 11. | **11a** | **11b** | 50 | 64 | 80 | 78 | 91 | 85 | R |
| 12. | **12a** | **12b** | 60 | 54 | 67 | 77 | 98 | 93 | S |

[a] Isolated yield of products **1b-12b** after 24h by column chromatography

[b] Enantiomeric excess determined indepenendently by HPLC and/or GC