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Immobilized baker's yeast reduction of ketones in an ionic liquid, [bmim]PF_6 and water mix

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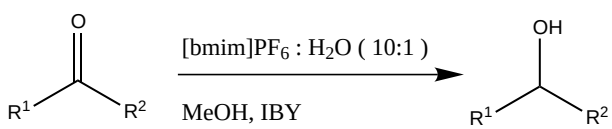
Abstract—The bioreduction with immobilized baker's yeast of several ketones was carried out in a 10:1 [bmim]PF_6 ionic liquid/water mix. The reductions produced alcohols with comparable enantioselectivities to baker's yeast reductions in alternative media.

Baker's yeast has been used to carry out a variety of transformations in synthetic organic chemistry. However, its use in this area has been limited by the necessity to employ aqueous solvent systems. For such bioreagents/catalysts to become more widely applicable in synthetic chemistry they need to operate and retain their selectivity in solvents more compatible with organic compounds. There have been several reports discussing alternative organic solvents such as liquefied petroleum gas,[1] hexane,[2] benzene,[3] toluene,[4] carbon tetrachloride,[4] and petroleum ether.[5] The main advantage of employing such solvents is the ease with which the product can be isolated. The drawbacks associated with the use of organic solvents are their potential toxicity to the bioreagent/catalyst, and problems associated with their disposal.

Concurrent research on ionic liquids has shown that they support a large and diverse range of organic reactions, these include amongst many others oxidations,[6] coupling reactions,[7] nucleophilic displacements,[8] reductions,[9] and alkylations.[10] There have also been reports of purified enzyme reactions in ionic liquids.[11] We speculated that it may be possible to carry out whole-cell biotransformations such as yeast mediated reductions of ketones in an ionic liquid. This would combine the advantages of whole cell bioreagents with the advantages of ionic liquids, principally their recyclable nature. For the purposes of the investigation we chose the readily available ketones **1**–**7** for reduction. It has been shown that the inactivation of enzymes in organic solvents can be avoided if the enzyme is surrounded by a layer of water.[12] Thus, we decided to add a quantity of water to the ionic liquid. The ionic liquid we chose to use was the moisture stable 1-butyl-3-methylimidazolium phosphorous pentafluoride, [bmim]PF_6, because like previous organic solvents explored[1–5] it is essentially immiscible with water. We also decided to employ immobilized baker's yeast (IBY) in order to simplify the work-up procedure whereby the IBY could be removed by filtration. The method of yeast immobilization that we chose was encapsulation in calcium alginate beads,[13] as this is a cost effective and facile method of immobilization. We also added a quantity of methanol as an energy source[2] to the reaction system, Scheme 1, (see Ref. 14 for details of reaction procedure).



Scheme 1.

It should be noted that, whilst in an aqueous system the coenzyme, NADPH, necessary for reduction is, through various metabolic pathways within the yeast, continuously recycled, this does not occur in non aqueous systems. Thus, the extent of the reaction is limited by the initial concentration of the coenzyme within the yeast.[5]

The results from our investigation are given in Table 1. We found that the yield of product varied over the range of substrates, some giving poor yields whilst

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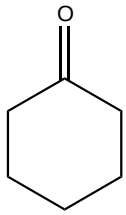
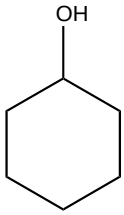
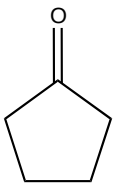
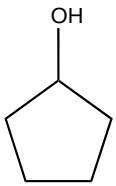
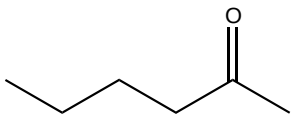
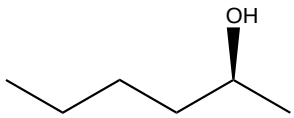
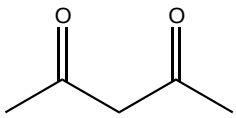
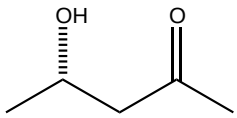
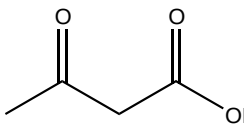
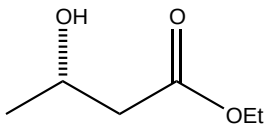
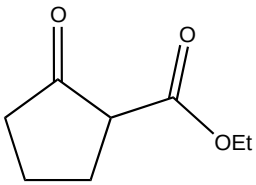
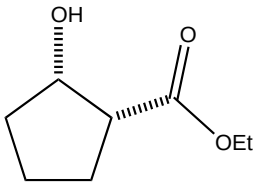
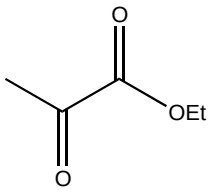
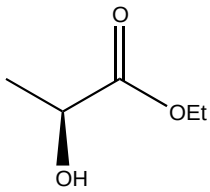
Table 1.

