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Continuous flow whole cell bioreduction of fluorinated acetophenone

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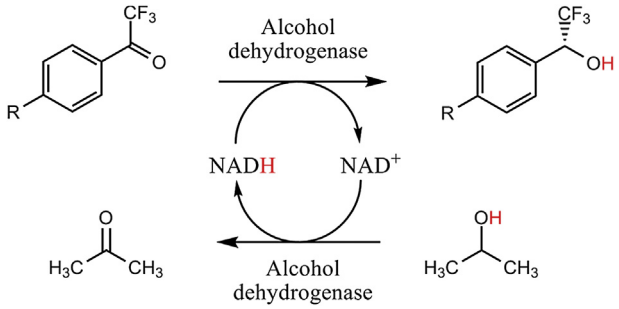
ABSTRACT

Several microorganism strains were used to reduce 2,2,2-trifluoroacetophenone (**1**) and 4′-Br-2,2,2-trifluoroacetophenone (**3**). Immobilized cells of *Geotrichum candidum* in calcium alginate led to conversion and enantiomeric excess higher than 99%. By using immobilized *G. candidum* cells under continuous flow conditions, the same conversion and enantiomeric excess were achieved in 90 min of residence time.

1. Introduction

Chiral trifluoromethyl alcohols play an important role in pharmaceutical, veterinary, agrochemical, and material sciences based on the influence of fluorine's unique properties. Of particular relevance is the emergence of drug candidates featuring fluorine atoms, which often present a favorable therapeutic profile.[1] Chiral trifluoromethyl alcohols can be produced by asymmetric hydrogenation of trifluoromethyl ketones catalyzed by organoboranes,[2–5] (*S*)-binap,[6] chiral organomagnesium amides,[7] chiral rhodium-(I)-complexes[8] or chiral ruthenium-complexes.[9,10] However, some chemical catalysts led to insufficient levels of enantioselectivity and low catalytic efficiencies.

On the other hand, chiral trifluoromethyl alcohols can also be produced by biocatalytic processes.[11–17] Biocatalysis often offers advantages over chemical synthesis, mainly due to higher enantioselectivity, milder and safer reaction conditions and lower environmental impact. Biocatalysts for reduction can be isolated enzymes and whole cells microorganisms, animals or plants. To exhibit catalytic activities, the enzymes require a coenzyme, such as NADH or NADPH from which a hydride is transferred to the substrate carbonyl carbon. Hydrogen sources, as ethanol, 2-propanol, glucose, formic acid or dihydrogen, are necessary to perform the reduction reaction. The reduction of the substrate accompanies the oxidation of the coenzyme from NADH to NAD^+. Then, the coenzyme has to be reduced to NADH, which can be driven by different hydrogen sources. After that, the next cycle of the ketone can occur (Scheme 1).[18–20]



Scheme 1. The cycle of asymmetric reduction of ketone to alcohol by alcohol dehydrogenase, the coenzyme (NADH) and cofactor regeneration (2-propanol).

In comparison to isolated enzymes, the whole cell methodology has distinct characteristics. Enzymes used as whole cells are stable once they are used in their natural environment. Furthermore, the cells have internal coenzyme and cofactor regeneration, so that the addition of cheap glucose is sufficient to drive the reaction.[21–25]

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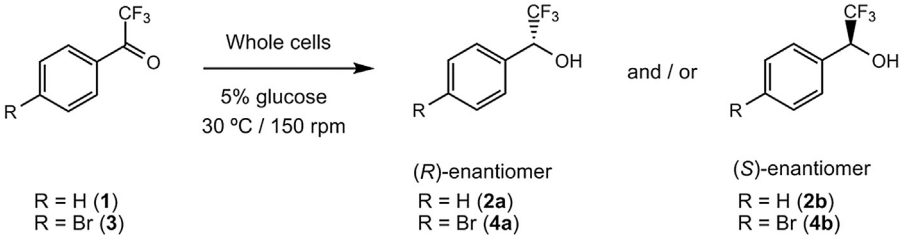
In this work, we report the use of eight different microorganisms (six yeasts strains and two filamentous fungi strains) for the asymmetric reduction of 2,2,2-trifluoroacetophenone (**1**) and 4′-Br-2,2,2-trifluoroacetophenone (**3**) (Scheme 2). Different concentrations of substrate were studied using free cells and immobilized cells (only yeasts strains) under batch and continuous flow conditions.



Scheme 2. Reduction of 2,2,2-trifluoroacetophenone (**1**) and 4′-Br-2,2,2-trifluoroacetophenone (**3**) using whole cells microorganisms.

2. Results and discussion

In the first step of this work, we tested eight different microorganisms (six yeasts strains and two filamentous fungi strains) for the asymmetric reduction of 2,2,2-trifluoroacetophenone (**1**) and 4′-Br-2,2,2-trifluoroacetophenone (**3**) using free cells and substrate concentrations of 7.2 and 14.4 mM (final concentration in 5% glucose solution). According to the results showed in Table 1, all microorganisms were able to reduce completely 2,2,2-trifluoroacetophenone (**1**), and some microorganisms, such as *Geotrichum candidum* and *Aspergillus niger* provided the *S*-enantiomer (**2b**) in enantiomeric excess higher than 90%. *Kluyveromyces marxianus* and *Rhodotorula minuta* were able to produce the *S*-enantiomer (**2b**) in 50% and 45% ee, respectively, while lower ee were obtained with *Hansenula* sp. (24%) and *Candida* sp. (29%). *Mucor ramannianus* and *Rhodotorula rubra* furnished the *R*-enantiomer (**2a**) in 13% and 45% ee, respectively. Interestingly, for some microorganisms the switch from hydrogen to bromine in the 4′ position leads to decreased conversions compared to the 2,2,2-trifluoroacetophenone (**1**). *G. candidum*, *R. minuta*, and *R. rubra* were able to reduce completely 4′-Br-2,2,2-trifluoroacetophenone (**3**). *G. candidum* provided the *S*-enantiomer in 96% ee, while *R. minuta* and *R. rubra* provided the *S*-enantiomer and *R*-enantiomer in 50% and 35% ee, respectively. All reactions were carried out for 24 h at 30 °C under orbital shaking speed of 150 rpm in the orbital shaker.

In order to improve the method, yeasts were immobilized in calcium alginate spheres and tested for the reduction of acetophenone **1** and **3**.[21–25] The immobilization can influence enantiomeric excess and conversion level,[26] however according to the results showed in Table 3, immobilized *Candida* sp., *G. candidum*, *R. minuta*, and *R. rubra* were able to completely reduce 2,2,2-trifluoroacetophenone (**1**). On the other hand, reduction with immobilized *Hansenula* sp. and *K. marxianus* presented lower conversions in comparison to free whole cells.

Considering the 4′-Br-2,2,2-trifluoroacetophenone (**3**), immobilized *Candida* sp., *G. candidum*, *R. minuta*, and *R. rubra* were able to reduce it preserving the same conversion achieved by using free whole cells. Immobilized *Hansenula* sp. and *K. marxianus* increased conversions compared with whole cells. All immobilized microorganisms preserved the enantiomeric excess achieved by using of free whole cells.

Based on Table 3, high ee were achieved for the reduction of 2,2,2-trifluoroacetophenone (**1**) and 4′-Br-2,2,2-trifluoroacetophenone (**3**) by using immobilized cells of *G. candidum*. Then, we decided to increase the substrate concentration to 14.4 mM for this microorganism. The immobilized cells of *G. candidum* were able to reduce the acetophenone **1** with high conversions and enantiomeric excess while for the reduction of acetophenone **3**, the conversion decreased to 56%.

Based on the results obtained on the bioreduction of acetophenones under bath conditions by using immobilized *G. candidum*

Table 1
Bioreduction of 2,2,2-trifluoroacetophenone (**1**) and 4′-Br-2,2,2-trifluoroacetophenone (**3**) by using free cells (with substrates in a 7.2 mM final concentration)

Microorganism	2,2,2-Trifluoroacetophenone (**1**)			4′-Br-2,2,2-trifluoroacetophenone (**3**)		
	Conversion (%)	ee (%)	Space-time yield [g/(L d)]	Conversion (%)	ee (%)	Space-time yield [g/(L d)]
A. niger	>99	90 (*S*)	127	79	94 (*S*)	145
Candida sp.	>99	29 (*S*)	127	62	1 (*S*)	114
G. candidum	>99	91 (*S*)	127	>99	96 (*S*)	183
Hansenula sp.	>99	24 (*S*)	127	39	74 (*S*)	72
K. marxianus	>99	50 (*S*)	127	68	18 (*S*)	125
M. ramannianus	>99	13 (*R*)	127	91	1 (*R*)	167
R. minuta	>99	45 (*S*)	127	>99	50 (*S*)	183
R. rubra	>99	28 (*R*)	127	>99	35 (*R*)	183

Reaction condition: Substrates were previously dissolved in 1 mL of ethanol and added to a mixture of microorganisms and 5% glucose solution to give 50 mL of a solution with final substrate concentration of 7.2 mM. Reactions were carried out for 24 h at 30 °C under a shaking speed of 150 rpm in the orbital shaker. Products were analyzed by (chiral) gas chromatography (GC).

Based on the table above, higher ee were achieved for the reduction of 2,2,2-trifluoroacetophenone (**1**) and 4′-Br-2,2,2-trifluoroacetophenone (**3**) by using *G. candidum* and *A. niger*. Then, in order to enhance the productivity of the reduction with and based on previous results obtained by our group on the bioreduction of ketones in continuous flow with immobilized cells, we decided to move forward towards a continuous flow methodology for such transformation. For such, cells of *G. candidum* immobilized