Entry	Ketone substrate	Entry	Alcohol product	Yield % Lit. ( )	Ee % Lit. ( )	$[\alpha]_D^{20}$ Lit. ( )
1		**1a**		35	–	–
2		**2a**		20	–	–
3		**3a**		40 (18)[15]	79 (82)[15]	+9.3 (+11.7)[16]
4		**4a**		22 (90)[17]	95 (74)[17]	+38.2 (+40.0)[17]
5		**5a**		70 (78)[5]	95 (97)[5]	+41.0 (+43.0)[18]
6		**6a**		75 (66)[19]	84 (99)[19]	+12.3 (+14.7)[19]
7		**7a**		60 (43)[20]	76 (91)[20]	−7.1 (−9.4)[21]

others gave good yields. When we attempted to reduce the two aromatic ketones, 4-bromoacetophenone and 4-methoxyacetophenone, we observed no reduction and only starting material was recovered. The literature values for enantiomeric excesses given in Table 1 are for yeast reductions carried out in alternative media. In general the enantiomeric excesses obtained in an ionic liquid medium were comparable to those obtained in other media, although entry **4a** was significantly higher and entry **7a** significantly lower.

We also found that under high vacuum that it was possible to distil the alcohols **1a** and **2a** directly from the [bmim]PF_6. This negated the extraction with organic solvents. The ionic liquid [bmim]PF_6 was recycled after use in the reactions. We also noted that whilst these reactions may be carried out in the absence of water the yields and enantiomeric excesses were extremely poor, probably because of the inactivation of the enzyme within the yeast responsible for the reduction as stated above.

As far as we are aware this is first example of the use of a whole-cell biotransformation in a moisture stable ionic liquid, in this case [bmim]PF_6, and it clearly expands the potential and possibilities for moisture stable ionic liquids as environmentally sound solvents to support a broad range of synthetic organic transformations. Furthermore it points to further development for the role of bioreagents, in particular yeast, in this field of synthetic chemistry.

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    Preparation of immobilized baker's yeast: Sodium alginate (5 g) was added to water (200 mL) and the mixture was stirred until the sodium alginate was completely dissolved. Baker's yeast (20 g) was added to water (80 mL) and the mixture was stirred for 2 h to produce a homogenous suspension. This suspension was then transferred to the sodium alginate solution. The combined mixture was stirred for a further 2 h. After this time the mixture was transferred to a dropping funnel with a 2 mm outlet. The sodium alginate and yeast mixture was added dropwise to aqueous calcium chloride (3% solution, 800 mL). The resultant beads that were formed were filtered and washed several times with water. They were then stored in a refrigerator until required.
14. **Bioreduction reaction procedure**: The ionic liquid [bmim]PF_6 (100 mL) and water (10 mL) were mixed together and warmed to a temperature of 33°C. Subsequently calcium alginate beads containing yeast (10 g) were added to the solution that was then stirred. After 10 min methanol (2 mL) was added and the whole system was stirred for a further 1 h. The ketone (10 mmol) was then added and the reaction was stirred at 33°C for 72 h. The beads were then filtered and the remaining filtrate was extracted with diethyl ether (5×100 mL). The extracts were combined and dried (anhyd. Na_2SO_4), filtered and the solvent was removed in vacuo. The resulting oil was purified by flash chromatography or short path distillation. All products **1a** to **7a** were characterized by ^1H, ^{13}C and IR spectroscopy.
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